Amino Acids (2013) 45:563–612
DOI 10.1007/s00726-013-1540-y

ABSTRACTS

Abstracts presented at the 13th International Congress on Amino Acids, Peptides and Proteins

Galveston, Texas

October 5–7, 2013

President: Guoyao Wu

Distinct set of kinases induced after retrieval of spatial memory discriminate memory modulation processes in the mouse hippocampus

Lin Li[1], Ajinkya Sase[1], Sudarshan Patil[1], Berta Sunyer[1], Harald Höger[2], Karl-Heinz Smalla[3], Oliver Stork[4,*], Gert Lubec[1,*]

[1]Department of Pediatrics, Medical University of Vienna, Vienna, Austria;
[2]Core Unit of Biomedical Research, Division of Laboratory Animal Science and Genetics, Medical University of Vienna, Himberg, Austria;
[3]Speziallabor Molekularbiologische Techniken, Leibniz Institute for Neurobiology, Magdeburg, Germany;
[4]Department of Genetics & Molecular Neurobiology, Institute of Biology, Otto-von-Guericke-University Magdeburg, Magdeburg, Germany

Protein phosphorylation and dephosphorylation events play a key role in memory formation and various protein kinases and phosphatases have been firmly associated with memory performance. Here, we determined expression changes of protein kinases and phosphatases following retrieval of spatial memory in CD1 mice in a Morris Water Maze task, using antibody microarrays and confirmatory Western blot. Comparing changes following single and consecutive retrieval, we identified stably and differentially expressed kinases, some of which have never been implicated before in memory functions. Based on these findings we define a small signalling network associated with spatial memory retrieval. Moreover, we describe differential regulation and correlation of expression levels with behavioural performance of polo-like kinase 1. Together with its recently observed genetic association to autism-spectrum disorders our data suggest a role of this kinase in balancing preservation and flexibility of learned behaviour.

Changes of hippocampal β-alanine and citrulline levels parallel early and late phase of retrieval in the Morris Water Maze

Ajinkya Sase[1], Sudath Dahanayaka,[2] Harald Höger,[1] Guoyao Wu[2], and Gert Lubec[1]

[1]Department of Pediatrics, Vienna Medical University, Vienna, Austria;
[2]Department of Animal Science, Texas A&M University, College Station, TX, USA

Although a series of amino acids have been associated with spatial memory formation there is limited information on brain levels of beta-alanine and citrulline in rodents. Given the importance of amino acid metabolism in cognitive functions it was the aim of the study to determine hippocampal levels of beta-alanine and the arginine metabolite citrulline in mice during two different phases of memory retrieval in a spatial memory paradigm. Ten mice were used per group and the first group was trained and sacrificed 5 min, the second 6 h following retrieval in the Morris Water Maze (MWM) and the third and fourth group were untrained, yoked controls that swam the same time in the MWM as their trained counterparts. Hippocampi were taken and free amino acids were determined using a well-established HPLC protocol. β-Alanine levels were higher in trained mouse hippocampi, during both, early and late phase of memory retrieval. Citrulline levels were lower in trained mouse hippocampi, during both, early and late phase of memory retrieval. Taurine, methionine, cysteine, lysine and ornithine levels were higher in yoked mice at the late phase while tyrosine was higher in yoked mice during the early phase. There were no significant correlations between time spent in the target quadrant, the key parameter for spatial memory and any of the amino acid levels. Herein, an amino acid pattern different between yoked and trained animals at early and late phase of animal retrieval is shown indicating probable involvement of different amino acid pathways in animals trained and untrained in the MWM. The results may be useful for the interpretation of previous studies and the design of future research to identify amino acids as possible targets for modulating spatial memory.

SSAT activity determines the speed of prostate cancer cell growth

Jun Li and Heather M Wallace

Division of Applied Medicine, University of Aberdeen, Scotland, UK

Polyamines are required in high amounts by the prostate gland under physiological conditions. In prostate cancer, the polyamine requirement is even higher to support tumour cell growth. Spermidine/spermine N^1-acetyltransferase (SSAT) is a key regulatory enzyme in polyamine metabolism acetylating intracellular polyamines prior to export or recycling. SSAT transgenic cross-bred mice models have shown inconsistent results with either inhibition or promotion of tumour growth occurring (Kee et al. 2004; Tucker et al. 2005). The aim of our study was to determine the influence of SSAT expression and activity on tumour cell growth and to establish whether this would alter the response of the cells to chemotherapy. WT and SSAT human cDNA transfected LNCaP prostate cancer cell lines were used as the model system to investigate the cellular responses to therapy. Aspirin and 5-fluorouracil were chosen and results were compared under conditions of high ($SSAT^+$), low ($SSAT^-$), and normal (WT) SSAT activity. Tetracycline-off (Tet-off) advanced inducible gene system was applied to control *Sat1* gene expression of the SSAT transfected LNCaP cell line (a kind gift from Dr Carl Porter; Roswell Park Memorial Institute, Buffalo, USA). The cells were cultured in RPMI1640 with 10 % (v/v) Tet free foetal bovine serum + 1 mM aminoguanidine + 50 mg/ml G418 + 150 μg/ml hygromycin B + 1 μg/ml Tet. A new method was developed to quantify intracellular and extracellular polyamines using Liquid Chromatography Mass Spectrometry (LC–MS). Cells were grown for 48 h prior to exposure to the drugs. In comparison with WT cell growth, $SSAT^+$ cells grew slower but $SSAT^-$ cells proliferated faster ($SSAT^- > WT > SSAT^+$). Growth of $SSAT^+$ cells was inhibited by aspirin or 5-FU to a greater extent than WT cells, with the least inhibition seen in $SSAT^-$ cells. $SSAT^+$ cells showed a lower rate of total protein synthesis than the others. The total intracellular polyamine content of the cell types differed with $SSAT^+$ cells having 2–3 fold higher content than $SSAT^-$ or WT cells. Enhanced polyamine efflux from $SSAT^+$ cells without treatment resulted in more than 10 fold increase in total extracellular polyamines. Treatment with aspirin or 5-FU decreased total intracellular polyamines further with a simultaneous increase in polyamine efflux. Interestingly, it was the first time, to our knowledge, that N^8-acetylspermidine and N^1,N^{12}-diacetylspermine were found in the culture medium of SSAT overexpressing LNCaP cells, which had only been found previously in the urine of cancer patients. This study suggests that increased polyamine acetylation by SSAT induction could provide a new perspective in understanding cancer cell survival in terms of polyamine homeostasis. SSAT induction could be considered as a non-toxic means of inhibiting cancer cell proliferation.

Cerebrolysin restores amino acid neurotransmitters balance in the brain following traumatic head injury: an experimental study in the rat

Dafin F Muresanu[1], Aruna Sharma[2], Ranjana Patnaik[3], Herbert Mössler[4], Hari S Sharma[2]

[1]Department of Clinical Neurosciences, University Hospital, University of Medicine & Pharmacy, Cluj-Napoca, Romania; [2]Laboratory of Cerebrovascular Research, Department of Surgical Sciences, Anesthesiology & Intensive Care Medicine, University hospital, Uppsala University, SE-75185 Uppsala, Sweden; [3]School of Biomedical Engineering, Department of Biomaterials, National Institute of Technology, Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India; [4]Ever NeuroPharma, Oberburgau, Unterach, Austria

Amino acid neurotransmitters play prominent roles in the pathophysiology of traumatic brain injury and in recovery process. An increased level of excitatory amino acid neurotransmitters e.g., glutamate and aspartate and a decrease concentration of inhibitory amino acids i.e., GABA and glycine leads to brain pathology after trauma. Since a balance between excitatory and inhibitory amino acids neurotransmitter is required for homeostasis in the CNS, drug therapy that induces restoration of normal levels of the excitatory and inhibitory amino acid neurotransmitters in the CNS following trauma may induce neuroprotection. Previous reports from our laboratory showed that Cerebrolysin® (Ever NeuroPharma, Austria) when administered in a dose of 2.5–5 ml/kg, i.v. 30 min before or 1 h after heat stress induces profound neuroprotection in rats. In heat stress increased glutamate and aspartate and a decrease in GABA and Glycine is associated with brain pathology. Cerebrolysin treatment restores this imbalance in amino acid neurotransmitters in hyperthermia induced brain injury. In this investigation we further extended our study on traumatic brain injury model in rats and examined amino acid neurotransmitters in the brains of normal and in cerebrolysin treated animals. Cerebrolysin is a balanced composition of several neurotrophic factors derived from neurons, glial and endothelial cells as well as selective fragments of active peptides involved in neurorepair. Experiments were conducted in Sprague–Dawley rats (200–250 g) under Equithesin anesthesia for injury purposes. Closed head injury (CHI) was delivered on the right parietal cortex of rats under anesthesia by dropping a metal rod of 114.6 g from a 20 cm height inducing 0.224 N force on the surface of parietal bone without inducing any fracture. After injury the animals were allowed to survive 8 and 24 h. In these animals brain tissues were analyzed for amino acid neurotransmitters using HPLC. Our results showed that CHI increased the glutamate and aspartate levels in the cortex by 230–280 % after 8 h injury that was further enhanced to 340–500 % 24 h after CHI in the control group. On the other hand GABA and glycine declined by 80–120 % after 4 h injury and about 167–204 % 24 h after CHI in untreated group. Cerebrolysin treatment (2.5 ml/kg, i.v.) 1 h and 2 h after CHI attenuated these changes by 30–40 % after 8 h CHI and 56–75 % after 24 h injury. Interestingly, 5 ml/kg dose of Cerebrolysin was able to reduce the changes in excitatory amino acid neurotransmitters by 50–60 % after 8 h and 78–87 % 24 h after CHI. The decline in inhibitory amino acids was also reduced by 40–60 % after 8 h CHI and 70–85 % 24 h after injury. Pathological investigations showed a tight correlation between neuronal damages and increase in glutamate and aspartate levels in the cortex in control or drug treated animals after CHI. This suggests that cerebrolysin could influence amino acid neurotransmitter metabolisms in CHI to induce neuroprotection, not reported earlier. The possible mechanism and significance of our finding in relation to strategic aspects of neuroprotection in military using cerebrolysin will be discussed.

Neurotoxicity of single walled carbon nanotubes (SWCNT, 2–10 nm) and neuroprotection by TiO_2 nanowired delivery of cerebrolysin

Hari S Sharma[1], Dafin F Muresanu[2], Ranjana Patnaik[3], Herbert Mössler[4], Aruna Sharma[1]

[1]Laboratory of Cerebrovascular Research, Department of Surgical Sciences, Anesthesiology & Intensive Care Medicine, University hospital, Uppsala University, SE-75185 Uppsala, Sweden; [2]Department of Clinical Neurosciences, University Hospital, University of Medicine & Pharmacy, Cluj-Napoca, Romania; [3]School of Biomedical Engineering, Department of Biomaterials, National Institute of Technology, Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India; [4]Ever NeuroPharma, Oberburgau, Unterach, Austria

Carbon nanotubes (CNT) are now-a-days used for enhanced drug delivery and cancer therapy. Functionalized CNTs may be loaded with drugs to target specific proteins, receptors and tissues in vivo for enhanced therapeutic efficacy. CNTs half-life of 3 h enables them to deliver drugs or other therapeutic agents safely for treatment or diagnostic purposes. However, recent reports suggest necrosis or tissue damage in liver or kidney by SWCNTs but the effects of CNTs per se on brain function are still not well characterized. In this investigation, SWCNTs (outer diameter 2–8 nm, length 0.5–2.0 μm, concentration 1 mg/ml, i.v.) was administered and breakdown of the blood–brain barrier (BBB) to protein tracers, edema formation and neuronal cell injuries were examined in a rat model. To induce possible neuroprotection Cerebrolysin® (Ever NeuroPharma, Austria) was administered either alone or tagged with TiO2 nanowires. SWCNT in a dose of 5 μg or 10 μg/kg/min in Equithesin (3 ml/kg, i.p.) anesthetized rats resulted in BBB breakdown to Evans blue albumin (EBA) and $^{[131]-}$Iodine after 24 h in various areas of the brain. The brain water content also increased in these regions by 2–4 %. Nissl histochemistry showed a tight correlation between neuronal damages and leakage of EBA in the brain 24 h after SWCNT infusion. Cerebrolysin (2.5 ml/kg, i.v.) given 4 h after CNTs infusion reduced these pathological changes by more than 60 %. However nanowired cerebrolysin (2.5 ml/kg, i.v.) given after 4–8 h of CNT infusion resulted in 76–89 % reduction in brain pathologies seen after 24 h Taken together our observations demonstrate that SWCNT induces brain damage and cerebrolysin treatment is able to reduce brain pathology of CNTs in vivo. This effect of cerebrolysin was most pronounced when the drug was delivered using nanowired technology. This suggests that co-administration or adjunct therapy of cerebrolysin is needed in CNTs induced drug delivery or its therapeutic use in cancer therapy. The functional significance of our findings is discussed.

Lipid metabolism in subcutaneous adipose tissue of *Angus steers* by abomasal infusion of conjugated linoleic acid and/or arginine

S. H. Choi, L. A. Gilmore, T. Wickersham, G. Wu, and S. B. Smith

Department of Animal Science, Texas A&M University, College Station, TX 77845, USA

Based on previous research with pigs, we hypothesized that infusion of arginine into the abomasum of Angus steers would stimulate lipogenic enzymes activities and adipogenic gene expression in bovine subcutaneous adipose tissue, and that this would be attenuated by conjugated linoleic acid (CLA). Twenty-four Angus steers were

assigned randomly to four treatments in a modified 2 × 2 factorial design with amino acids (L-arginine or L-alanine) and CLA as the main effects. Amino acids and CLA were infused into the abomasum: CLA, 100 g/day; arginine, 50 g/day; alanine (isonitrogenous control), 100 g/day. During the first period (14 days), steers were infused with arginine or alanine; during the second period (14 days), steers were infused amino acids with and without CLA. Body weight gain and average daily gain were decreased ($P = 0.001$) by infusion of CLA into the abomasum. Abomasal infusion of arginine increased plasma arginine ($P = 0.02$) and ornithine ($P = 0.05$) but decreased threonine ($P = 0.04$). CLA isomers in plasma ($P = 0.001$) and subcutaneous adipose tissue ($P = 0.003$) were increased by infusion of CLA. NADPH-malate dehydrogenase activity in subcutaneous adipose tissues was decreased ($P = 0.01$) by CLA infusion. Lipid synthesis in vitro from glucose and acetate was increased by infusion of arginine plus CLA. Arginine also tended to decrease expression of CPT1-β ($P = 0.067$) and increase *C/EBP-β* expression ($P = 0.067$) in subcutaneous adipose tissue. The increases in lipogenesis and adipogenic gene expression caused by arginine infusion were consistent with our original hypothesis, that arginine promotes adipogenesis in beef cattle. However, CLA infusion had no effect on adipogenic gene expression.

Nanotechnology and central nervous system drug delivery

G. Tosi[1,*], B. Ruozi[1], A. Vilella[1], D. Belletti[1], P. Veratti[1], E. Baraldi[1], M. Zoli[2], A. Grabrucker[3], A. Sharma[4], H.S. Sharma[4], F. Forni[1], M. Vandelli[1]

[1]Pharmaceut. Sci., Te.far.t.I, Dept of Pharmaceut. Sciences, Univ. of Modena and Reggio Emilia, Modena, Italy;
[2]Biomed. Sci., Dept of Biomed. Sciences, Univ. of Modena and Reggio Emilia, Modena, Italy;
[3]Dept. of Psychiatry and Behavioral Sci., Univ. of Ulm, Ulm, Germany

In line with the overall increase in knowledge and nanotechnologies, surface engineering of nano-sized carriers is now representing the cutting edge of nanomedicine, leading to the production of selectively targeted therapies based on targeted nanocarriers. In fact, achieving nanocarriers able to be stable in the blood-stream, to protect the drug from metabolism and to promote a long-lasting release of the drug is still a pivotal pre-requisite for nanomedicine, but it is now to be considered as "not enough". Active targeting to specific pathological cells is now the challenge of pharmaceutical nanotechnologists, who are facing with difficulties in colloidal chemistry and most of all in the characterization of the engineered nanocarriers from a technological and physiological points of view. As an example, the application of nanotechnology to brain-related disorders, called nanoneuromedicine, is surely representing one of the most stimulating challenge as well as one the most difficult due to the presence of biological barriers (BBB) and the great variability in BBB permeability depending on the chosen disease. Encouraging results have been obtained demonstrating the possibility of targeting the CNS up to an important percentage of brain localization. In this contest, polymeric nanoparticles (NPs) and liposomes (LPs) were formulated and specifically engineered to cross the BBB and arrive to CNS and proposed to encapsulate some drugs able to rescue from neurodegeneration, to the CNS. Our attention point on the use of polymeric nanoparticles engineered on surface by a selective ligand able to promote the NPs crossing of BBB. In fact, preliminary studies demonstrated the ability of new targeted polymeric poly-lactide-co-glycolide (PLGA) NPs modified with a short peptide (H_2N-Gly-L-Phe-D-Thr-Gly-L-Phe-L-Leu-L-Ser(O-β-D-Glucose)-$CONH_2$ (g7-NPs) to create BBB interaction and trigger an efficacious BBB crossing delivering of active. In particular, several in vivo biodistribution studies and pharmacological proof-of-evidence of brain delivery of model drugs (not able by themselves to reach the brain) demonstrated the ability of g7-NPs to create BBB interaction and trigger an efficacious BBB crossing. A total biodistribution of g7-NPs, obtained after i.v. administration in rats, evidenced a strong and significant localization of the g7-NPs into CNS in a quantity about two orders of magnitude greater (10–15 %) than that found with the other known NP drug carriers. More recently, the g7-NP BBB crossing mechanism was investigated, pointing out an interaction between g7-NPs and BBB and endocytosis/macropinocytosis pathways for BBB crossing. Same results were pointed out also in vitro on neurons/glia cell cultures, evidencing the endocytotic pathways as g7-NPs cell entrance as well as the assessing of the safety of g7-NPs not creating any damage to cells even at high doses. Notwithstanding these outputs, it is our opinion that in order to obtain a real update of neurological disorders' therapy based on innovative and non invasive protocols (i.e. nanomedicine), a team work is strongly needed. The interdisciplinary competences and skills of all the experts in Neuro-diseases and Nano-Technology (from neurobiologists to neurophysiologist, from nanotechnologists to physicians) must be shared, discussed, considered and applied, thus opening the pave to new vistas in treatments and most of all for the correct development of the research.

Identification of carboxypeptidase E in human semen which binds to heparin and exhibits antibacterial property

Sanjay Kumar[1], Anil Kumar Tomar[1], Sudhuman Singh[2], Kamaldeep Gill[1], Sharmistha Dey[1], Sarman Singh[3], Savita Yadav[1,*]

[1]Department of Biophysics, All India Institute of Medical Sciences, New Delhi, India;
[2]School of Life Sciences, Jawaharlal Nehru University, New Delhi, India;
[3]Department of Laboratory Medicine, All India Institute of Medical Sciences, New Delhi, India

Carboxypeptidase E (CPE) cleaves basic amino acids residues at C-terminal end and involves in the biosynthesis of numerous peptide hormones and neurotransmitters. It was purified from human seminal plasma by ion exchange, heparin affinity and gel filtration chromatography followed by identification through SDS-PAGE and MALDI-TOF/MS analysis, which was further confirmed by western blotting. CPE was characterized as glycoprotein by Periodic Acid Schiff's (PAS) staining and treating with deglycosylating enzyme N-glycosidase F. The interaction of CPE with heparin was determined by Surface Plasmon Resonance (SPR) and also by in silico structure analysis. The association constant (K_A) and dissociation constant (K_D) of CPE for heparin was determined by SPR and found to be 1.06×10^{-4} and 9.46×10^{-5} M respectively. A cavity formed by amino acid residues including ASN172, LYS175, MET195, PRO198, SER292, ASN293, ASN348, THR361, ALA363, LYS364, ARG33, ILE34, TYR35, THR36, HIS58 and LEU169 in CPE were predicted as heparin binding site by in silico structure analysis. It was further detected in human spermatozoa lysates by western blotting using mouse anti CPE primary antibody. The localization of CPE in whole head region of spermatozoa was visualized by Indirect Immunofluorescence (IIF). 20–100 μg/ml concentration of CPE was observed as highly effective in killing *E. coli* by Colony Forming Unit (CFU) assay. Scanning Electron Microscope (SEM) experiment has demonstrated the membrane dependent bacterial killing by CPE. We suggest that CPE might act not only in the innate

immunity of male reproductive tract but also regulate sperm fertilization process by interacting heparin.

L-Amino acids as chiral auxiliaries to develop reagents for enantioseparation of DL-selenomethionine by reversed–phase high-performance liquid chromatography

Hariom Nagar and Ravi Bhushan

Department of Chemistry, Indian Institute of Technology Roorkee, Roorkee – 247667, India

Optically pure amino acids L-Ala, L-Val, L-Leu and S-methyl-L-Cysteine were used to synthesise chiral derivatizing reagents with fluoro dinitro benzene as the chromophore; these are FDNP-L-Ala, FDNP-L-Val, FDNP-L-Leu and FDNP-SMLC). The reagents were characterised using UV, IR, CHN, and ^{1}H NMR. Diastereomers of selenomethionine were synthesized with the nucleophilic substitution of remaining fluorine atom in these CDRs under microwave irradiation for 55 s at 75 % (of 800 W) and also by stirring for 50 min at 45 °C. The diastereomers were enantioseparated by reversed-phase high-performance liquid chromatography on a C_{18} column with detection at 340 nm using gradient elution with mobile phases containing *aq* TFA (0.1 %)-MeCN and by reversed-phase thin layer chromatography with mobile phases containing *aq* TEAP (50 mM)-MeCN in different compositions. The conditions of derivatization and chromatographic separation were optimized. The method was validated for accuracy, precision, limit of detection and limit of quantification.

Computational strategies to design new highly potential BSAO polyamine substrates

Flavio Ballante[‡,*], Giampiero Tempera[†], Enzo Agostinelli[†], Rino Ragno[‡]

[‡]Rome Center for Molecular Design, Department of Drug Chemistry and Technologies, "Sapienza" University of Rome, P.le A. Moro 5, 00185 Rome, Italy;
[†]Istituto Pasteur Fondazione Cenci Bolognetti, Department of Biochemical Sciences "A. Rossi Fanelli", "Sapienza" University of Rome and CNR, Biology and Molecular Pathology Institute, Piazzale Aldo Moro 5, 00185 Rome, Italy

Natural polyamines putrescine, spermidine and spermine are ubiquitous polycationic compounds present in significant amounts in nearly every prokaryotic and eukaryotic cell type. Spermidine and spermine primarily exist in aqueous solution at pH 7.4 as fully protonated polycations. Such ubiquitous chemical entities play an important role in cell growth and proliferation, in the synthesis of proteins and nucleic acids, in both normal and cancer cells. Preliminary structure based (SB) studies through the AutoDock suite were performed on 25 among natural polyamines and newly synthesized and biologically assayed polyamine analogs in order to clarify their binding modes. Further investigations through a combined approach of docking and 3-D QSAR and COMBINE procedures, named 3-D QSAutogrid/R and COMBINEr respectively, are in due course to rationalize in a multi-informative scenario the different activity profiles and derive a useful pharmacophoric frame able to weight the different ligand-residues interactions magnitudes. Such approach will be useful for the development of novel compounds endowed of both higher potency and selectivity. As future perspective, these molecules will be assayed alone or in combination with BSAO on several cancer cells, with the aim to evaluate their cytotoxic effects that could be taken into consideration as new approach in anti-cancer therapy. Details and methodologies will be reported.

Protective effect of glutamic acid against oxidative stress in rats

N.O. Salyha

Institute of Animal Biology NAAS, V. Stus street, 38 Lviv, Ukraine 79034

Metabolic processes occurring in animal and human organisms at the times of stresses and diseases lead to the use of a large amount of L-glutamic acid. White male Wistar rats (3 months old), 200–220 g body weight, were divided in three groups. Each group consisted of 10 animals. Each animal received 20 g of food per day. Animals of all groups were healthy. The rats were sacrificed under anesthesia 4 weeks after. This study was conducted to determine effects of supplementing L-glutamic acid to the standard rodent diet (containing 17 % crude protein) on activities of antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase) and the intensity of lipid peroxidation in tissues of rats. The supplemental doses of L-glutamic acid were determined on the basis of crude protein content in the basal diet. Animals received dietary supplementation with 0 % (control group), 10 and 25 % of L-glutamic acid (second and third group respectively). The obtained data are treated statistically. To determine the probable differences between mean values using Student's test. Results indicated that dietary supplementation with 25 % of L-glutamic acid increased the concentrations of reduced glutathione and glutathione peroxidase activity in the liver, spleen and kidneys, while decreasing the concentrations of lipid hydroperoxides and TBA-active products in these tissues. Supplementation with 10 and 25 % L-glutamic acid enhanced catalase and superoxide dismutase activities in erythrocytes. In all the measured variables, L-glutamic acid supplementation elicited dose-dependent responses. Collectively, dietary supplementation has beneficial effects on antioxidative system, thereby reducing lipid peroxidation.

Ageing: a public health concern and an opportunity for ICAAS

Luc Cynober

Dept of Clinical Chemistry, Cochin and Hôtel-Dieu Hospitals, AP-HP, Paris, and
Dept of Experimental, Metabolic and Clinical Biology, Laboratory of Biological Nutrition EA 4466,
Faculty of Pharmacy, Paris Descartes University, Paris, France

In France, a third of the population will be aged over 60 in 2050 *versus* 20 % in 2005. In the USA, the number of people aged $\geq$65 is forecast to double between 2010 and 2050 (from 40 to 88 million). At least a third of this population is very healthy, and 25 % get regular physical exercise. Those who are healthy or relatively healthy want to stay that way, and so are frequently dietary supplement users, although the benefits and risks remain uncertain, particularly at high intake levels. Among the dietary supplements available, amino acids (AAs) are of special interest in this subpopulation, as they target functions like muscular and cognitive function that typically decline with age, and some AAs have

anti-oxidant properties—oxidative stress appears to be involved in these overriding signs of aging and is a major factor in other alterations such as ageing of the skin and development of fat mass. Leucine, which will be largely discussed during this symposium, plays is lead signalling role for muscle protein synthesis and could be a useful tool for preventing sarcopenia. Short-term studies clearly demonstrate that leucine is able to improve protein synthesis, but the results are not so clear on long-term administration in humans, suggesting metabolic adaptations occur with time and/or with aging. Work sponsored by the International Council on Amino Acid Sciences (ICAAS) recently established the upper level of safe intake (ULSI) of leucine for healthy adults. Establishing the ULSI in the ageing sub-population is the next urgent task for the ICAAS. Citrulline, like leucine, regulates muscle protein synthesis through stimulation of the mTOR pathway. Long-term studies in old rats show that citrulline increases muscle mass and function and decreases fat mass. In addition, citrulline, likely through its anti-oxidant properties, also blunts the age-related weight increase of lipid rafts in the brain, which may be important for preserving cognitive functions. Citrulline looks to be very safe, but no ULSI has yet been established, making it another urgent task.

Amino acid metabolism in aging

Naomi K. Fukagawa

Gerontology Unit, University of Vermont, Burlington, VT 05405, USA

The aging process is a continuum throughout adult life and often associated with a deterioration of body function as well as the accumulation of chronic disabilities and of disease. There is general agreement that some of the physiologic, metabolic, and biochemical processes that change with advancing age can have adverse effects on the nutritional status of the elderly. For example, changes in taste acuity, smell and vision may interfere with eating and the enjoyment of food. Lower metabolic rates and a decline in carbohydrate tolerance may render the older person more vulnerable to obesity and diabetes. The impact of nutritional status on morbidity and mortality is unquestioned. Malnutrition increases the risk for frailty and nutritional deficits can influence immune status, response to medical treatments and recovery from acute illnesses, including surgery. Health-promoting interventions implemented individually, such as exercise programs, preventive home visits, comprehensive geriatric evaluation and management, and attention to adequate nutrition with or without nutritional supplements, have been shown in separate studies to be both feasible and effective in reducing deterioration as one ages. However, it is imperative that dietary recommendations be based on scientific data that take into consideration individual variability and the importance of aligning the needs, preferences and values of people, including families involved in the care of their elders. Protein and its constituent amino acids are key components of any healthy diet. Sarcopenia, the slow but progressive loss of lean muscle mass associated with advancing age, has been the focus of many studies related to nutrition and aging but there is no clear-cut answer to the question of how to restrain the process. The more general question of how the requirements for protein and specific amino acids change with age continues to be investigated. However, the over the last few decades, research began to shift towards studying the efficacy and safety of specific amino acids or combination of amino acids that may sustain and/or enhance physiologic processes, ranging from specific tissue metabolism to overall function (e.g. exercise performance, immune function, cognition, and chronic disease development). This review will focus on the use of specific amino acids in older individuals as supplements designed to attenuate common age-related changes or to enhance specific functions that may help older individuals to remain healthy and independent as long as possible.

Essential amino acids for sarcopenia: what is the evidence?

Elena Volpi

University of Texas Medical Branch, Galveston, TX, USA

Sarcopenia, the loss of muscle mass and function with aging, is a significant contributor to frailty and mobility disability in older adults. Reduced muscle protein anabolism in response to nutrient intake has been identified as one of the mechanisms that underlie sarcopenia. Specifically, a reduced response of muscle protein synthesis to low doses of amino acids as well as insulin resistance has been reported in otherwise healthy older adults. Nonetheless, evidence is accumulating suggesting that larger doses of amino acids or leucine can effectively stimulate muscle protein synthesis and improve overall protein anabolism in older adults. Initial data from clinical trials suggest that amino acid or leucine supplementation may also improve muscle size and function. Further studies will be necessary to determine the impact of such interventions on overall physical functioning, mobility and overall health in older persons.

Essential amino acid requirements of elderly adults

Wayne W. Campbell

Department of Nutrition Science, Purdue University, West Lafayette, IN, 47907, USA

The essential or indispensable amino acids (IAA) (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan and valine) must be obtained from the diet because they cannot be synthesized de novo in humans. National and international consensus reports on dietary protein and amino acid requirements (2002/2005 Food and Nutrition Board, Institute of Medicine; 2007 WHO/FAO/UNU) provide reasonably similar estimates of IAA requirements for adults, based on currently accepted assessment techniques. Both reports acknowledge variability of findings among research studies, which softens certainty of the estimated values. Researchers have speculated that metabolic and physiological changes with advancing age, including changes in splanchnic handling of amino acids, whole body and skeletal muscle amino acid use for protein synthesis, and body composition, might alter the metabolism and dietary requirement for IAA. Results from very limited isotope kinetics studies performed with apparently healthy adults suggest aging does not appreciably influence amino acid turnover, and presumably dietary requirement. The influences of age-associated morbidities, e.g., impaired glucose tolerance, hyperinsulinemia, and diabetes; chronic inflammation; sarcopenia (with and without obesity) and frailty; compromised organ function; and other catabolic states require investigation. The glaring paucity of data on the effects of age on IAA requirements prompted the Food and Nutrition Board and WHO/FAO/UNU to conclude that IAA intake recommendations specifically for older or elderly adults were not possible. By default, IAA recommendations established primarily from research conducted with young adults were also extrapolated to apply to older and elderly people.

Protein, calories and sarcopenia

John E. Morley

Division of Geriatric Medicine and Division of Endocrinology, Saint Louis University Medical Center, St. Louis, MO, USA

Loss of muscle mass in older persons (i.e. sarcopenia) is a major problem leading to functional deterioration. There is increasing evidence that a leucine enriched essential amino acid supplement may play a key role in preventing and treating sarcopenia. The SCWD Society has recommended at least 1.2 g/kg of protein a day for persons with sarcopenia. There is evidence that protein supplementation may act synergistically with resistance exercise to improve muscle function. By having a direct effect on *m*TOR essential amino acids act not only as building blocks but also accelerate protein synthesis. Protein supplements should be given between meals and in juxtaposition to exercise to be optimally effective. Anorexia and weight loss are common problems in older persons. Depression is a major cause. For persons suffering from weight loss, protein enriched calorie supplements play an important role in reversing weight loss.

Effects of amino acids on skeletal muscle protein metabolism in young and older aged individuals: past, present and future directions

John Atherton

School of Clinical Sciences and Faculty of Medicine & Health Sciences, University of Nottingham, Uttoxeter Road, Derby, DE22 3DT, UK

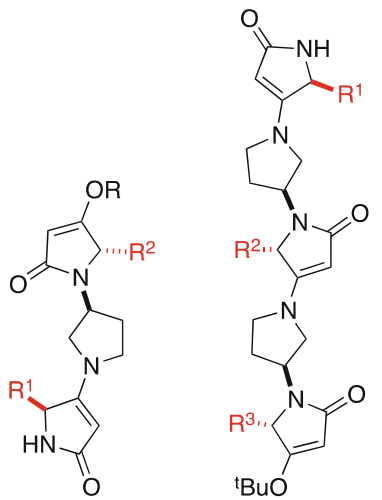
Increasing the plasma availability of essential amino acids (EAA) by exogenous supply (orally or intravenously) or via oral protein sources stimulates skeletal muscle anabolism. Mechanistically, EAA-induced increases in muscle protein synthesis (MPS) are initiated after AA-transportation into the muscle cell whereupon leucine in particular (but not exclusively) indirectly activates the mammalian target of rapamycin complex-1 (mTORc1), independently of proximal insulin signaling [phosphatidylinositol 3-kinase (PI3K)] pathways. Subsequent downstream signaling enhances translational initiation via activating mTORc1 substrates (e.g. p70S6K1, 4EBP1) culminating in ribosomal assembly, polyribosome formation and increased MPS. In terms of kinetics, skeletal muscles are receptive to the anabolic effects of EAA for only a short period, equating to ~2 h in the rested state (even after ~50 g EAA-rich protein), thereafter becoming refractory despite continued EAA availability and mTORc1 signalling. This phenomenon has been termed "muscle-full", whereby muscles intrinsically sense "excess" EAA and divert them to oxidation. Episodes of physical activity (i.e., exercise) are able to delay the muscle full signal to facilitate muscle adaptation (e.g. cellular remodelling/hypertrophy), in accordance to the nature of the exercise activity e.g. resistance vs. endurance. Intake of EAA also provides a second route for muscle anabolism via suppression of muscle protein breakdown (MPB). This occurs independently to *direct* effects of EAA on MPB since EAA supply under insulin clamped conditions (postabsorptive ~5μU/ml) is insufficient to suppress MPB. Instead, the release of insulin in response to nutrition causes suppression of MPB (~15μU/ml provides ~50 % suppression). On this basis, the robust insulin secretagogue properties of EAA are likely sufficient to increase plasma insulin enough to supress MPB, and maximise nutrient-driven muscle anabolism, even in the absence of carbohydrates. To facilitate delivery of AA and insulin to the cell-capillary interface, nutrients also modulate peripheral vascular function. Indeed, while insulin is known to increase blood flow to skeletal muscle, certain AA (e.g. arginine, the precursor for nitric oxide) can also enhance muscle macro/microvascular flow. Therefore, an appreciation of prandial coordination between the endocrine pancreas, peripheral vasculature, and muscle cells is needed to fully understand the effects of AA on muscle protein anabolism. In terms of aging, it has been demonstrated that the transient anabolic effects of EAA are blunted in older age (vs. younger groups) providing evidence for the existence of 'anabolic resistance'. This phenomenon has been proven in both pre-clinical and clinical scenarios seemingly as an "all-cause" mechanism of muscle atrophy; though how much this relates to inactivity/sedentarism in older people remains to be definitively determined. Nonetheless, this has lead to suggestion that increasing dietary EAA and/or enhancing the anabolic effects to EAA would represent an important strategy to offset sarcopenia. To conclude, optimal EAA-feeding strategies for older individuals need to be viewed in the context of the vascular/endocrine pancreas/muscle- axis and in terms of interactions with physical activity; comprehension of all these facets will likely be key to exploiting the anabolic efficacy of EAA and minimizing impacts of sarcopenia in older age.

EKO: a method to discover small molecules to perturb protein–protein interactions

Kevin Burgess

Department of Chemistry, Texas A&M University, PO Box 30012, College Station, TX, USA 77842-3012

Small molecules that perturb protein–protein interactions (PPIs) have enormous potential in the treatment of diseases, but they are difficult to discover or design. For instance, high throughput screening, and the various fragment-based approaches have limitations that are so severe that in some cases the data obtained does not justify the cost and time invested in these methods. Very recently, our group has devised a new approach to the problem of finding molecules that perturb specific PPIs; we call this *Exploring Key Orientations.* It involves defining a set of chemotypes for molecules that are ideally suited to this function (two examples are shown below), then matching their preferred conformations with structural features of PPI-interface regions on a massive scale. Significantly, it is a *chemistry-centered* method where small molecule design takes priority over bioassay considerations.



The pH sensitive bioactive peptides for wide biomedical applications

Long Chen, Riddhi Kharidia, Zhigang Tu, Nicole Patrone, Jun F. Liang, Charles V. Schaefer

Departments of Chemistry, Chemical Biology, and Biomedical Engineering,
School of Engineering and Sciences, Stevens Institute of Technology, Hoboken, NJ 07030, USA

Bioactive peptides with pH-dependent activities are of great importance for biomedical research and disease treatments. For examples, it has been found that the extracellular pH of some solid tumor are consistently lower as compared to the pH = 7.2–7.4 in normal tissues/organs. Therefore, if bioactive peptides with appropriate pH sensitive toxicity that fit the extracellular pH difference between tumor and normal tissues, they should be able to kill tumors in acidic environments selectively but spare normal tissues with physiological pH. We had developed a general approach to construct pH-sensitive peptides by replacing corresponding arginine and lysine residues in bioactive peptides with histidines. Resulting peptides could respond environment pHs to show 3–30 times activity changes. The secondary structures and chemical/physical properties of pH-sensitive peptides were studied using CD spectropolarimeter, atomic force microscopy, scanning electron microscopy. We also studied the acting mechanisms of pH-sensitive peptides on artificial cells by using Langmuir–Blodgett trough, microbalance, and fluorescence quenching technology. In addition, potential applications of pH-sensitive peptides in the treatment of cancers and various bacterial infections were evaluated through in vitro and in vivo studies.

Rational design of peptidomimetics for mimicking protein helices and targeting androgen receptor in prostate cancer

Jung-Mo Ahn

Department of Chemistry, University of Texas at Dallas, Richardson, TX 75080, USA

Protein–protein interactions are one of the fundamental processes that regulate numerous key cellular pathways. Since α-helical structures are frequently found in protein complex formations, short helical peptides have been considered as a valuable tool for research and clinical applications. However, peptides in general have drawbacks that severely compromise their effective in vivo use, such as rapid enzymatic degradation, poor bioavailability, and lack of membrane penetration. Thus, small molecules that mimic functions of helical peptides would be of great interest. To the end, we have designed oligo-benzamides as versatile scaffolds to emulate protein helical surfaces. The rigid oligo-benzamide scaffolds can present 2–3 functional groups corresponding to the side chains found at the *i*, *i* + *4*, and *i* + *7* positions in a helix. In addition to the outstanding α-helix mimicry, oligo-benzamides can be efficiently synthesized by following high-yielding and iterative reaction steps in solution- and solid-phase. Furthermore, the oligo-benzamide scaffolds can be easily modified to mimic helical amphiphilicity that may improve binding affinity and selectivity to target proteins. In order to evaluate the oligo-benzamide scaffolds, we have designed peptidomimetics of helical segments in various proteins including androgen receptor (AR). AR interacts with its coactivator proteins to exert its functions by using helical LXXLL motifs. These helix-mimicking small molecules have successfully demonstrated their utilities in disrupting AR-coactivator protein complex formation, inhibiting AR-mediated gene transcription, and blocking AR-mediated cell proliferation. These exciting results indicate that oligo-benzamides are effective tools to mimic functions of α-helices and have a high therapeutic potential.

Purification and antioxidant activity analysis of solid state fermentation sesame meal peptide

Li Lv-mu, Peng Hui-hui

College of Tea & Food Science and Technology, Anhui Agricultural University, Hefei 230036, China

This experiment was performed to analyses antioxidant activity of peptide from solid state fermentation sesame meal. The isolation of peptide was from aqueous extract of sesame meal fermented by microorganism. Three components could be got when extracting solution of sesame peptide was separated by Sephadex-G15 gel chromatographic column. The peptides distributions were 3, 4 and 6. Antioxidant activities of the sesame peptide were analyzed. The scavenging ability of DPPH and ·OH was determined by the 2,2′-diphenyl-1-picrylhydrazyl (DPPH) assays and the salicylic acid method respectively. The total reducing power was measured by ferric reduction ability. At 1 mg/ml, the DPPH scavenging activity of the extracting of sesame peptide, 3 peptide and 4 peptide were 90 %, 6 peptide only was 80 %. At 0.6 mg/ml, the ·OH scavenging activity of the extracting of 3 and 4 peptide were 100 %, the extracting of sesame peptide and 6 peptide only were 90 %. At 4 mg/ml, the reducing powers of the extracting of the extracting of sesame peptide, 4 and 6 peptide were corresponded to glutathione whose concentrations was 0.5 mg/ml, and the same to 3 peptide at 2 mg/ml. The results were as follow: the sesame peptide has stronger reducing activity and the ability of scavenging DPPH and ·OH. The antioxidant activity was enhanced with sesame peptide molecular weight reduced.

Liquid chromatographic enantioseparation of fifteen DL-amino acids using new reagents having *(S)*-(+)-1-benzyl-3-aminopyrrolidine as chiral auxiliary on cyanuric chloride platform

Ravi Bhushan and Manohar Lal

Department of Chemistry, Indian Institute of Technology Roorkee, Roorkee – 247667, India

Diastereomers of fifteen DL-proteinogenic amino acids were synthesized under microwave irradiation using two new chiral derivatizing reagents. The diastereomers of 15 amino acids were separated using C_{18} column and a linear gradient of mobile phase from 100 % A (Water-MeCN, 90:10) to 100 % B (Water-MeCN, 10:90), both containing TFA, in 50 min (flow rate 1.0 ml/min, detection at 230 nm); concentrations of organic modifier, TFA and flow rate were optimized. Total 30 pairs of diastereomers were separated. The peaks of a diastereomeric pair, captured by PDA detector, and the areas obtained from the system software (found in the ratio of 1:1) demonstrated that derivatization reaction and separation of diastereomers had successfully occurred. The recovery studies of the two eluted diastereomers also served as a measure of their yields of the order of 98 %. It was observed that the *k* values and *Rs* obtained on linear gradient elution were higher than those observed under isocratic conditions. Retention times and resolution showed a good correlation with the decreasing hydrophobicity of amino acids. The

elution order of diastereomers was confirmed. The diastereomers were found to be stable for 90 days under the refrigeration conditions (3–5 °C). Enantiomerically pure *(S)*-(+)-1-benzyl-3-aminopyrrolidine was introduced as chiral auxiliary in cyanuric chloride (CC) and its 6-butoxy derivative providing two chiral derivatizing reagents (CDR 1 and 2). These were characterized and their optical purity was ascertained.

Separation and analysis of thirty component mixture of diastereomers of proteinogenic amino acids by liquid chromatography using two new cyanuric chloride based reagents having *(R)*-(+)-naphthylethyl amine as chiral auxiliary

Manohar Lal and Ravi Bhushan

Department of Chemistry, Indian Institute of Technology Roorkee, Roorkee – 247667, India

A sensitive method was developed for separation of 19 diastereomers from a mixture of thirty and determination of D-amino acids in scalemic mixtures in the absence of pure D-enantiomers. Diastereomers of fifteen DL-proteinogenic amino acids were synthesized under microwave irradiation using two new chiral derivatizing reagents (CDRs). The mixture of 30 diastereomers was separated on C_{18} column in a single run using a linear gradient of mobile phase from 100 % A (Water-MeCN, 90:10) to 100 % B (Water-MeCN, 10:90), containing TFA, in 50 min (flow rate 1.0 ml/min, detection at 230 nm). Chromatographic conditions were optimized. UV spectra of each pair of diastereomers were captured with PDA detector. Comparison of chromatogram of the multicomponent mixture with the chromatograms of the diastereomeric pairs of individual amino acids revealed that a few of the diastereomers co-eluted. Yields and stability of diastereomers were established. Enantiomerically pure *(R)*-(+)-naphthylethyl amine was introduced as chiral auxiliary in cyanuric chloride and its 6-butoxy derivative by nucleophilic substitution of one chlorine atom in each of them. CDRs were characterized; their optical purity was ascertained. Conditions of derivatization were optimized. Possibility of racemization of amino acids during the course of the derivatization was investigated. Peak areas corresponding to the two diastereomers (obtained from the system software) served as a measure/indicator of completion of derivatization reactions.

Expression and characterization of the soluble domain of gaur [*Cyamopsis tetragonoloba* (L.) Taub.] β-mannan synthase in a heterologous system

Pallavi Gahlot[1], Shilpi Kumari[1], Kanwarpal Singh Dhugga[2], and G. S. Randhawa[1]

[1]Department of Biotechnology, Indian Institute of Technology Roorkee, Roorkee-247667, India;
[2]Crop Research & Development, 7300 NW 62nd Avenue, Johnston, IA 50131, USA

Mannan synthase, a Golgi protein of guar with five predicted transmembrane domains, makes the mannan backbone of galactomannan. Galactomannan is a hemicellulosic cell wall storage polysaccharide, which constitutes more than 90 % of the seed endosperm at maturity. The mannan synthase gene, which encodes a polypeptide of 526 amino acids, is specifically expressed in the endosperm of developing seeds with peak expression at 25–30 days after flowering. A cytosolic domain of 296 amino acids between the first two transmembrane domains contains all the conserved residues known to occur in polymerizing β-glycosyltransferases. In this study, the region encoding this cytosolic domain alone and in different configurations with one or two transmembrane domains bracketing it on either side was cloned and expressed in a heterologous protein expression system. This study will aid in determining whether the cytosolic domain catalyses the enzyme activity in vitro in the absence of the remaining protein as well as the membrane environment. Furthermore, we will attempt to crystallize the expressed protein to determine its structure.

Polyamines added to the culture medium of *Xenopus* fertilized eggs induce massive cell dissociation at the blastula stage by interfering with Ca^{2+}-dependent cell adhesion

Koichiro Shiokawa[1,2], Takeshi Kondo[2], Senji Takahashi[2], and Kazuei Igarashi[3]

[1]Department of Judo Therapy, Faculty of Medical Technology, Teikyo University, 1-1 Toyosatodai, Utsunomiya 320-8551, Tochigi, Japan;
[2]Department of Biosciences, School of Science and Engineering, Teikyo University, 1-1 Toyosatodai, Utsunomiya 320-8551, Tochigi, Japan;
[3]Graduate School of Pharmaceutical Sciences, Chiba University, 1-8-1 Inohana Chuo-ku, Chiba 260-8675, Japan

Polyamines (putrescine, spermidine, and spermine) occur ubiquitously in both prokaryotic and eukaryotic cells, but their biological functions in the course of animal development have not been clarified yet. We have been interested in elucidating the effects of polyamines exogenously added into the culture medium of *Xenopus* embryos. We cultured *Xenopus* fertilized eggs in the 0.1× Steinberg's solution that contained different amounts of putrescine (1–50 mM), spermidine (0.5–10 mM), or spermine (0.1–5 mM). All these polyamines at the higher concentration induced cell dissociation followed by embryo death at the blastula stage, whereas at the lower concentrations they did not induce cell dissociation, but instead induced bending of the embryo axis into the ventral side at the tadpole stage. Analyses of polyamine contents revealed that exogenously added polyamines were somehow uptaken by embryos. We suspected that the cell dissociation was induced by the execution of the maternal program of apoptosis by the toxic effect of polyamines. However, we detected neither DNA ladder formation, nor activation of caspases in the dissociated embryos, and furthermore, we found that embryos were not dissociated and developed into normal tadpoles when they were similarly treated with polyamines in 1× Steinberg's solution. We then searched the component within the Steinberg's solution that abolished the polyamine-induced cell dissociating effects. We found here that only Ca^{2+} (0.3 mmol), but not Mg^{2+} (even at 10 mmol) and others, was effective in abolishing the polyamine effects. Since polyamine-induced cell dissociation was almost completely suppressed by the elevated level of Ca^{2+}, we assumed that polyamines added into 0.1× Steinberg's solution inhibited Ca^{2+}-dependent cell adhesion to induce

cell dissociation, probably by interfering with binding of Ca^{2+} to cadherin.

Glutamate-glutamine outflow from fresh human cortical slices

Katharina Prauss, Marlen Haegele, Andreas Moser

Department of Neurology, University of Luebeck, Ratzeburger Allee 160, D-23538 Luebeck, Germany

Glutamate is thought to be the most important excitatory neurotransmitter in the CNS, while glutamine predominantly serves as a precursor and metabolite in the glutamine-glutamate cycle. To study the effect of intrinsic extracellular glutamate levels on glial glutamine outflow, human cortical slices were incubated in superfusion chambers in vitro. Fresh human neocortical tissue of frontal and temporal areas was obtained during surgical treatment of drug-resistant epilepsy or of subcortical brain tumors. For superfusion experiments, the white matter was discarded from the gray matter, which finally contained all six neocortical layers. Gray matter was chopped and tissue slices were transferred to superfusion chambers. Outflows of endogenous glutamate and glutamine were established after a 40-min washout period and amounts were quantified after two-phase derivatization by high performance liquid chromatography with electrochemical detection. Under basal conditions glutamate:glutamine ratio of extracellular levels was approximately 1:2. After addition of the voltage-gated sodium channel (vgNaCh) blocker tetrodotoxin glutamate levels decreased to 70 % and the ratio was still 1:2. After activation of vgNaCh by veratridine, glutamate markedly increased to 13 pmol/mg/min during the whole incubation time. Glutamine also rapidly increased to 25 pmol/mg/min but decreased afterwards during veratridine incubation. When the excitatory amino acid transporter (EAAT) blocker TBOA was employed, glutamine remained nearly unchanged whereas glutamate significantly enhanced. These results led us to suggest that glutamine release through glial glutamine transporter SN1 is related to EAAT activity that can be modulated by intrinsic extracellular glutamate in human cortical slices.

Therapeutic trials for HCV and diabetic patients type II using sprouting seeds

Emad Fawzy Eskander[1] and Hanna Hamdy Ahmed[1], Ashraf El-Sherbiny[2]

[1]Dept. of Hormones,
[2]Dept. of Internal Medicine, Medical Division, National Research Center, Cairo, Egypt

The current study introduces novel therapeutic trials for two public health diseases; Hepatitis C virus (HCV) and Diabetes mellitus (DM) using germinating seeds. Egypt has one of the world's highest prevalence of HCV infection, estimated nationally at 14.7 %. Diabetes mellitus type II is a metabolic disorder that is characterized by high blood glucose and relative insulin deficiency. The onion (*Allium cepa*) is the most widely cultivated species of the genus *Allium*. Fenugreek (*Trigonella foenum-graecum*) is a plant in the family Fabaceae are thought to be a galactagogue that is often used to increase milk supply in lactating women. It has been reported that *Nigella Sativa* has immunostimulant and hepatoprotective effects. We aimed to use medical alternatives to the combined interferon–ribavirin treatment for HCV infection, insulin and the original treatments for DM through the application of some Sprouting seed treatments individually and in combinations such as: onion (*Allium cepa*), fenugreek (*Trigonella foenum-graecum),* black seeds (*Nigella Sativa).* Our study population was divided into four groups: HCV infected patients group, Diabetic patients group, HCV/Diabetic patients group, and healthy controls group. HCV chronically infected Egyptian patients group was subdivided into two subgroups according to type of treatment: IFN/RBV combination therapy with PEG-IFN/RBV subgroup and germinating seed treatments subgroup. Also, Diabetic patients group was subdivided into two subgroups according to type of treatment: insulin and original treatment subgroup and germinating seed treatments subgroup, laboratory tests will be performed. Sprouts are believed to be highly nutritious and rich in enzymes which promote good health. Wide-ranging claims have been made for the effectiveness of onions against diabetes. They contain chemical compounds believed to have anti-inflammatory, anticholesterol, anticancer, and antioxidant properties. Several human intervention trials demonstrated the antidiabetic effects of fenugreek seeds. Additionally, *Nigella Sativa* has protective effects against nephrotoxicity. Also, we can propose that individual/combination therapies with these seeds can be used as alternatives for IFN based therapy in HCV patients and insulin in diabetic patients. Thus, the medical alternatives using the previously described sprouting seeds may provide an effective treatment for HCV and Diabetes mellitus type II.

Chloroquine enhances cytotoxicity on human cancer cells induced by polyamines metabolites: nanotechnological approaches for anticancer agents delivery

Giampiero Tempera[1], Martina Meringolo[1], Marco Coccia[1], Pietro Matricardi[2], Agnese Molinari[3] and Enzo Agostinelli[1]

[1]Istituto Pasteur Fondazione Cenci Bolognetti, Departments of Biochemical Sciences;
[2]Drug Chemistry and Technologies, "Sapienza" University of Rome and CNR, Biology and Molecular Pathology Institute, P.le Aldo Moro 5, 00185 Rome;
[3]Department of Technology and Health, Italian National Institute of Health, Viale Regina Elena 299, 00161 Rome, Italy

The cytotoxic effect of chloroquine diphosphate (CQ) was studied on both wild-type (WT) and multidrug resistant (MDR) melanoma and colon adenocarcinoma (LoVo) cells. CQ has been used as an antimalarial and an anti-inflammatory drug. It might cause distinct cell death in different cell lines. We explored the cytotoxic effect on tumour cells induced by CQ, administered alone or in association with bovine serum amine oxidase (BSAO) and spermine. Cell survival experiments performed on LoVo and M14 cells treated with CQ alone clearly showed that this drug induced a slight cytotoxicity on both WT and MDR cells. In combination with BSAO/spermine, LoVo cells were treated with different concentrations of CQ for 24 h and then with spermine in the presence of BSAO. The clonogenic assay showed that the quinine derivative was able to sensitize both WT and MDR cells to the spermine metabolites (H_2O_2 and aldehydes). It was observed greater cytotoxicity in cells pre-treated with CQ than in samples only treated with BSAO/spermine. Transmission electron and confocal microscopy observations showed that pre-treatment with CQ induced the formation

of numerous cytoplasmic vacuoles and increased the number of lysosomal structures. To increase the stability of the enzyme and the release of cytotoxic products, BSAO was conjugated on a new injectable nanohydrogel (NHs), obtained derivatizing hyaluronic acid (HA) with cholesterol (CH). The HA-based NHs system is a useful controlled delivery system for future therapeutic enzymes application.

Effect of ammonia and glutamine on branched-chain amino acid oxidation and protein metabolism in healthy and endotoxemic rats

M. Holeček, M. Kovařík, and L. Šišpera

Department of Physiology, Charles University in Prague, Faculty of Medicine in Hradec Kralove, Czech Republic

Enhanced oxidation of branched-chain amino acids (BCAA; valine, leucine and isoleucine) is a typical metabolic alteration in sepsis coupled with activated synthesis of glutamine and muscle wasting. Two separate experiments were performed in which the effects of inhibition and stimulation of glutamine synthesis on BCAA and protein metabolism were evaluated. In the first study, glutamine synthesis was inhibited by alanyl-glutamine infusion in healthy and endotoxin treated rats. In the second study, glutamine synthesis was stimulated by infusion of ammonium acetate/bicarbonate mixture. The parameters of protein and BCAA metabolism were measured using L-[1-^{14}C]leucine. Infusion of alanyl-glutamine induced a decrease in plasma BCAA levels, a decrease in leucine oxidation, and an improvement of protein balance due to the decrease in proteolysis both in intact and endotoxemic rats. Ammonium infusion induced an increase in ammonia and glutamine, an increase in BCAA oxidation, a decrease in BCAA and alanine levels in blood plasma, and a decrease in whole-body protein turnover and protein synthesis in muscle. It is concluded that changes in glutamine synthesis induced by alanyl-glutamine and by ammonia infusion are associated with significant alterations in BCAA and protein metabolism. The results also demonstrate that the cause of decreased plasma BCAA levels in liver cirrhosis is hyperammonemia. Supported by PRVOUK P37/02.

Role for glutamine in adipocyte metabolism and obesity

Malcolm Watford, and Samantha Dori

Department of Nutritional Sciences, Rutgers University, New Brunswick, NJ 08901, USA

Adipocytes express very high glutamine synthetase (GS) activity and during the differentiation of both mouse 3T3 L1 cells and human pre-adipocytes into adipocytes GS protein content is induced many fold. Although differentiation of these cells does not require exogenous glutamine we have found that limiting glutamine availability by culture in the absence of glutamine and knock-down of GS activity prevents differentiation. We identified monoclonal expansion of the cells and expression of C/EBP beta, PPAP gamma and C/EBP alpha, as the sites in differentiation where glutamine is required. In addition, glutamine availability is required for maximal rates of lipid accumulation in mature adipocytes and thus glutamine, as in some other cell types, may be a substrate for lipid synthesis. Given the relatively poor blood supply in adipose tissue we propose that adipocyte GS provides glutamine required to maintain adipocyte differentiation, growth and function. During obesity however, adipocyte glutamine synthesis may be detrimental in that it would fuel invading macrophages and thus would lead to inflammation. Macrophages cannot survive in culture in the absence of glutamine but they do survive when cultured over mature, GS expressing, 3T3 L1 adipocytes. Furthermore, co-culture of macrophages and adipocytes results in changes in cytokine production, such as higher IL6, compared with macrophages or adipocytes cultured alone. Thus adipocyte glutamine synthesis is involved in the development of obesity and inflammation, through roles in adipocyte differentiation and lipid accumulation, and by providing glutamine as a fuel for macrophages.

Human epididymal protein-4 and cystatins: novel cross class protease inhibitors from human seminal plasma

Nirmal Chhikara[1], Vikash Kumar Yadav[1], Mayank Saraswat[1], Anil Kumar Tomar[1], Kamaldeep Gill[1], Sharmistha Dey[1], Sarman Singh[2], Savita Yadav[1]

[1]Department of Biophysics, and
[2]Department of Lab. Medicine, All India Institute of Medical Sciences, New Delhi INDIA

HE-4, an epididymal protein, is a member of whey acidic protein four-disulfide core (WFDC) family with no known function. The cystatins form a super family of structurally related proteins with highly conserved structural folds. They are all potent, reversible, competitive inhibitors of cysteine proteinases (CPs). Proteins from this group present differences in proteinase inhibition despite their high level of structural similarities. In this study, HE-4 and three cystatins (cystatin 9, cystatin SN, and SAP-1) were purified by systematic chromatographic methods. Here, we show that HE-4 is secreted in the human seminal fluid as a disulfide-bonded homo-trimer and is a cross-class protease inhibitor inhibits some of the serine, aspartyl and cysteine proteases tested using hemoglobin as a substrate. Using SPR we have also observed that HE-4 shows a significant binding with all these proteases. Disulfide linkages are essential for this activity. Moreover, HE-4 is N-glycosylated and highly stable on a wide range of pH and temperature. Taken together this suggests that HE-4 is a cross-class protease inhibitor which might confer protection against microbial virulence factors of proteolytic nature. All three CPIs suppressed the activity of papain potentially and showed remarkable heat stability. Interestingly SAP-1 also inhibits the activity of trypsin, chymotrypsin, pepsin, and PSA (prostate specific antigen) and acts as a cross-class protease inhibitor in in vitro studies. Using Surface Plasmon Resonance, we have also observed that SAP-1 shows a significant binding with all these proteases. These studies suggest that SAP-1 is a cross-class inhibitor that may regulate activity of various classes of proteases within the reproductive systems.

Structural basis and molecular mechanism of metal specificity to superoxide dismutase from human pathogen C*lostridium difficile*

Wei Li[1], Hongfei Wang[2], Chengli Wang[1], and Xiangshi Tan[1]

[1]Department of Chemistry & Institutes of Biomedical Sciences, Fudan University, Shanghai 200433, China;
[2]State Key Laboratory of Quantum Optics and Quantum Optics Devices, Shanxi University, Taiyuan 030006, China

The superoxide dismutase from *Clostridium difficile* (SOD_{cd}) is a cambialistic SOD with Mn ion as the host of the active site although other metal ions can substitute the Mn ion. $MnSOD_{cd}$ showed an optimal SOD activity while Fe-sub-$MnSOD_{cd}$ showed 10-fold less activity and Co-sub-$MnSOD_{cd}$ revealed undetectable SOD activity. The structural basis and molecular mechanism of the metal specificity to the SOD_{cd} were investigated. Crystal structures of $MnSOD_{cd}$ (2.20 Å), Fe-sub-$MnSOD_{cd}$ (1.80 Å) and Co-sub-$MnSOD_{cd}$ (1.95Å) reveal a similar metal coordination geometry of distorted trigonal bipyramidal with His111, His197 and Asp193 providing the equatorial ligands and with His56 and a hydroxide or water forming the axial ligands. A striking feature from electrochemical studies revealed that $MnSOD_{cd}$ possesses an optimal E_m value of 0.48 V *vs* NHE, which lies at the middle of the two E_m values for the redox couples of O_2/O_2^- (−0.16 V) and O_2^-/H_2O_2 (0.89 V). Fe-sub-$MnSOD_{cd}$ exhibits a much lower E_m value (0.12 V), while Co-sub-$MnSOD_{cd}$ shows much higher E_m value (>0.7 V) than $MnSOD_{cd}$. The distinct redox potentials, which are mainly governed by the intrinsic redox chemistry of the metal ions, are specifically tuned by the micro-environment including the H-bond network around the metal center. The H-bond network including the coordinated solvent, Gln178, Tyr64, and His60, tunes the SOD_{cd} activity through adjusting the metal redox potential, substrate binding, relaying the labile proton. These results suggest that the cognate metal redox potential tuned by the metal micro-environment determines the metal specificity of the SOD_{cd}.

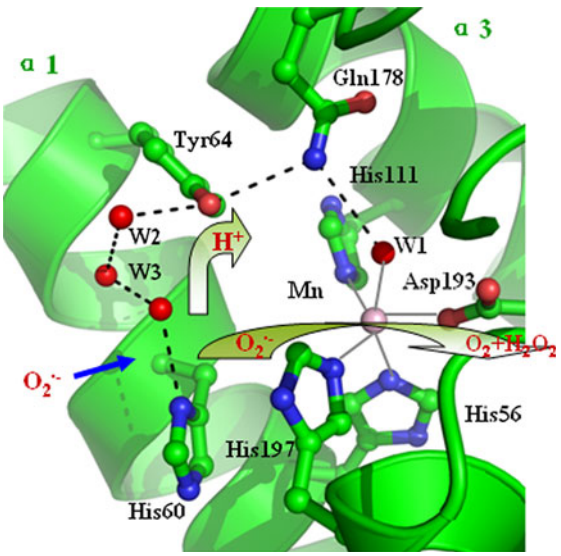


Figure 1 The H-bond network around the metal active site of $MnSOD_{cd}$ with the substrate channel and the proton relay pathway beginning from His60, via waters and gatekeeper Tyr 64, to Gln 178 and ending at the coordinated solvent

A novel chemical strategy for the synthesis of RGDX (X: amino acid) tetrapeptide

Yi Guo, Yao Fu, Sen Yang, Li Xu

Key Laboratory for Molecular Enzymology and Engineering, The Ministry of Education, College of Life Science, Jilin University, Changchun 130012, China

In this paper, a new route is described to synthesize RGDX (X, Amino acid) tetrapeptide. To better understand the method, the tetrapeptide RGDCySS was chosen as a model target for X. First, GDCySS was obtained in four steps, comprising the chloroacetylation of L-aspartic acid, synthesis of chloroacetyl L-aspartic acid anhydride, formation of $ClCH_2COAsp$–CySS and ammonolysis of $ClCH_2COAsp$–CySS. Second, Arg-NCA was reacted with GDCySS to synthesize RGDCySS by the NCA method. In the developed approach, less protected amino acids were used compared to conventional solid-phase synthesis. The new route offers advantages of low cost, simplicity and rapid synthesis with a reasonable yield of 63.0 % (calculated according to arginine content). In addition, compared with this method, a conventional solid phase method was also used to synthesize RGDCySS, the yield was 70 % calculated from the first amino acid anchored to resin. In addition, many other RGD containing peptides with sequence RGDX (X being any amino acid residue) can readily be synthesized using this strategy.

[This work is supported by the National Natural Science Foundation of China (No. 81271697, 21043002), Science Foundation of Changchun City (No. 09SF02), Specialized Research Fund for the Doctoral Program of Higher Education (20100061120077) and Social Development Project of Science and Technology Department of Jilin Province, China (No. 20106031, 20120967, YYZX2012)].

Myelstones—intrinsically-disordered basic proteins of central nervous system myelin

George Harauz

Molecular and Cellular Biology, University of Guelph, N1G 2W1, Canada

The classic isoforms of myelin basic protein (14- to 21.5-kDa) are essential to formation of the multilamellar myelin sheath of the mammalian central nervous system. They are intrinsically-disordered proteins that interact with many partners (membrane surfaces, cytoskeletal and SH3-domain proteins, Ca^{2+}-calmodulin, phosphoinositides, and Zn^{2+}) via **Mo**lecular **R**ecognition **F**ragments (MoRFs), which undergo local disorder-to-order transitions upon association. The conformations and specific interactions of these proteins are modulated by a dynamic barcode of post-translational modifications, including phosphorylation by mitogen-activated protein and other kinases. The MBPs are thus to myelin what basic histones are to chromatin. Originally thought to be merely structural proteins forming an inert spool, the histones are now known to be dynamic entities involved in epigenetic regulation and diseases such as cancer. Analogously, the MBPs are not mere markers of compact, mature myelin, but active participants in oligodendrocyte proliferation, and in membrane process extension and stabilization during myelinogenesis. A central segment of these proteins is pivotal both structurally and functionally in membrane-anchoring and SH3-domain interaction, all controlled by MAPK phosphorylation. During myelin development (the first 20 years of life in humans), mis-signalling or mis-structuring due to incorporation of toxic proline analogues obtained from the diet, or infection with viruses (such as HHV-6) containing motifs found also in MBP, may lead to

inherently unstable regions of the sheath, that may later be triggered to become foci of demyelination.

Protein profiling in plant extracts using chip-based capillary electrophoresis for identification of genetically modified organisms (GMO)

Marek Trojanowicz[1], Ewa Poboży[1], Monika Filaber[1], Anna Koc[1], Juan F. Garcia-Reyes[2]

[1]Department of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland;
[2]Department of Physical and Analytical Chemistry, University of Jaén, 23071 Jaén, Spain

A rapidly increasing cultivation of different genetically modified (GM) plants, and numerous social controversies concerning their use in food production, require continues improvement of analytical methods for the determination of transgenic material in foods. This is carried out simultaneously with the examination of biological and biomedical aspects of genetically modified organisms (GMO) in foods, as well as the safety assessment of genetically modified foods. The most commonly used methods for analysis of GMO are PCR techniques, and also immunochemical determination the encoded new proteins. In our studies the capillary gel electrophoresis in a gel format with laser-fluorescence detection was applied in protein profiling of fractionated and total extracts of maize standards. The sensitivity of such determinations can be enhanced by lyophilization of extracts or using cut-off filters. Especially effective pretreatment step in the determination of low abundance proteins was using combinatorial peptide ligand library for sample processing prior to the electrophoretic analysis. Several reproducible differences were observed for protein profiles between maize standards non-containing the genetically modified organisms (GMO) and those containing GMO, what can be potentially employed for identification of GMO in maize samples and foods of maize origin.

Dietary valine supplementation improves immune and endocrine function of lactating sows

Peng Yi, Chen Yi, Fu Shao-Hui, Chen Li-Xiang, and He Jian-Hua

College of Animal Science and Technology, Hunan Agricultural University, Changsha, Hunan 40128, China

Dietary valine supplementation improved sow performance in our previous study, it may relate to immune and endocrinology function improvement. Thirty-two healthy sows (Landrace × Yorkshire, similar parity, and body condition) were assigned to fed one of the four diets: basal diet (0.93 % Lysine, 0.84 % Valine) or basal diet supplemented with 0.10 %, 0.25 %, and 0.40 % valine respectively from 107 days of gestation to 21 days of lactation, blood samples were collected at 10 and 18 days of lactation, milk samples were collected at 1 and 10 days of lactation. The results showed that dietary valine supplementation significantly increased serum albumin concentrations at 10 and 18 days of lactation ($P < 0.05$), and increased serum Immunoglobulin M, Immunoglobulin G and Prolactin concentrations at 18 days ($P < 0.05$) of lactation. Valine supplementation also significantly increased milk INS (Insulin) concentrations at 14 days of lactation ($P < 0.05$), and increased milk GH (Growth Hormone) concentrations at 1 and 14 days of lactation. Dietary valine supplementation tended to increase milk ALB and IgM concentrations ($P > 0.05$) and tend to reduce diarrhea rate in suckling pigs ($P > 0.05$). It was suggested that better performance of sows fed valine supplemented diet mainly related to improved sows' immune function and increased milk-borne growth factors concentrations.

Carbonic anhydrases: new insights from studies in *Xenopus* oocytes

Joachim W. Deitmer

General Zoology, University of Kaiserslautern, P.O. Box 3049, D-67653 Kaiserslautern, Germany

Carbonic anhydrases (CAs) are ubiquitous zinc metalloenzymes, which catalyze the reversible hydration of carbon dioxide to yield protons and bicarbonate anions, and which are involved in many patho-physiological processes. Some isoforms of the 15 members including alpha subfamily are located in the cytosol, while others are catalytically active at the extracellular membrane face. Therefore, it has been difficult to discriminate the functional role of individual CA isoforms. We have employed *Xenopus* oocytes, which do not express intrinsic CA activity themselves, to express single and multiple CA isoforms, in particular isoforms II, IV, and IX, alone and together with other proteins, suspected to interact with CAs. Evidence is presented on the role of different CA isoforms to form 'transport metabolons' with acid/base-coupled membrane transporters, some of which require the catalytic activity and other do not. CA isoforms may directly or indirectly interact with transporters to enhance their activity. Here, I summarize some new insights on CA function, location, and interaction with other proteins obtained recently with the heterologous expression of CAs in oocytes.

[*Supported by the Deutsche Forschungsgemeinschaft (DFG), DE 231/24-1*].

Enhanced polyamine turnover after arginine supplementation in an Atlantic salmon model—implications on growth and animal welfare

Synne M. Andersen, Elisabeth Holen, Anders Aksnes, Ivar Rønnestad, Jens-Erik Zerrahn, Marit Espe,

[1]National Institute of Nutrition and Seafood Research (NIFES), Po Box 2029, N-5817 Bergen, Norway;
[2]Ewos Innovation As, N-4335 Dirdal, Norway;
[3]Department of Biology, University of Bergen, Po Box 7803, N-5020 Bergen, Norway;
[4]Evonik Degussa Int. AG, 7100 Vejle, Denmark

Arginine supplementation to several animal models has shown promising effects on reducing adiposity, simultaneously enhancing muscle deposition. Here we aimed to investigate whether arginine supplemented beyond requirement to Atlantic salmon would favor muscle gain rather than fat deposition. Two feeding trials were carried out, in juvenile and adult Atlantic salmon, for 8 and 12 weeks, respectively. For each trial, four plant-protein based diets were supplemented with graded doses of arginine, and fed to quadruplicate tanks of salmon. In the juveniles, the highest arginine inclusion (37.4 g/kg) resulted in the highest weight gain, representing gain of both protein and fat, while no effects were observed in the adult salmon. The arginine inclusion levels significantly affected amino acid metabolism and polyamine turnover.

The profiles of free amino acids in muscle and plasma clearly indicated higher production of arginine metabolites in both trials. Increased abundance and activity of spermidine/spermine-acetyltransferase (SSAT) in liver indicated higher turnover of polyamines, possibly explaining why no or little differences on polyamine concentrations in liver, muscle or white adipose tissue (WAT) were observed between the treatments. Increased expression of lipolytic genes was observed in WAT of juvenile but not in adult salmon supplemented with high arginine levels. Overall, our results indicates that juvenile salmon benefit from a high arginine diet, but that long term supplementation to healthy animals might lead to adaptation.

Mental retardation and isoleucine metabolism

Xue-Ying He, Carl Dobkin, Charles Isaacs, Manfred Philipp, W. Ted Brown, and Song-Yu Yang

[1]Department of Developmental Biochemistry and
[2]Department of Genetics, NYS Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY 10314, USA,
[3]Department of Chemistry, Lehman College, City University of New York, Bronx, NY 10486, USA

Several isoleucine metabolic disorders can result in mental retardation. Although the newborn screening has reduced mortality of patients, the incidence of mental retardation has not yet been significantly reduced. Among these disorders, males carrying a mutation in the *HSD17B10* gene all suffer from mental retardation since this gene encodes a unique mitochondrial hydroxysteroid (17β) dehydrogenase, namely HSD10. This multifunctional enzyme was previously known as 2-methyl-3-hydroxybutyryl coenzyme A dehydrogenase (MHBD) whose catalytic activity is essential to the oxidative degradation of isoleucine. It is likely that HSD10 has yet uncharacterized functions. Both enzymatic and non-enzymatic functions of HSD10 may be involved in mental retardation. Such new concepts may lead to the design of more effective treatments for this inborn error of isoleucine metabolism.

L-Amino acids as chiral selectors for enantioseparation of (±)-Bupropion using chiral ligand-exchange thin layer chromatography

Sonika Batra, and Ravi Bhushan

Department of Chemistry, Indian Institute of Technology Roorkee, Roorkee-247667, India

One of the most significant aspects of amino acid research includes their use in the form of chiral selectors as impregnating reagents, as mobile phase additive or for developing chiral ligand exchange reagent for direct chromatographic resolution of several racemates. Besides, they are used as auxiliaries to prepare chiral derivatizing reagents. The present paper deals with preparation of different Cu (II)-L-amino acid complexes as chiral ligand exchange reagent (LER) for enantioresolution of (±)–Bupropion by complexation chiral thin layer chromatography (TLC) using Cu(II) acetate and four L-amino acids (viz., L-proline, L-histidine, L-phenylalanine, and L-tryptophan). Four different approaches were adopted for impregnating the plate/loading with the LER. These are (1) by mixing the LER with silica gel slurry before making the plates, (2) using the Cu (II)-L-amino acid complex as chiral mobile phase additive, (3) ascending development of plain plates in the LER solutions, and (4) using a solution of Cu (II) acetate as mobile phase additive for the TLC plates impregnated with ascending development of plain plates in the solutions of amino acid. Formation of Cu(II)-amino acid complexes is pH-dependent, because complexation requires an unprotonated amino group and an anionic acid group in the amino acids. Spots were located using iodine vapour. The results obtained with all the approaches have been compared in terms of resolution. Effect of concentration of Cu(II)-acetate and amino acids has also been studied.

Towards the modulation of the negative HLH transcription factor Id by chemically modified peptides

Chiara Cabrele

University of Salzburg, Dept. of Molecular Biology, Div. of Chemistry and Bioanalytics, Billrothstr. 11, 5020 Salzburg, Austria

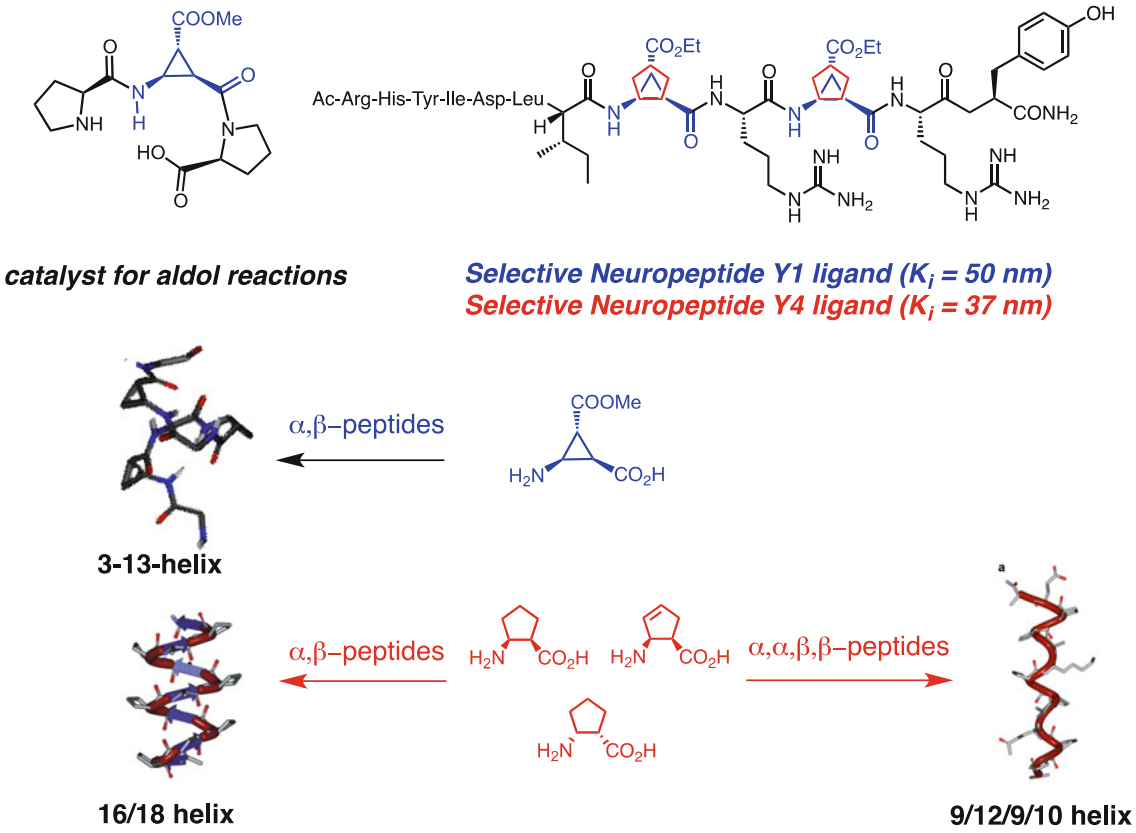
Protein–protein interactions represent an important mechanism of regulation of cellular events. For example, the two families of the E bHLH and Id HLH proteins positively and negatively modulate DNA transcription by forming DNA-binding dimers and non-DNA-binding E/Id heterodimers, respectively. Such mechanism is critical for the control of cell proliferation and differentiation during development as well as in vascular diseases and cancer (Perk, J.; Iavarone, A.; Benezra, R. Nat. Rev. Cancer 2005, 5, 603–614). The Id HLH domain (about 40-residue long) is essential for protein–protein interaction; indeed, the fold of the Id/E HLH heterodimer is assumed to be a parallel four-helix bundle (Wibley, J.; Deed, R.; Jasiok, M.; Douglas, K.; Norton, J. Biochim. Biophys. Acta 1996, 1294, 138–146). To prevent the formation of the Id/E heterodimer, we have designed peptide-based compounds targeting the Id HLH domain and inhibiting the Id-protein function in cells in the low micromolar range (Pellegrino, S.; Ferri, N.; Colombo, N.; Cremona, E.; Corsini, A.; Fanelli, R.; Gelmi, M.L.; Cabrele, C. Bioorg. Med. Chem. Lett. 2009, 19, 6298–302). The design, synthesis and biological properties of these and other novel anti-Id-protein molecules will be discussed at the 13th ICAPP.

Cyclic *cis*-β-amino acids: unique building blocks for bioactive ligands, organocatalysts and foldamers

Oliver Reiser

Universität Regensburg, Institut für Organische Chemie, Universitätsstr. 31, 93059 Regensburg, Germany

We will discuss the enantioselective synthesis and application of cyclic *cis-β-amino acids* as constituents in peptides, especially in α,β- and α,α,β,β-foldamers, which has led to the successful development of organocatalysts, helical foldamers stable in aqueous media, or selective ligands for neuropeptide Y receptors.



Helical Foldamers in Alcoholic and Aqueous Media

Arginine production in the neonate

Juan C. Marini, Murali H. Premkumar, Inka C. Didelija, Barbara Stoll and Doug G. Burrin

Baylor College of Medicine, Houston, TX, USA

Endogenous arginine synthesis in adults is a complex multiorgan process, in which citrulline is synthesized in the gut, enters the general circulation, and is converted into arginine in the kidney, by what is known as the intestinal-renal axis. In neonates, the enzymes required to convert citrulline into arginine are present in the intestines. Thus, the prevailing dogma is that this axis is not functional in neonates and that arginine, but not citrulline, is exported by the gut. Paradoxically, plasma citrulline is also used as a marker for gut health and development in the neonate. We have determined citrulline and arginine kinetics in piglets (6–10 days) using tracers. Whereas arginine flux increased during feeding (293 vs. 366 ± 44 μmol/kg/h, $P < 0.0001$), citrulline flux remained unchanged (74 ± 7 μmol/kg/h, $P = 0.432$). The fractional contribution of citrulline to arginine synthesis remained constant (15.7 ± 2.6 %, $P = 0.818$), but the absolute amount increased during feeding (44 vs. 62 ± 11 μmol/kg/h, $P < 0.0001$). Whereas 56 ± 7 % of the citrulline flux was accounted for as plasma arginine during fasting, a larger fraction (74 ± 6 % $P < 0.0001$) was accounted for during the feeding phase. The presence of argininosuccinate synthase and lyase in the gut was verified by immunohistochemistry. Although these two enzymes were present in the tip of the villi, the enzymes involved in citrulline synthesis were present in the crypts. Our results in neonatal pigs demonstrate a substantial citrulline flux that can be explained by the lack of co-localization of the enzymes for citrulline synthesis and further conversion into arginine.

Fate of proteins and amino acids in the small and large intestine: what consequences for the mucosa?

Francois Blachier

UMR 914 INRA/AgroParisTech, Nutrition Physiology and Ingestive Behavior, 16 rue Claude Bernard, 75005 Paris, France

Hyperproteic (HP) diets are used for body weight reduction but consequences on the intestinal physiology have been little investigated. We recently shown that HP diets ingestion in the rat model modifies the microbiota composition in the colon when compared with normoproteic isocaloric diet. Also, HP diet increase the amount of protease activities, ammonium, short-chain fatty acids (SCFAs) and other organic acids as well as the mass of the colonic content. In the meantime, the monocarboxylate transporter expression and metabolic capacity of isolated colonocytes towards butyrate and other energy substrates were similar in the NP and HP groups. Consequently, the amount of butyrate and other SCFAs was increased in feces recovered from HP animals. Our results are compatible with the view that increased transfer of proteins to the large intestine after HP diet ingestion increases monocarboxylic acid production from amino acids by a modified microbiota with the maintenance of their luminal

concentrations due to an increase of the colonic mass content. This allows the homeostasis of absorption and metabolism of butyrate and other monocarboxylic acids in colonocytes, such homeostasis being believed to be central for the control of the epithelial renewal process. The cost of such homeostasis is the loss of energy in the form of SCFAs in the feces.

Specificity of citrulline metabolism in the intestine: causes and consequences

Luc Cynober

Department of Experimental, Metabolic and Clinical Biology EA 4466. Faculty of Pharmacy, University Paris Descartes, France

Citrulline (CIT) is synthesized by enterocytes from mainly arginine and glutamine, and in the adult, it is not catabolized in situ because argininosuccinate synthase + lyase are not expressed. Hence CIT is released in the portal vein, is not taken up by the liver and so appears in the systemic circulation. Since there is no other significant source of circulating CIT at the whole body level, as CIT is not a component of proteins and is almost absent from food (only present at significant levels in watermelon), the concept emerged at the turn of the millennium that plasma CIT could be a biomarker of intestinal functional mass. This concept has been further supported in all situations (acute or chronic) where gut failure is documented. Further studies have clearly shown that CIT production by the gut is upregulated by low protein diet or during fasting, and serendipitous results indicate that CIT is a signaling molecule stimulating protein synthesis through mTOR-dependent and -independent mechanisms. This anabolic action mediated by CIT supplementation has been documented in various experimental models, and in healthy subjects fed a low protein diet. Finally, not only does CIT increase protein mass, but it also decreases fat mass. This opens an avenue of clinical research, in particular for fighting sarcopenia in the elderly. Lastly, we can advance the hypothesis that CIT is a leucine counterpart, the former acting to sustain protein synthesis in cases of low or nul protein intake, the latter stimulating protein synthesis in the fed state in conjunction with insulin.

Study on MCE-LIF method for the hydrolysis of L-glutamine with L-asparaginase enzyme reactor based on GNPs

Li Qi, Juan Qiao

Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry, Chinese Academy of Sciences, 100190 Beijing, China

Because L-asparaginase (L-Asnase) can suppress the growth of malignant cells by rapid depletion of two essential amino acids, L-glutamine (L-Gln) and L-asparagine (L-Asn), for studying the cytotoxic effect and the secondary complications of L-Asnase in the treatment of acute lymphoblastic leukemia (ALL), a novel enzyme reactor of L-Asnase for the hydrolysis of L-Gln, employing the enzyme-gold nanoparticle conjugates in capillary, was developed in this work. Firstly, a microchip electrophoresis-laser induced fluorescence method (MCE-LIF) was established for the separation of L-amino acids (L-Gln and L-Glu) and the hydrolysis of L-Gln by using L-Asnase enzyme reactor. Then, employing L-Gln as target analyte, the enzyme kinetics of L-Asnase in free solution (E–F), enzyme-gold nanoparticle conjugates (E-GNP) and the enzyme-gold nanoparticle conjugates immobilized in capillary (E-GNP-C) were investigated in detail with the proposed MCE-LIF method. Owing to the high specific activity, the E-GNP-C enzyme reactor exhibited the best performance for the hydrolysis of L-Gln. Therefore, the application of GNP and the conjugates immobilized in capillary for construction of L-Asnase enzyme reactors would provide a good strategy for understanding its side effect in the treatment of ALL.

A novel antioxidative mechanism mediated by proline/arginine/NO metabolism in yeast

Hiroshi Takagi

Graduate School of Biological Sciences, Nara Institute of Science and Technology, Japan

Nitric oxide (NO) is a ubiquitous signaling molecule involved in the regulation of many cellular functions. In the unicellular eukaryote yeast, NO may be involved in stress response pathways, but its role is poorly understood due to the lack of mammalian NO synthase (NOS) orthologues. Previously, we have proposed the oxidative stress-induced L-arginine synthesis and its physiological role under stress conditions in yeast *Saccharomyces cerevisiae*. Recently, we indicated that increased conversion of L-proline into L-arginine led to NO production in response to elevated temperature. We also showed that the flavoprotein Tah18, which was previously reported to transfer electrons to the Fe–S cluster protein Dre2, was involved in NO synthesis in yeast. Gene knockdown analysis demonstrated that Tah18-dependent NO synthesis confers high-temperature stress tolerance on yeast cells. Currently, we focus on the antioxidative mechanism by NO, such as cGMP-mediated signal transduction and protein activation via *S*-nitrosylation found in mammals. Our microarray analysis revealed that NO up-regulates the expression of genes involved in copper uptake, which are regulated by the transcriptional activator Mac1. Our hypothesis is that NO can directly modify the Cys residue in Mac1 via *S*-nitrosylation, leading to a conformational change for its activation. In fact, NO increases intracellular copper level. Interestingly, NO activates the superoxide dismutase Sod1 that requires copper for its activity in the presence of copper, probably due to an increase in copper uptake. As it appears that such a cell protection mechanism is specific to yeasts and fungi, it represents a promising target for antifungal activity.

Multiple detection of thermophilic exoenzymes by transfer zymography

Marleny Fernández, Zully Hernández, Liliana Kurz, Lellys M. Contreras, and Jeff Wilkesman

Universidad de Carabobo, FACYT, Laboratorio de Investigaciones Bioquímicas

Thermophiles are a source of heat stable enzymes, attractive for biotechnological and industrial purposes. To enhance the enzymatic analysis, a method to determine simultaneously carbohydrases, lipases and proteases from *Bacillus stearothermophilus* isolated from the hot springs Las Trincheras, Venezuela, was developed. The strain was cultured in nutritive broth for 120 h at 55 °C. Cell-free fractions (CFF) were extracted from the culture and tested for enzymatic activity. Lipase and protease present in CFF were positively tested by zymography.

Electrophoretic transfer protein zymography, employing a single polyacrylamide gel containing a substrate mixture (olive oil and gelatin copolymerized plus addition of starch in the reactivation buffer) was used to test the enzyme content from CFF and commercial controls. Controls were all detected, whilst CFF showed no enzymatic activity, perhaps because poor protein transference during electroblotting. Results indicate feasibility in detection of the three enzymes by transfer zymography. The lipases or proteases activity from controls or CFF, detected by individual zymograms was quantified by densitometry and compared with the activity determined by photometric methods, showing that both methods of quantification have similar tendencies.

Protease characterization in duck liver steatosis by 1- and 2-dimensional zymography

Liliana Kurz[1], María Fernanda Padrón[2], Sahar Awde[3,4,5], Hervé Rémignon[3,4,5], Jeff Wilkesman[2]

[1]Universidad de Carabobo, Facultad de Ingeniería, Escuela de Química, Valencia, 2005 Venezuela;
[2]Universidad de Carabobo, Facultad de Ciencias y Tecnologia, Departamento de Quimica, Valencia, 2005 Venezuela;
[3]INRA, UMR1289 Tissus Animaux Nutrition Digestion Ecosystème et Métabolisme, F-31326 Castanet-Tolosan, France;
[4]Université de Toulouse INPT ENSAT, UMR1289 Tissus Animaux Nutrition Digestion Ecosystème et Métabolisme, F-31326 Castanet-Tolosan, France;
[5]Université de Toulouse INPT ENVT, UMR1289 Tissus Animaux Nutrition Digestion Ecosystème et Métabolisme, F-31076 Toulouse, France

Protease activity in liver cells under a state of steatosis was electrophoretically characterized. From a healthy and a fatty duck liver, four separate extracts were obtained: a simple homogenate, a metalloprotease extract, a cathepsin extract and calpain extract. Protein concentration was higher in all fatty liver extracts. Proteolytic activity employing casein as substrate was determined at 280 nm, evidencing an activity increase in the fatty liver extracts. Zymography allowed semi-quantitative results, successfully detecting cathepsin- and metalloprotease-activity using polyacrylamide gels copolymerized with gelatin and quantified by densitometry. The use of specific inhibitors confirmed the identity of the proteins studied. These results confirmed the presence of hydrolases in liver cells under steatosis and a significant increase in their activity. Metalloprotease activity was further characterized by 2D zymography, evidencing a p*I* 5.55 with a M_r of $\sim$89,000. While cathepsin showed a p*I* 4.40 with a M_r of $\sim$38,000. The analysis of liver proteases activities may be extrapolated to improve the understanding of the mechanisms underlying steatosis development exploited in *foie gras* production. Additionally, the final quality of the product may be enhanced provided a higher knowledge behind the proteases related to liver steatosis in force fed palmipeds.

Amino acids, peptides and proteins as histotroph affecting embryonic development: discoveries and roles in reproductive health

Fuller W. Bazer

Department of Animal Science, Texas A&M University, College Station, TX 77843-2471, USA

The mammalian uterus includes the serosa, myometrium and endometrium from the outer covering to the lumen. The endometrium includes the luminal (LE), superficial glandular (sGE), and glandular (GE) epithelia, each with a unique phenotype, as well as supporting stromal cells, vascular elements, nerves and immune cells. Importantly, the uterine epithelia secrete or selectively transport molecules into the uterine lumen that is collectively known as histotroph that is required for growth and development of the conceptus (embryo and its associated extra-embryonic membranes). Components of histotroph include nutrients such as amino acids and glucose, enzymes, growth factors, cytokines, lymphokines, transport proteins for vitamins and minerals, extra-cellular matrix molecules. Amino acids, particularly arginine, stimulates the mechanistic target of rapamycin pathway to stimulate proliferation, migration and protein synthesis of cells of the conceptus, and arginine serves as a substrate for synthesis of nitric oxide and polyamines required for growth and development of the conceptus. Hormones from conceptus trophectoderm that act on the uterine epithelium include interferon tau (IFNT) in ruminants and estrogens in swine that signal pregnancy recognition. Those hormones act in concert with progesterone from the corpus luteum to increase expression of genes including those for nutrient transport, proteases, protease inhibitors, growth factors, hematopoiesis and angiogenesis and molecules critical for implantation and placentation. The plieotropic effects of IFN tau will be discussed, as will effects of uteroferrin [phosphatase, acid, type 5, tartrate-resistant (ACP5) and tartrate resistant acid phosphatase (TRAP)] for transport of iron and for stimulation of erythropoiesis.

Monosodium glutamate: effects on appetite, food intake/selection and body weight

John D. Fernstrom[1], John T. Brosnan[2], Mark I. Friedman[3], and Adam Drewnowski[4]

[1]University of Pittsburgh, Pittsburgh, USA;
[2]Memorial University of Newfoundland, St. Johns, NF, Canada;
[3]Monell Chemical Senses Center, Philadelphia, PA, USA;
[4]University of Washington, Seattle, WA, USA

The session on monosodium glutamate (MSG) chaired by J.D. Fernstrom consisted of three talks: (1) metabolic actions of glutamate and ingested MSG in relation to energy and fat metabolism, and body weight (J.T. Brosnan); (2) effect of MSG ingestion on appetite, food intake and body weight in animals (M.I. Friedman); and (3) effects of MSG ingestion on appetite, food intake and body weight in humans (A. Drewnowski); as well as discussions. MSG is ingested as a flavoring agent, but handled metabolically in the gut, liver and elsewhere as the non-essential amino acid glutamic acid. Glutamic acid is a key nodal point connecting amino acid and carbohydrate metabolism in the body. Recently, glutamate has also been suggested to influence fat metabolism, and will be a key focus of discussion. Monosodium glutamate ingestion is reported to increase or decrease food intake and body weight in animals. Older literature suggests there is no impact on weight gain. This talk will examine the influence of MSG on food intake in both acute and chronic paradigms, and the impact on caloric balance and body weight. Monosodium glutamate is a taste described as savory or meaty in western cultures. It's inclusion in some foods markedly improves overall taste and flavor, and for this reason is often included in prepared and processed foods. However, it is also reputed to promote excessive food intake and promote weight gain. Some recent epidemiologic studies have also linked MSG intake to body weight. This talk will review these issues, and assess what conclusions can be drawn from published findings.

Nitrogen metabolism in the gut microbiota: impact on host nutrition and health

Zhaolai Dai[1], Zhenlong Wu[1], Weiyun Zhu[2], Junjun Wang[1], and Guoyao Wu[1,3]

[1]State Key Laboratory of Animal Nutrition, China Agricultural University, Beijing, China 100193;
[2]Laboratory of Gastrointestinal Microbiology, Nanjing Agricultural University, Nanjing, China 210095;
[3]Department of Animal Science, Texas A&M University, College Station, TX, USA 77843

Emerging evidence shows that the microbiota along the digestive tract plays an import role in the metabolism and recycling of nitrogenous compounds in the body. In the small intestine, luminal microorganisms can degrade proteins of both dietary and endogenous origin to generate amino acids (AA) for use by the host. At the same time, the degradation and *de novo* synthesis of AA by the intestinal bacteria regulate the availability of dietary AA to enterocytes and extra-intestinal tissues. Microbial AA metabolites, such as ammonia, hydrogen sulfide, short chain fatty acids, amines, and polyamines, have enormous physiological impacts on host health both locally and systematically. A variety of factors, such as diets and the physiological conditions of the host, influence the composition, abundance and activity of the gut microbiota, thereby affecting the bacteria-host co-metabolism of nitrogenous compounds. Elucidating the characteristics and the underlying regulatory mechanisms of nitrogen metabolism in intestinal microbes and their contribution to the host's AA homeostasis will improve the nutritional value of dietary proteins and AA, as well as the health of both humans and animals.

(Supported by the National Basic Research Program of China, NSFC, China Postdoctoral Science Foundation, and Texas A&M AgriLife Research).

Allyl sulfur compounds conjugates in cancer inhibition and tissue repair

Ashif Iqbal Bhuiyan[1], Felicia Carotenuto[2], Emanuela Vitale[1], Arianna Mauretti[1], Annalisa Neri[1], Francesca Nanni[3], Paolo Di Nardo[2] and Sonia Melino[1]

[1]Department of Chemical Sciences and Technologies,
[2]Department of Clinical Sciences and Translational Medicine,
[3]Department of Industrial Engineering, University of Rome Tor Vergata, Rome Italy

The allyl sulfur compounds, either alone or in association with other antitumor drugs, may be considered as potential ideal agents in the anticancer therapy. Natural organ sulfur compounds (OSCs) have shown chemopreventive effects and to suppress the proliferation of tumour cells in vitro by inducing apoptosis. The biochemical mechanisms underlying the antitumorigenic and anti-proliferative effects of garlic-derived OSCs are not fully understood. Very likely, the rate of clearance of allyl sulfur groups from cells is a determinant of the overall response and several modes of action have been proposed. Different effects of water- and oil-soluble OSCs on enzymes involved in the detoxification system have been observed. The biochemical transformations of these compounds in the cell and their forming adducts with thiol functional groups of the proteins could constitute the relevant events to uncover the anticancer properties of the allyl sulfur compounds. We have synthesized and characterized OSCs conjugates and protein-OSCs-microemulsions, and investigated on their effects on both apoptosis induction in MCF-7 and HuT78 cancer cells. Preliminary studies have been also performed in order to investigate on the proliferation and differentiation of Sca-1 cardiac progenitor cells for tissue repair. The results suggested that these antioxidant-microemulsions might have a potential use in cancer and regenerative therapy.

Glutamine enhances intestinal epithelial barrier function by modulating tight junction protein expression

Zhenlong Wu[1], Bin Wang[1], Zhaolai Dai[1], Junjun Wang[1], and Guoyao Wu[1,2]

[1]State key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing, 100193, P. R. China;
[2]Department of Animal Science, Texas A&M University, College Station, TX 77843, USA

Barrier function is essential for the maintenance of normal intestinal function and health in humans and animals. The disruption of the intestinal barrier is associated with a wide range of disorders. As one of the primary metabolic fuels for the small intestine and a precursor for DNA and protein synthesis, glutamine is essential for intestinal growth, integrity, and function. In recent years, there is growing interest in glutamine signaling in the intestine due to its actions on activating key protein kinases, including the mammalian target of rapamycin and extracellular signal-regulated kinases. Our recent study shows that glutamine deprivation inhibits protein synthesis and cell growth, leads to a decrease in transepithelial electrical resistance (TEER) and an increase in the intestinal permeability. This effect was also observed in intestinal porcine epithelial cells in response to oxidative stress. Results of the western blot analysis indicated that oxidative stress-induced down-regulation of tight junction protein was reversed by supplementation with 0.5–5 mM glutamine in a dose-dependent manner. Collectively, these findings support the notion that physiological levels of glutamine are required to protect intestinal epithelial cells from oxidative stress-induced barrier dysfunction.

(Supported by the National Basic Research Program of China, NSFC, China Postdoctoral Science Foundation, and Texas A&M AgriLife Research).

Alpha-ketoglutarate enhances protein synthesis in intestinal porcine epithelial cells

Kang Yao[1,2], Chenxing Fu[3], Yulong Yin[1], Youngqing Hou[4], Junjun Wang[5], Zhenlong Wu[5], and Guoyao Wu[2]

[1]The Chinese Academy of Science, Changsha, China;
[2]Texas A&M University, College Station, TX, USA;
[3]Hunan Agricultural University, Changsha, China;
[4]Wuhan Polytech University, Wuhan, China;
[5]China Agricultural University, Beijing, China

Abstract a-Ketoglutarate (AKG) is an intermediate of the Krebs cycle that can be produced from glutamine catabolism. Emerging evidence shows beneficial effects of AKG on improving intestinal growth, integrity, and function. We have used intestinal porcine epithelial cells (IPEC-1) to elucidate mechanisms responsible for AKG to regulate intestinal protein turnover and amino acid metabolism. Our experimental protocol involved culture of IPEC-1 cells for 3 days in Dulbecco's modified Eagle's-F12 Ham medium (DMEM-F12) containing 0, 0.2, 0.5 or 2 mM of AKG. At the end of the 3-day culture, cells were then used to determine glutamine metabolism (using L-[U-^{14}C]glutamine as the tracer), protein concentration, protein synthesis, proteolysis, and the total and phosphorylated levels of the

mammalian target of the rapamycin (mTOR), ribosomal protein S6 kinase-1 (S6K1) and eukaryotic initiation factor (eIF) 4E-binding protein-1 (4E-BP1). Compared with 0 mM of AKG (control), AKG (0.2–2 mM) dose-dependently reduced glutamine degradation and the production of glutamate, alanine and aspartate in IPEC-1 cells. Addition of 0.5 and 2 mM of AKG to culture medium enhanced protein synthesis without affecting protein degradation, compared to the control group. The stimulatory effect of AKG on protein synthesis was attenuated by addition of 50 nM rapamycin (a potent inhibitor of mTOR). Consistent with these data, 0.5 or 2 mM of AKG increased the phosphorylated levels of mTOR, S6k1 and 4E-BP1 proteins in IPEC-1 cells. Thus, AKG can spare glutamine and activate the mTOR signaling pathway to stimulate protein synthesis in intestinal epithelial cells.

(Supported by the National Basic Research Program of China, NSFC, and Texas A&M AgriLife Research).

Multi-phased acyltransferase crystal structures lead to discovery of new chemistry and chemicals

Tsung-Lin Li

Genomics Research Center, Academia Sinica, Taipei 115, Taiwan

Teicoplanin/A40926 is the last line lipoglycopeptide antibiotics to treat multidrug-resistant gram-positive pathogens, e.g. MRSA and VRE. This class of antibiotics is empowered by the *N*-acyltransferase attaching a long aliphatic chain on the glucosamine moiety at the central residue of teicoplanin pseudoaglycone. High-resolution crystal structures for the enzyme in unary, binary and ternary complexes revealed a multistage conformational change responds to binding of acyl-CoA, enabling upload of teicoplanin pseudoaglycone as well as the acyltransfer reaction in the occlusion of the *N*- and *C*-halves of the protein. With few limitations the acyl group can be considerably diverse. Both vancomycin and acyl-NAC can serve as an acceptor and donors respectively. Beyond acyltransferase, the enzyme was serendipitously discovered able to carry out oxidation reactions, in which the oxidation reaction is mediated by the CoA cofactor through an unusual sulfur–peroxide mechanism evidenced by the complexed crystal structures and delicate biochemical assays.

Novel antimicrobial peptides rich in Val/Arg residues and the biological activities in vitro and in vivo

Q.Q. Ma, and A.S. Shan

Institute of Animal Nutrition, Northeast Agricultural University, Harbin 150030, China

Antimicrobial peptides (AMPs) are major components of the innate self-defense system and were an alternative to conventional antibiotics because of the distinct mechanism of action. Here, we developed a series of peptides with Val/Arg residues and determined the activities in intro and in vivo. The peptides were produced by segregating Arg residues on the polar side and Val residues on the opposite side. The results showed that Val-containing peptides showed strong antimicrobial activity and weak haemolysis or cytotoxicity. CD spectra showed that the peptides formed α-helical-rich structure in the presence of negatively charged membranes. The tryptophan fluorescence and quenching experiments indicated that the peptides bound preferentially to negatively charged phospholipids over zwitterionic phospholipids, which corresponds well with the biological activity data. A novel α-helical antimicrobial peptide was screened and the results showed that the peptides administered 2.5 and 5.0 mg/kg BW significantly ($P < 0.05$) decreased the mortality of the mice and the peritoneal bacterial counts. Collectively, the peptides rich in Val/Arg residues may provide a useful alternative to antibiotic agents to treat or prevent bacterial infections.

Creatine supplementation of Zucker fatty rats attenuates hepatic steatosis

M.E. Brosnan, S.P. Waghmare, K. Clow, R.J. Brown, and J.T. Brosnan

Department of Biochemistry, Memorial University of Newfoundland, St John's NF, A1B 3X9, Canada

We have shown that supplementation with creatine monohydrate prevents hepatic steatosis caused by feeding a high-fat diet (70 % of calories) in rats. We now examine whether creatine supplementation could prevent hepatic fat accumulation in genetically obese Zucker rats fed a moderate-fat diet. Seven-week-old male lean (*Fa/?)* and obese (*fa/fa*) rats were sacrificed at the start of the study to obtain baseline data. Baseline liver histology with Oil-Red-O revealed a minor degree of hepatic fat accumulation in *fa/fa* as compared to *Fa/?* rats. Additional groups of *fa/fa* rats were fed either Purina 5008 chow (7 % fat w/w) or the same diet supplemented with 1 % w/w creatine monohydrate for 6 weeks. Creatine supplementation down-regulated endogenous creatine synthesis, as evidenced by decreased levels of guanidinoacetate in both groups of rats. It also reduced total hepatic fat by 55 % and hepatic triglyceride content by 66 %. This was confirmed by Oil-Red-O staining. Hepatic S-adenosylmethionine levels were increased by the creatine supplementation. There was also a trend towards decreased hepatic cholesterol in the creatine-supplemented rats. At baseline, creatine levels in livers of *fa/fa* rats were significantly lower than those in the *Fa/?* rats while liver guanidinoacetate concentration was higher, suggesting an impaired synthesis of creatine. These data extend the reach of creatine's effect in decreasing liver fat to genetically obese rats fed a moderate-fat diet.

(Supported by CIHR, NSERC and Memorial University of Newfoundland).

Consequences of supplementation with specific amino acids on the gut-associated lymphoid tissue

Megan R. Ruth, and Catherine J. Field

Department of Agricultural, Food and Nutritional Sciences, 4-126A Li Ka Shing Health Research Innovation Centre, University of Alberta, Edmonton, AB T6G 2E1, Canada

The intestine and gut-associated lymphoid tissue (GALT) function to protect the body from foreign antigens and pathogens, while permitting tolerance to commensal bacteria and dietary antigens. The requirement for protein to support the immune system is well established. Less is known regarding the immune modifying properties of individual amino acids on GALT. Oral and parenteral feeding studies have established convincing evidence that not only the total protein intake, but the availability of specific amino acids [in particular, glutamine (Gln), glutamate (Glu), and arginine (Arg), and perhaps methionine (Met), cysteine (Cys) and threonine (Thr)] are essential to optimizing the immune functions of the intestine and proximal immune cells. These amino acids each have unique properties that include, maintaining the integrity, growth and immune related functions of the intestine, normalizing inflammatory cytokine secretion and improving T-lymphocyte numbers, specific T cell functions, and the secretion of IgA by lamina propria cells. Our understanding of this

area has come from studies where single amino acids are supplemented to a mixed protein diet or amino acid mixture in total parenteral nutrition and the effect on select immune parameters were measured. The evidence and mechanisms through which these amino acids modify GALT will be discussed in this presentation and may include serving as immunotransmitters (Glu), as energy substrates for enterocytes and leukocytes (Gln & Glu), and as precursors for bioactive molecules, including glutathione (Cys, Met, Glu & Gln), nitric oxide (Arg), and mucins (Thr).

Adaptation of colonocyte mitochondria to lumen borne hydrogen sulfide

Frédéric Bouillaud[1], M Andriamihaja[2], S. Mimoun[2], C. Ransy[1], C. Chaumontet[2], and F. Blachier

[1]Institut Cochin INSERM, CNRS, Université Paris Descartes Paris France;
[2]INRA-AgroParisTech, Paris France

Sulfide (H_2S, HS^-, S^{2-}) is highly toxic because it is an inhibitor of the mitochondrial complex IV similar to cyanide. When intact cells are studied sulfide concentration above 10 μM cause a significant inhibition of cellular respiration. The target is the complex IV of the mitochondrial respiratory chain (cytochrome oxidase). Sulfur containing amino acids as well as other sulfur containing molecules feed the anaerobic metabolism of the bacteria in the colonic lumen, which raises concentrations of free sulfide to 60 μM or even to millimolar concentrations if one considers the bound sulfide. However, sulfide is also used as a substrate by the mitochondrial respiratory chain. The enzyme involved is a sulfide quinone reductase (SQR), which reduces coenzyme q (quinone) in the mitochondrial respiratory chain. Therefore, according to concentrations sulfide stimulates (high nM, low μM) or inhibits (>10 μM) cellular respiration. Clearly the lumen borne sulfide puts colonocytes in jeopardy of a severe sulfide poisoning. Colonocytes are adapted to maximize their sulfide oxidation capacity: (1) The use of carbon containing substrates by cells involves mitochondrial complex I (NADH coenzyme Q oxidoreductase) or II (succinate dehydrogenase) also reducing quinone but SQR takes priority over the use of other substrates. (2) The activity of SQR makes it able to saturate the mitochondrial respiratory chain (complex III and IV) with electrons from sulfide. (3) Moreover, a reverse electron transfer in mitochondrial complex I increases further the sulfide disposal rate. However, when the sulfide exposure is too high the colonocytes switch towards a glycolytic metabolism.

Dipeptidyl peptidase IV inhibitory properties of milk protein-derived peptides

Alice B. Nongonierma, and Richard J. FitzGerald

Department of Life Sciences and Food for Health Ireland (FHI), University of Limerick, Limerick, Ireland

The increasing incidence of type 2 diabetes (T2D) worldwide, with projections of 366 million T2D subjects in 2030, has led the scientific community to investigate different strategies for the management of this disease. Such strategies include inhibition of dipeptidyl peptidase IV (DDP-IV). This aminodipeptidase activity hydrolyses glucoregulatory incretin hormones such as glucose dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) to inactive components. Food protein derived peptides represent natural sources of DDP-IV inhibitors. However, only a limited number of peptide sequences have been identified to date. Milk proteins contain regions within their primary sequences possessing peptide motifs with preferred DPP-IV substrate characteristics (a Pro residue at the penultimate position). New milk protein derived DDP-IV inhibitory peptide sequences were identified herein using an in silico approach following milk protein digestion with gastrointestinal enzymes. The DPPIV inhibitory potential was subsequently evaluated using an in vitro synthetic peptide approach. Furthermore, the stability of selected milk protein derived sequences in the presence of DPP-IV was also evaluated in silico, some of the selected peptides were predicted to be DPP-IV substrates. Evaluation of the interaction between milk peptides and a DPP-IV inhibitory drug (Sitagliptin) showed a combined DPP-IV inhibition in vitro, which may have implications for the pharmacokinetics of this drug in vivo. Overall milk-protein derived peptides show potential as dietary ingredients in the management of T2D to extend the half-life of incretin molecules.

Glutamate and *N*-carbamylglutamate supplementation for swine production

Xin Wu[1], Zikui Liu[2],Yulong Yin[1,*], François Blachier[3], and Guoyao Wu[4]

[1]Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, 410125, China;
[2]Veterinary Faculty, Hunan Agricultural University, Changsha, Hunan, 410128, China;
[3]AgroParisTech, CNRH-IdF, UMR914 Nutrition Physiology and Ingestive Behavior, 75005 Paris, France;
[4]Department of Animal Science, Texas A&M University, College Station, TX 77843, USA

Dietary glutamate is a major energy source and an important substrate for the synthesis of glutathione and other amino acids in the gut. Almost all dietary glutamate, both in free form and as a protein constituent, is metabolized in the intestinal mucosa. Results of our previous study showed that intestinal *N*-acetylglutamate (NAG) synthase mRNA levels were lower in 7- to 28-day-old than in 1-day-old pigs. *N*-carbamylglutamate (NCG), an analogue of NAG, is a metabolically stable activator of CPS-I. NCG helps overcome the practical limitation of arginine delivery to the neonates. We found that dietary supplementation with NCG to gestating sows: (1) increased the number of piglets born alive; (2) decreased the number of piglets born dead; and (3) affected the levels of microRNAs targeting the VEGFA and eNOS genes in the umbilical vein. Metabolic data show that arginine is synthesized from dietary glutamate in enterocytes of neonatal pigs. In our studies, dietary supplementation with glutamate plus NCG increased plasma arginine in piglets surgically fitted with a catheter in the jugular and portal vein. A combination of glutamate and NCG also has a greater effect on intestinal epithelial cell proliferation than glutamate alone. Collectively, glutamate plus NCG can provide a new and effective way to enhance intestinal synthesis of citrulline and arginine, leading to the sparing of protein resources in swine production.

Low dose GYKI-52466: a prophylactic approach to neuroprotection

D. Steven Kerr, and Prasanta K. Nayak

Department of Pharmacology & Toxicology, University of Otago Medical School, Dunedin, New Zealand

Preconditioning with low dose kainic or domoic acid induces tolerance against the excitotoxic actions of higher doses of these

toxins. Given the lasting nature of the tolerance effect, we examined G-protein coupled receptor function and found, quite unexpectedly, that a number of classical AMPA and KA agonists and antagonists (such as NBQX, GYKI 52466, NS-102, and GAMS) act as strong inverse agonists of GPCR function. Subsequently, we showed that the most potent inverse agonist, GYKI-52466, induces tolerance to high-dose KA both in vitro and in vivo. Here we assessed low-dose GYKI-52466 preconditioning in a rat model of hypoxic-ischaemic (HI) stroke. Young Sprague–Dawley rats were administered GYKI-52466 (0.5–3 mg/kg) 90–180 min prior to unilateral carotid artery ligation. Animals were allowed to recover for 2 h and placed in a hypoxia chamber for 1 h. Sensorimotor tests were performed pre-surgery and at 1, 7, 14 and 90 days post-HI. At sacrifice, brains were fixed and sectioned for histological assessment of damage. Low-dose GYKI-52466 significantly reduced infarct volume and ventricular enlargement at day 14 and 90 post-HI and dramatically improved motor function in all treatment groups. Pharmacokinetic analyses indicated brain levels of only 0.6 μM GYKI-52466 at 90 min post-administration. Our results show that GYKI-52466 is effective at doses well-below, and at pre-administration intervals well-beyond previous studies, and suggest that classical antagonism of ionotropic AMPA receptors does not underlie its neuroprotective efficacy. GYKI-52466 preconditioning represents a novel, prophylactic strategy for neuroprotection in a field almost devoid of effective pharmaceuticals.

N-Acetylcysteine stimulates proliferation and prevents lipopolysaccharide-induced death of intestinal porcine epithelial cells

Minhui Long[1], Wanjin Ouyang[1], Dan Yi[1], Yongqing Hou[1], Guoyao Wu[2]

[1]Hubei Key Laboratory of Animal Nutrition and Feed Science, Wuhan Polytechnic University, Wuhan 430023, China;
[2]Department of Animal Science, Texas A&M University, College Station, TX, USA 77843

We previously reported the protective effects of *N*-acetylcysteine (NAC) on intestinal functions of lipopolysaccharide (LPS)-challenged piglets. To further investigate the underlying mechanisms, in vitro studies were performed with intestinal porcine epithelial cells (IPEC-1). The cells were cultured for 4 days in cysteine-free Dulbecco's modified Eagle's-F12 Ham medium (DMEM-F12) containing 0, 100, 200, or 400 μM NAC and 0 or 10 μg/ml LPS in a 3 × 2 factorial arrangement. Cell numbers and proliferation, expression and activation of mammalian target of rapamycin (mTOR), signal transducer and activator of transcription 3 (STAT3), and epidermal growth factor receptor (EGFR) signaling molecules were determined. Without LPS, cells exhibited a time- dependent growth curve, and 100 μM NAC stimulated cell growth. LPS treatment reduced cell number, the mRNA level for B cell lymphoma-extra large (Bcl-xl), Ras, Akt, insulin-like growth factor 1 (IGF-1), insulin-like growth factor 1 receptor (IGF-1R), sodium-dependent glucose co-transporter 1 (SGLT-1), and porcine β-defensin-1 (pBD-1), while increasing expression of the genes for Bcl-2 associated X protein (Bax), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-a), STAT3, and heat shock protein 70 (HSP70). Addition of 100 μM NAC to culture medium attenuated the LPS-induced cell death and restored the mRNA levels of these genes to the values for the control group. These results indicate a protective effect of NAC on LPS-induced apoptosis of enterocytes via mechanisms involving EGFR and STAT3 signing pathways.

Role of D-amino acids in a deep-sea bacterium *Shewanella violacea* DSS12

Shota Watanabe[1], Yasumasa Shiraishi[1], Minoru Tanigawa[1], Hideyuki Tamegai[2], Chiaki Kato[3], and Katsushi Nishimura[1]

[1]Department of Materials and Applied Chemistry, College of Science and Technology, Nihon University, Tokyo, Japan;
[2]Department of Chemistry, College of Humanities and Science, Nihon University, Tokyo, Japan;
[3]Japan Agency for Marine-Earth Science and Technology, Yokosuka, Japan

Shewanella violacea DSS12 (*S. violacea*) is a psychrophilic and facultatively piezophilic bacterium which was isolated from the mud of the Ryukyu Trench. The expression of respiratory components in this organism is regulated by pressure and aerobic conditions. A hypothetical gene encoding D-amino acid dehydrogenase (DAD) exists in the genome of *S. violacea*, and the activity of DAD has been detected. In this study, to gain a better understanding of the role of D-amino acids in *S. violacea*, we investigated effects of D-amino acids on the growth of the organism and characterized DAD. *S. violacea* was cultivated in a Marine Broth containing 10 mM D- or L-amino acid (Ala, Glu, Pro and Ser). Of these amino acids, only D-serine showed inhibitory effect on the growth. *S. violacea* cells grown in Marine Broth in aerobic or microaerobic condition were harvested. The cells were disrupted by ultrasonication and centrifuged to yield a supernatant which was used as the cell-free extract. Free amino acids in the cell free extract were determined with an amino acid analyzer after removal of proteins. *S. violacea* contained, in order of abundance, Pro, Glu and Ala in both culture conditions. DAD activity was spectrophotometrically assayed with 2,6-dichlorophenolindophenol using D-amino acids (Ala, Asp, Glu, Met, Pro, Ser and Val) as substrates. The cell-free extracts of aerobic and microaerobic conditions exhibited DAD activity for D-Pro and D-Ser, respectively. DAD had a temperature optimum of 55 °C and a pH optimum of 7.0. In the case of addition of D-Pro, the reduction of cytochromes was observed indicating an electron transfer from DAD to cytochromes.

Physiological roles of glutamate signaling as taste and visceral information via gut-brain Axis due to efficient digestion and metabolism for homeostasis

Kunio Torii, Tokyo, Japan

Dietary glutamate (Glu) stimulates the digestive tract to evoke umami taste, one of the five basic tastes, enhancing food palatability. Glutamate is also a major energy source for the small-intestinal mucosa. Thus, this amino acids is crucial for the absorption and metabolism of dietary nutrients. Because nearly all Glu in the enteral diet is catabolized in the small intestine, only a trace amount of free Glu reaches the general circulation. Recently, we demonstrated a unique gut sensing system for free Glu (glutamate signaling). Glu is the only one nutrient among amino acids, sugars and electrolytes in the stomach that activates rat gastric vagal afferents from the luminal side specifically via metabotropic Glu receptors type1 (mGluR1) on mucosal cells releasing mucin and nitric oxide (NO). Then, NO stimulates serotonin (5HT) release from the enterochromaffin cell. Finally, the released 5HT stimulates $5HT_3$ receptor at the nerve end of gastric vagal afferent fiber. Glu signaling in the stomach with food digests may play a role in recognizing food intake and, subsequently, in initiating digestion processes in the gut. Functional magnetic resonance imaging (f-MRI, 4.7T) analysis revealed that luminal sensing with most preferable 60 mM MSG (monosodium L-glutamate) in the rat stomach activates

the lateral hypothalamic area (LHA, feeding center), enhancing appetite for the presented food, and separately does both the medial preoptic area (mPOA, body temperature controller) and the dorsomedial hypothalamus (DMH, basic metabolic rate regulator), resulting in diet-induced thermogenesis and higher energy expenditure during eating without changes in appetite for food. Interestingly, when rats were forced to consume a high-fat and high-sugar diet with free access to 60 mM MSG and drinking water in a choice paradigm, they showed a strong preference for MSG solution and exhibited reduced rates of fat deposition in the body, weight gain and blood leptin. Interestingly, brain functional changes, which were revealed by f-MRI signals, after administration of 60 mM MSG into the stomach were abolished in the case of total vagotomized rats, suggesting that luminal glutamate signaling contributes to efficient digestion and thermogenesis without development of obesity. On the other hand, glutamate signaling induces digestive juice cascade-type secretion through mGluR1 and mucin release (phospholipase C system) for the mucosal barrier system against auto-digestion and bacterial invasion. Some portion of Glu signaling is directly stimulating this function but its major action is controlled via the gut-brain axis due to maintenance of the normal alimentary tract function without any disorder of unpleasant symptoms. Collectively, free Glu in foods serves as an essential signal for food intake recognition and the digestion and metabolism of dietary nutrients to maintain whole-body homeostasis in mammals.

Role of D-amino acid dehydrogenases of three microorganisms in different environments

Koichiro Suzumoto[1], Hitomi Usui[1], Katsuya Sato[1], Shota Watanabe[1], Minoru Tanigawa[1], Hideyuki Tamegai[2], Chiaki Kato[3], and Katsushi Nishimura[1]

[1]Department of Materials and Applied Chemistry, College of Science and Technology, Nihon University, Tokyo, Japan;
[2]Department of Chemistry, College of Humanities and Science, Nihon University, Tokyo, Japan;
[3]Japan Agency for Marine-Earth Science and Technology, Yokosuka, Japan

D-Amino acid dehydrogenase (DAD) is a membrane-bound enzyme that catalyzes dehydrogenation reaction of neutral free D-amino acids without using oxygen. We have previously found DAD activities in a deep-sea bacterium *Shewanella violacea* DSS12 (*S. violacea*), a hyperthermophilic archaeon *Pyrobaculum oguniense* (*P. oguniense*) and a sulfur-oxidizing chemolithotroph *Starkeya novella* (*S. novella*). The present study is an attempt to reveal the role of DAD in these microorganisms. *S. violacea, P. oguniense* and *S. novella* were cultivated in aerobic and microaerobic conditions, in aerobic condition and in autotrophic and heterotrophic conditions, respectively. Microbial cells were disrupted and centrifuged to yield a supernatant which was used as the cell-free extract. DAD activity was spectrophotometrically assayed with 2,6-dichlorophenolindophenol using D-amino acids (Ala, Asp, Glu, Met, Pro, Ser and Val) as substrate. In *S. violacea*, the cell-free extracts from the aerobic and microaerobic conditions exhibited DAD activity for D-Pro and D-Ser, respectively, and addition of D-Pro caused a spectral change indicating reduction of cytochromes. *P. oguniense* exhibited DAD activity for D-Pro, D-Val and D-Ala. In *S. novella*, the cell-free extracts from the autotrophic and heterotrophic conditions showed DAD activity for D-Pro and D-Ala, respectively, and addition of the D-amino acids caused a spectral change indicating reduction of cytochromes only in the case of heterotrophic condition. These observations demonstrated an electron transfer from DAD to cytochromes in some microorganisms suggesting that DAD plays a role in ATP production. The expression of DAD in these microbes is being further investigated.

Amino acid signaling: mechanisms and translational relevance in medicine and nutrition

Teresa A. Davis, Agus Suryawan, Renan A. Orellana, and Marta L. Fiorotto

USDA/ARS Children's Nutrition Research Center, Dept. Pediatrics, Baylor College of Medicine, Houston, TX, USA

Food ingestion stimulates the synthesis of muscle proteins and this response is triggered by the postprandial rise in amino acids, particularly leucine, and insulin. Although the amino acid signaling pathway is incompletely characterized, the consensus is that amino acids and insulin induce protein synthesis by activating independent signaling pathways that converge at mechanistic target of rapamycin complex 1 (mTORC1), leading to the activation of key regulators of translation. Using the neonatal pig as a model of the human neonate, we have identified components of the amino acid and insulin signaling pathways that regulate the high rate of growth during the neonatal period and are using this information to develop strategies to optimize growth of low birth weight infants by improving their nutritional management. For example, we have shown that intermittent bolus feeding, as compared to continuous feeding, can enhance muscle growth by eliciting a pulsatile pattern of amino acid- and insulin-induced translation initiation. Additionally, our studies have shown that leucine supplementation can enhance protein synthesis in muscle of the newborn pig by stimulating mTORC1-dependent translation initiation. Further studies are needed to establish whether the anabolic effects of leucine can be sustained chronically to promote lean growth.

(Supported by NIH AR444474, NIH HD072891, USDA NIFA 2013-67015-20438, and USDA/ARS 6250-51000-055).

The regulation of mTORC1 signaling by amino acids in skeletal muscle of neonatal pigs

Agus Suryawan, Hanh V. Nguyen, and Teresa A. Davis

USDA/ARS-CNRC, Pediatrics, Baylor College of Medicine, Houston, TX, USA

The activation of insulin (I) and amino acid (AA) signaling pathways towards mTORC1 is enhanced in skeletal muscle of the neonate leading to an elevated rate of protein synthesis. Studies show that Ragulator (LAMTOR1, ROBDL3) and v-ATPase are crucial new components for AA-induced activation of mTORC1. Rheb and Grb10 also control mTORC1 activation. Rheb-induced mTORC1 activation involves interaction of Rheb with mTORC1 and Ragulator. In this study we determined the effects of the postprandial rise in I and AA on the activation and/or abundance of Rheb, Grb10, LAMTOR1, ROBDL3, and v-ATPase in muscle and whether the responses are modified by development. Overnight fasted 6- and 26-day-old pigs were infused for 2 h with saline or with I or AA to achieve fed levels. Rheb-mTORC1 complex abundance in muscle was increased by both I and AA and the response was greater in 6-day old pigs. The abundance of LAMTOR1, ROBDL3 and v-ATPase was higher and Grb10 was lower in 6-day-old pigs. Neither I nor AA altered the abundance of these components. Both I and AA increased Grb10 phosphorylation but the response was not affected by age. These results suggest that the enhanced mTORC1 activation in the neonate is in part due to regulation by Rheb, LAMTOR1, ROBDL3, v-ATPase, and Grb10.

(Supported by NIH AR444474 and USDA/ARS 6250-51000-055).

The control of proline metabolism in cancers by oncogenic and tumor suppressor signalings

Wei Liu, Meredith P. Harman, Chad Hancock, James M. Phang

Metabolism and Cancer Susceptibility Section, Basic Research Laboratory, Center for Cancer Research, National Cancer Institute at Frederick, National Institutes of Health, Frederick, MD 21702, USA

Growing tumors alter their metabolic profiles to meet the bioenergetic and biosynthetic demands of increased cell growth and proliferation. It has been well appreciated that multiple metabolic pathways can be reprogrammed by different oncogenic and tumor suppressor signalings. Proline metabolism is a critical component of metabolic reprogramming in tumors. Proline dehydrogenase/oxidase (PRODH/POX), the first enzyme in proline catabolism, has been identified as a tumor suppressor protein, which can initiate apoptosis, inhibit tumor growth and block the cell cycle by ROS signaling. During tumor progression, PRODH/POX is under the control of various tumor-associated factors. It can be induced by tumor suppressor p53 and inflammatory factor peroxisome proliferator-activated receptor gamma (PPARγ), and suppressed by onco-miRNA miR-23b* and oncogenic transcription factor c-MYC. However, under metabolic stress such as oxygen and glucose deprivation, proline catabolism can be induced by AMPK signaling to promote tumor survival through ATP production or ROS-induced autophagy. Our recent studies show that c-MYC not only suppresses proline catabolism, but also promotes proline biosynthesis from glutamine. Blockade of the proline biosynthetic pathway markedly decreased aerobic glycolysis, and thus ATP production and cell growth. These findings further established a critical role for proline biosynthesis in linking the reprogramming of glutamine and glucose metabolism in tumors. Further studies of the roles of proline metabolism in tumorigenesis and tumor development will provide a deeper understanding of tumor metabolic reprogramming and novel therapeutic strategies in cancer.

pH and redox buffering of mitochondrial oxidation

Svend Høime Hansen[1], Niels Grunnet[2]

[1]Department of Clinical Biochemistry, 3-01-1, Rigshospitalet, Copenhagen University Hospital, 2100 Copenhagen Ø, Denmark; [2]Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, 2200 Copenhagen N, Denmark

The mitochondria are the powerhouses of the cells. The majority of the cellular ATP production can be ascribed to the coupled mitochondrial processes formed by the tricarboxylic acid cycle, the electron transport chain and the ATP synthase. The tricarboxylic acid cycle produces NADH that acts as substrate for the electron transport chain, which subsequently creates a proton gradient across the mitochondrial membrane. Finally ATP synthase subsequently utilises the proton gradient for the ATP production. To ensure robustness of these coupled systems for the oxidative phosphorylation, some basic support systems exist to stabilise these processes, e.g. as pH or pH buffers. Consequently, understanding the coupling between of the oxidative and support processes is pivotal for studying the mitochondrial function. Taurine is found in especially high concentrations in oxidative tissue due to an up-concentration of taurine inside the mitochondria. The slightly alkaline pK value for the amino group in taurine and its general resistance towards oxidation makes taurine an ideal pH buffer for stabilising the oxidative environment in the mitochondrial matrix, although only in animal cells. The thiol groups from the tripeptide glutathione (GSH) and the thioredoxin proteins can be considered as redox buffers in the mitochondria, and thus involved in the scavenging of the reactive oxygen species (ROS) formed as by-products in the mitochondrial oxidation. At slightly alkaline pH due to taurine pH buffering it seems possible to establish a redox equilibrium between the redox pairs NADH/NAD^+ and thiol/disulfide.

Optimization of magnetic bead enzyme-linked immunosorbent assay for detection of specific antibodies against Amyloid beta representing biomarkers of Alzheimer's disease

Pavla Krulisova, Barbora Jankovicova, Jana Kucerova, Zuzana Bilkova

Department of Biological and Biochemical Sciences, Faculty of Chemical Technology, University of Pardubice, Studentska 573, 532 10 Pardubice, Czech Republic

Alzheimer's disease (AD) is the most common cause of dementia worldwide with an increasing prevalence and incidence in the aging population. AD diagnostics are rapidly evolving, the goal is to create a method that is accurate, relatively non-invasive and inexpensive. Naturally occurring plasma autoantibodies relating to Amyloid beta (Aβ), which is one of the main hallmarks of AD pathology, are now attracting significant interest in this field. We optimized method for determination of these antibodies based on magnetic bead enzyme-linked immunosorbent assay (ELISA). Surface of amino-functionalized superparamagnetic microparticles represented a solid phase to which recombinant Aβ peptide (1–42) was immobilized through the C-terminus. Horseradish peroxidase (HRP) assisted colorimetric detection was applied in the assay. Procedure was firstly optimized to increase assay sensitivity. Surface modification of magnetic carrier with poly(ethylene glycol) (PEG) was chosen as the best compared to non-treated beads and beads treated with bovine serum albumin (BSA) as conventional blocking reagent. Significant increase of immobilized antigen amount and suppression of non-specific adsorption was observed in this case. Finally, the antibodies against Aβ from the sera of AD patients and healthy controls were quantified.

(This work was supported by MSMT project POSTDOK No. CZ.1.07/2.3.00/30.0021, project GACR P206/12/0381 and 7. FP EU project NADINE No. 246513.)

Immunodominant epitope of carbonic anhydrase I identified by phage display peptide library

Barbora Jankovicova[1], Petr Muller[2], Jan Lakota[3], Borivoj Vojtesek[2], Zuzana Bilkova[1]

[a]Department of Biological and Biochemical Sciences, University of Pardubice, Pardubice, Czech Republic; [b]Department of Oncological and Experimental Pathology, Masaryk Memorial Cancer Institute, Brno, Czech Republic; and [c]Laboratory of Molecular Oncology, Cancer Research Institute, Slovak Academy of Sciences, Bratislava, Slovakia

Carbonic anhydrase I (CAI) is zinc metalloenzyme found at the highest level in erythrocytes where catalyses reversible hydration of carbondioxide which is essential to many biological processes. CAI and antibodies against CAI have been reported in some cancer diseases as reliable diagnostic indicators. In mice we generated a set of monoclonal antibodies (mAbs) against CAI from human erythrocytes. Using dot-blot technique mAb with the strongest immunoreaction was

chosen and epitope characterization by phage display peptide library followed. Phage-displayed dodecapeptides were incubated with target mAb in complex with Protein A/G bound on solid phase. The specifically bound phage were eluted, amplified in *E. coli* and taken through additional two cycles. Finally, individual clones were characterized by DNA sequencing. Selected mAb recognized best amino acid sequence DFW corresponds to amino acids 190–192 of CAI and additionally also last three amino acids of CAI (sequence ASF, amino acids 259–261). Alignment analysis indicated that these amino acids are in close contact and form surface conformational epitope. This information could be useful for diagnostic or therapeutic purposes.

(This work was supported by MSMT project POSTDOK No. CZ.1.07/2.3.00/30.0021, project GACR P301/11/1678 and 7. FP EU project NADINE No. 246513.)

Large-scale method of blood plasma fractionation for sulfur amino acids transport evaluation

Alexandr A. Zhloba, Tatiana F. Subbotina

Biochemical Department of Scientific Centre, Pavlov State Medical University of St.-Petersburg, Russia. Proteomics Group, Almazov Federal Heart, Blood and Endocrinology Centre, St.-Petersburg, Russia

Protein-bound mixed homocysteine-disulfide can be formed not only with albumin, but also especially under pathological conditions with other proteins, including apolipoprotein B and alpha 2- macroglobulin. The distribution assessment of homocysteine (Hcy) between proteins is made by a gel-filtration method. An assessment of Hcy redistribution between fractions of proteins by means centrifugal ultrafiltration is offered. We used blood plasma from healthy donors and cardiovascular patients including a group with elevated d-dimer level. Work represents a method of evaluation the tHcy and its fraction after ultrafiltration, allowing cut off proteins with mass above 300 kDa. Ultrafiltrates were obtained using Vivaspin 500 (Sartorius, Germany) 100 MWCO PES (~100-kDa cutoff) и 300 MWCO PES(~300-kDa cutoff) centrifugal devices. The data provide evidence the Hcy elevation in macromolecular fraction of plasmas after activation of alpha 2-macroglobulin. The proposed Hcy evaluation in plasma preparations prior and after ultrafiltration may be performed in any laboratory with kits for tHcy evaluation in plasma. Evaluation of Hcy fraction associated with >300 kDa plasma proteins by means of centrifugal device Vivaspin 300,000 MWCO PES « Sartorius » provides information about Hcy transport by an additional way in macrophages, endothelial cells, smooth muscle cells and others in vascular wall by receptorial endocytosis.

Fibrinolysis as an additional source of basic amino acids in plasma

Tatiana F. Subbotina, and Alexander A. Zhloba

Biochemical Department of Scientific Centre, Pavlov State Medical University, Proteomics Group, Almazov Federal Heart, Blood and Endocrinology Centre St.-Petersburg, Russia

Exopeptidase activity may appear during coagulation and subsequent fibrinolysis including thrombin-activatable fibrinolysis inhibitor, carboxypeptidase N, and small amounts of carboxypeptidase B. All these enzymes can cleave the C-terminal lysine and arginine residues from fibrin. The aim was the development of a method for detection of blood carboxypeptidase activity associated with coagulation/fibrinolysis using the natural substrate fibrin by means of detection of reaction products, lysine and arginine. Plasma samples from 35 patients with cardiovascular diseases, 20 donors and 14 plasma pools have been investigated. Coagulation and subsequent fibrinolysis were initiated by addition of thrombin (or tissue factor) and tissue plasminogen activator, respectively. Arginine and lysine concentrations before and after completion of fibrinolysis were determined by RP-HPLC using C18 column. The parameters of fibrinolysis were evaluated by the clot turbidity assay. The coagulation/fibrinolysis was accompanied by a significant (near twofold) increase in concentrations of arginine and lysine. The degree of increase significantly correlated with time of fibrinolysis initiation. Therefore, fibrinolysis can be considered as a local source of the essential amino acids originated from fibrin clot degradation. This on-site increase in arginine concentration can be important for endothelial nitric oxide synthase activity, subsequent increase in NO production, and vasodilation. Thus, fibrinolysis provides not only clot lysis but also restoration of circulation.

How to choose the best dietary precursor of cysteine for glutathione synthesis?

D. Rémond, I. Savary-Auzeloux, C. Buffiere, K. Vidal, M. Faure, D. Dardevet, and D. Breuillé

INRA de Clermont-Fd/Theix, UMR 1019, Unité de Nutrition Humaine, Clermont-Ferrand, France

Due to its important protective functions, the maintenance of glutathione status in pathological situations is an important nutritional target. Cysteine is considered as the limiting amino acid for its synthesis. Unfortunately, its bioavailability from enteral solutions was shown to be low. We used a model of multicatheterized minipigs, maintained under enteral nutrition, in order to evaluate the bioavailability of cysteine, after an intragastric bolus of different cysteine precursors: free cysteine (Cys), cystine (Cyss), *N*-acetylcysteine (NAC), or S,N-diacetylcysteine monoethylester (DACE). Portal recovery and hepatic uptake of total cysteine (tCys = Cys + Cyss) were greater after Cys bolus than after Cyss bolus: 68 ± 5 % of the dose vs. 54 ± 4 % ($P < 0.01$,) and 61 ± 6 % of the dose vs. 43 ± 4 % ($P < 0.01$), respectively. Portal recovery of NAC and DACE were similar (22 ± 5 %), but lower than that of Cys and Cyss. Both derivatives were partly converted into cysteine within the gut, and tCys portal net flux accounted for 33 ± 4 % of NAC or DACE bolus. Their removal by the liver was less efficient than that of tCys (20 vs. 85 % of the portal release). The splanchnic release of tCys + NAC or tCys + DACE accounted for 22 % of NAC or DACE bolus vs. 10 % of tCys with Cys bolus. In conclusion, oral free cysteine ensures the greatest availability of cysteine for hepatic metabolism. However, if peripheral availability of cysteine is targeted, NAC or DACE could be more efficient because of their lower splanchnic extraction.

Intestinal amino acid sensing: role of dietary monosodium glutamate in preterm pigs

Barbara Stoll

Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA

L-Glutamate is the most abundant amino acid in dietary protein and occurs in free form in many natural products. Monosodium

glutamate also is added to prepared foods to enhance flavor since it elicits the *umami* taste. Dietary glutamate is a major oxidative fuel for the gut and is extensively metabolized in first pass by the intestine. Glutamate also is an important precursor for bioactive molecules, such as glutathione. The dominant role of glutamate as an oxidative fuel may have therapeutic potential for improving function of the infant gut, which exhibits a high rate of epithelial cell turnover. Our studies suggest that at high dietary intakes, free glutamate may be absorbed by the stomach as well as the small intestine, thus implicating the gastric mucosa in the metabolism of dietary glutamate. In addition to its nutritional role, glutamate is a key excitatory neurotransmitter. The recent discovery of nutrient-sensing cells and mechanisms in the gastric and intestinal mucosa that resemble those of the taste buds of the tongue provide a molecular link between specific dietary nutrients and various physiologic functions. The emerging evidence of gastrointestinal sensory cells interacting with luminal nutrients suggests that free glutamate may play a functional role in the gut physiology. We will present recent evidence from studies with premature pigs that adding monosodium glutamate to partial enteral nutrition slowed the gastric emptying rate.

Increased consumption of methionine as methionine hydroxy analog is positive to alleviate early weaning induced growth retardation in piglets

Zhengfeng Fang[1], De Wu[1], Lianqiang Che[1], Yan Lin[1], Shengyu Xu[1], Yves Mercier[2]

[1]Key Laboratory for Animal Disease Resistance Nutrition of the Ministry of Education of China, Animal Nutrition Institute, Sichuan Agricultural University, Ya'an 625014, China; [2]Adisseo France S.A.S., CERN, Commentry, France

To determine whether the early weaning induced growth retardation could be attenuated by increased inclusion of methionine in the diets of lactating sows and weaned piglets, DL-methionine (DLM) and its hydroxyl analog DL-2-hydroxy-4-methylthiobutyrate (HMTBA) were supplemented at 25 % of the total sulfur amino acids in the control (CON) diet to form the DLM and HMTBA diets. Primiparous sows (Landrace × Yorkshire) with similar bodyweight and backfat thickness were fed the CON, DLM or HMTBA diet ($n = 6$) from the postpartum day. Piglets from three of the six sows in each group were weaned at day 21 and 28, respectively. Overall, the HMTBA diet resulted in increased piglet bodyweight ($P < 0.05$ or $P = 0.11$) at postnatal day 35, and enhanced feed intake ($P < 0.05$ or $P = 0.09$) during the second week post-weaning compared with the CON and DLM diets. The HMTBA- and DLM-fed sows had increased plasma methionine ($P < 0.10$) and taurine ($P < 0.05$ or $P < 0.10$) levels, but plasma lysine ($P < 0.05$ or $P < 0.10$) and isoleucine ($P < 0.05$) levels were lower in the DLM-fed than in the CON- and HMTBA-fed sows. The HMTBA-fed piglets had up-regulated ($P < 0.05$) expression of intestinal FZHUI fatty acid-binding protein in jejunum, higher ($P < 0.05$) villus height and the ratio of villus height to crypt depth in jejunum and ileum, and more ($P < 0.05$) goblet cells in duodenum and ileum than the CON- and DLM-fed piglets. Taken together, increased consumption of methionine as HMTBA contributed to alleviated growth retardation in early weaned piglets, which may have important implications for the nutritional management of lactating sows and weanling piglets.

Increased consumption of methionine as methionine hydroxy analog promotes sow milk synthesis without compromise of lysine availability

Zhengfeng Fang[1], De Wu[1], Lianqiang Che[1], Yan Lin[1], Shengyu Xu[1], Yves Mercier[2]

[1]Key Laboratory for Animal Disease Resistance Nutrition of the Ministry of Education of China, Animal Nutrition Institute, Sichuan Agricultural University, Ya'an 625014, China; [2]Adisseo France S.A.S., CERN, Commentry, France

Dietary essential amino acids (AA) play an important role in mammalian milk synthesis. However, the extensive catabolism of dietary essential AA by the intestine results in decreased availability of these AA for mammary tissues. Here, we used primiparous sows as the model and showed that increased consumption of methionine as DL-2-hydroxy-4-methylthio butanoic acid (HMTBA) by lactation mothers was positive to infant growth due to the elevated fat, cystine and taurine levels in milk and increased milk synthesis as suggested by higher lactose levels. Metabonomic results based on ^{1}H nuclear magnetic resonance spectroscopy in conjunction with multivariate data analysis revealed significant associations of methionine sources-induced change of systemic AA profile and milk compositions with marked alterations in lipid, glycogen and AA metabolisms, which were manifested in that HMTBA and DL-methionine (DLM) consumption both resulted in increased levels of methionine and valine, but DLM consumption also led to lower lysine, tyrosine, glucose and acetate levels and greater citrate, lactate, formate, glycerol, *myo*-inositol and *N*-acetyl glycoprotein levels. The elevated level of plasma acetate may explain the higher level of milk fat and milk synthesis in the HMTBA-fed than in the CON- and DLM-fed sows. The decreased level of plasma lysine may explain the lower levels of milk protein and lysine in the DLM-fed than in the CON- and HMTBA-fed sows. These observations offered novel insights into the mechanisms of sulfur AA metabolism and milk synthesis regulated by methionine sources, which may have important nutritional implications for lactation animals and perhaps humans.

Effect of ageing and oxidative stress on the glutathione concentration in different kidney regions

Noor Riyadh Thiab, Graham L. Jones, and Nicola King

School of Science and Technology, University of New England, Armidale, Australia NSW 2351

Oxidative stress has been implicated in ageing and the pathogenesis of chronic kidney disease. Glutathione (GSH) is the major intracellular thiol in the kidney and an indicator of this organ's redox status. The aim of this study was to investigate how the GSH concentration is affected by age and oxidative stress in different kidney regions. Kidneys were dissected from Male Wistar rats of 5, 12, 36 and 60 weeks-old. Slices of superficial cortex, outer or inner medulla were incubated for 30 min ± 0.2 mM H_2O_2 prior to homogenisation and centrifugation. GSH concentrations were measured colormetrically using a kit.

Data are presented in nmol/mg protein, are means ± SE of n = 5 and statistical comparisons were carried out using ANOVA with an appropriate post-test. In all regions and all conditions the GSH concentration showed a similar pattern with 12 weeks-old > 36 weeks-old > 60 weeks-old and 5 weeks-old. The greatest concentration was measured in the 12 weeks-old with the superficial cortex (1509.16 ± 27.34) significantly greater than outer (851.67 ± 47.39, $p < 0.01$) and inner medulla (700.17 ± 20.57, $p < 0.01$). H_2O_2 exposure significantly reduced the GSH concentration in all ages and all regions except the inner medulla which had the lowest concentrations overall and where oxidative stress did not affect the 5 and 60 weeks-old. These results suggest that the antioxidant capacity in different kidney regions peaks at 12 weeks old and then reduces with increasing age.

Supplementation with L-glycyl-L-glutamine improves functional recovery following ischaemia reperfusion in the isolated rat heart

Amer Almashhadany, Shatha Ahmad, Pierre Moens, Graham L. Jones, and Nicola King

School of Science and Technology, University of New England, Armidale, Australia NSW 2351

The amino acids, glycine and glutamine have been implicated in myocardial protection against ischaemia reperfusion. This study investigated whether such protection could be enhanced by simultaneously delivering these amino acids as the dipeptide, L-glycyl-L-glutamine (gly-gln). Hearts from 36 week-old male Wistar rats were perfused in the Langendorff mode. For measurements of functional performance and reperfusion damage the perfusion protocol comprised 20 min' baseline perfusion, 40 min' global normothermic ischaemia and 40 min' reperfusion. In separate experiments small biopsy samples of the left ventricle were collected at the beginning and end of ischaemia for lactate measurements and from the right ventricle at the end of ischaemia for measurement of thiobarbituric acid reactive substances (TBARs). Where used, 2 mM gly-gln was added to the perfusate 10 min into baseline perfusion and was washed after 10 min' reperfusion. Data presented are means ± SE of n = 7 and were compared using student's unpaired T tests. The presence of 2 mM gly-gln significantly improved the recovery of left ventricular developed pressure from 18.49 ± 3.01 to 33.9 ± 4.85 % ($p < 0.02$) and lengthened the time to ischaemic contracture from 14.02 ± 1.4 to 23.63 ± 1.63 min ($p < 0.01$) compared to control. There was also a trend towards a reduced lactate and TBARs concentration in gly-gln exposed samples, although this did not quite reach significance. These results suggest that gly-gln shows good potential as a combatant against ischaemia reperfusion injury.

A new use of the biosensor DiS-C_3-(3) to measure dipeptide transport across the apical membrane of the renal proximal tubule

Othman A. Alghamdi, Pierre Moens, Graham L. Jones, and Nicola King

School of Science and Technology, University of New England, Armidale, Australia NSW 2351

PEPT1 and PEPT2 are H^+-dependent cotransporters found in the kidney. They are important for the reabsorption of small peptides and are also capable of transporting a broad range of peptidomimetic drugs. Due to the electrogenic nature of these carriers, transport of individual substrates should be accompanied by a positive change in the membrane potential difference (pd). The aim of this study was to investigate a novel use for the potential sensitive fluorescent dye 3,3′-dipropylthiacarbocyanine iodide [DiS-C_3-(3)] as a biosensor for dipeptide transport across the renal proximal tubule apical membrane. Brush border (BBMV) and outer medulla (OMMV) membrane vesicles were isolated from male Wistar rats using standard techniques. Fluorescence changes were measured when extracellular media at pH 6.6 containing 0–3 mM L-glycyl-L-glutamine (gly-gln) was added to a cuvette containing vesicles pre-equilibrated at pH 7.4 and 7.5 μM DiS-C_3-(3). Data presented are means ± SE of n = 6. A positive change in fluorescence occurred upon gly-gln addition reflecting the expected positive change in membrane pd. In addition a faster rate of fluorescence increase indicating a quicker rate of transport was measured when 0.1 mM gly-gln was added to BBMV ($4.5 \times 10^{-3} \pm 9 \times 10^{-5}$ arbitrary units per second) compared to OMMV ($3.6 \times 10^{-3} \pm 1.12 \times 10^{-4}$, $p < 0.001$, student unpaired T-test). These measurements were consistently repeatable indicating that DiS-C_3-(3) can be used as an innovative tool for measuring dipeptide transport in membrane vesicles.

Impacts of maternal protein nutrition on fetal programming in rats

Haijun Gao, Chandra Yallampalli

Department of Obstetrics & Gynecology, the University of Texas Medical Branch, Galveston TX 77555, USA

Maternal protein restriction (PR) induces hypertension and other cardiovascular diseases in adulthood offspring. However, the physiological mechanisms responsible for fetal programming on hypertension, particularly those residing in maternal aspects, remain unclear. Our studies attempted to explore the role of maternal RAS (renin-angiotensin system) in fetal programming on hypertension in response to gestational PR. Pregnant rats fed a low protein diet during gestation were used as a model in our studies. The major findings in our recent research are as follows. First, plasma levels of AngII (angiotensin II) were elevated by gestational PR during mid- and late pregnancy and the elevation in AngII levels was mainly attributed to the increased expression and activity of ACE (angiotensin I converting enzyme (1) in maternal lung, not those in kidney and plasma. Second, gestational PR induced an increase in *Agtr1*(AngII receptor, type 1) expression in uterine artery in late pregnancy, and correspondingly, vasoconstriction to AngII in uterine artery was exaggerated. Third, the expression of *Ace2* (angiotensin I converting enzyme (2), but not *Ace1* in rat placental labyrinth zone was reduced by gestational PR in late pregnancy; ACE protein was mainly present in syncytiotrophoblasts, whereas ACE2 protein was found predominantly in fetal mesenchymal tissue and fetal capillaries in rat placenta. Thus, gestational PR may impair the feto-placental blood flow by activating RAS and predispose hypertension in offspring.

Phencyclidine: it ain't excitin', but it sure is toxic

Kenneth M. Johnson

Department of Pharmacology and Toxicology, UTMB, Galveston, TX, USA

Acute administration of phencyclidine (PCP) causes effects similar to schizophrenia. Though PCP inhibits biogenic amine reuptake, its

primary mechanism of action is an open channel blockade of *N*-methyl-D-aspartate-operated glutamate receptors. In immature rats (~postnatal (PN) days 3–15), PCP administration results in neuronal death in layers II-IV of the retrosplenial and cingulate cortex, and more random death in the striatum, hippocampus, and ventral thalamus. In animal studies, we found that PCP administration on PN 7, 9, 11 results in a cumulative cortical apoptosis and behavioral similar to those found in schizophrenia. These include an exaggerated locomotor response to acute PCP challenge (PN28-35), in which pretreatment with either olanzapine (1 mg/kg) or risperidone (0.25 mg/kg) significantly blunted the sensitized response to PCP challenge. We also observed deficits in pre-pulse inhibition (PPI) of acoustic startle, social interaction (PN28, in males only), and novelty discrimination (PN56-58, both genders). Post-treatment with olanzapine (PN13-23) completely reversed the PPI deficit, suggesting schizophrenia-like characteristics of this model. We have now used PCP treated rat pups, as well as organotypic cortical slices cultures, to investigate the mechanisms leading to neuronal apoptosis, as well as mechanisms for neuroprotection. In cortical slices, we found that PCP blunted the PI-3K/ERK1/2/GSK-3β and MEK/ERK survival pathways, resulting in neuronal death. Interestingly, this effect was completely blunted by lithium, which in turn, as prevented by the trkB antagonist, K252a. We speculate that other mechanisms leading to increased BDNF may be therapeutic in schizophrenia.

Some aspects of L-cysteine transsulfuration in human brain

Wróbel Maria

Bronowicka-Adamska Patrycja, Czubak Jerzy, Poland

We characterized cysteine, cystine, cystathionine, reduced and oxidized glutathione together with γ-cystathionase and 3-mercaptopyruvate sulfurtransferase activities across multiple regions of the human brain (cortex, thalamus, hypothalamus, hippocampus, cerebellum and subcortical nuclei). Depending on the region, the values of cystathionine content expressed in nmol/mg protein were from 4 (cerebellum) up to above 55 (thalamus), cysteine from 0.6 (cerebellum) to 1.9 (thalamus) and reduced glutathione contents from 0.4 (cortex) to 1.5 (thalamus). The level of cystathionine reflects the activity of cystathionine β-lyase and γ-cystathionase. The human brain regions showed low γ-cystathionase and a negative correlation between the amount of cystathionine and the activity of γ-cystathionase was found. The activity of cystathionine β-synthase was examined in the thalamus homogenates, in the presence of homoserine and DL-propargylglycine (an inhibitor of γ-cystathionase). The difference in the cystathionine level between the homogenates with and without DL-propargylglycine was used to estimate the activity of this enzyme in the tissue homogenates. The determined value in the thalamus homogenate was 4.13 pmol mg^{-1} min^{-1}. Another pathway that can be involved in the generation of sulfane sulfur and/or hydrogen sulfide utilizes cysteine aminotransferase activity in combination with 3-mercaptopyruvate sulfurtransferase. The highest activity of 3-mercaptopyruvate sulfurtransferase was found in the hippocampus, hypothalamus and thalamus, and sulfane sulfur levels were correlated with the activity of this enzyme. The results confirm the crucial role of cystathionine β-synthase in hydrogen sulfide generation in the brain and suggest a supporting role of 3-mercaptopyruvate sulfurtransferase.

Imaging of collagen I fibers in primary breast cancer to predict metastasis

Kristine Glunde

Division of Cancer Imaging Research, JHU ICMIC, The Russell H. Morgan Department of Radiology and Radiological Science, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

Collagen I (Col1) fibers are the major structural extracellular matrix (ECM) component in breast tumors, and increased stromal Col1 has been shown to facilitate breast tumor formation, invasion, and metastasis. Cancer cell invasion starts at the tumor–stromal interface in primary breast tumors along radially aligned Col1 fibers, which can serve as a prognostic signature for survival of breast carcinoma patients. We are currently testing the relationship between lymph node metastasis and Col1 fiber texture and density in primary human breast cancers using second harmonic generation (SHG) microscopy, which detects an intrinsic signal generated by the noncentrosymmetric physical properties of Col1 fibers. Figure 1A demonstrates that the primary breast tumor of a representative lymph node negative (LN-) patient contains less dense, curly-shaped Col1 fibers in areas adjacent to cancer cells, whereas dense, straight-shaped Col1 fibers are detected in the primary tumor of a representative lymph node positive (LN+) patient in Fig. 1B. In animal models, hypoxic tumor regions contained fewer and structurally altered Col1 fibers than normoxic tumor regions, indicating that hypoxia can induce the degradation and restructuring of Col1 fibers, which is likely mediated by matrix metalloproteases 2 and 9 and lysyl oxidase. In summary, Col1 fiber restructuring in tumors may impact cancer cell dissemination as well as drug delivery into the tumor. Visualizing Col1 fibers in breast tumors may provide new biomarkers for cancer diagnosis, metastasis prediction, and prognosis of survival. Funded by NIH P50 CA103175.

Soy proteins with knockout of the α' subunit of β-conglycinin and the A1–5 subunits of glycinin remain hypolipidemic in rats

Chao Wu Xiao[1], Qixuan Chen[1,2], Carla Wood[1], Jiajie Liu[1], Christine Gagnon[2], Elroy R. Cober[2], Judith A. Frégeau-Reid[2], Stephen Gleddie[2]

[1]Nutrition Research Division, Food Directorate, Health Products and Food Branch, Health Canada, Banting Research Centre, Ottawa, Ontario, Canada K1A 0K9;
[2]Eastern Cereal and Oilseed Research Centre, Central Experimental Farm, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada K1A 0C6

This study examined the effects of soy isoflavones and different soy protein (SP) subunits on the prevention of non-alcoholic fatty liver disease, liver lipids, abdominal fat, blood lipids, thyroid hormones and enzymatic activities. Weanling Sprague–Dawley rats were fed diets containing either 20 % casein with or without supplemental isoflavones or alcohol-washed SP isolate (SPI) or SP concentrates (SPC) prepared from six different soy bean lines for 8 weeks. Feeding of SPC diets significantly lowered relative liver weights, blood total, free and

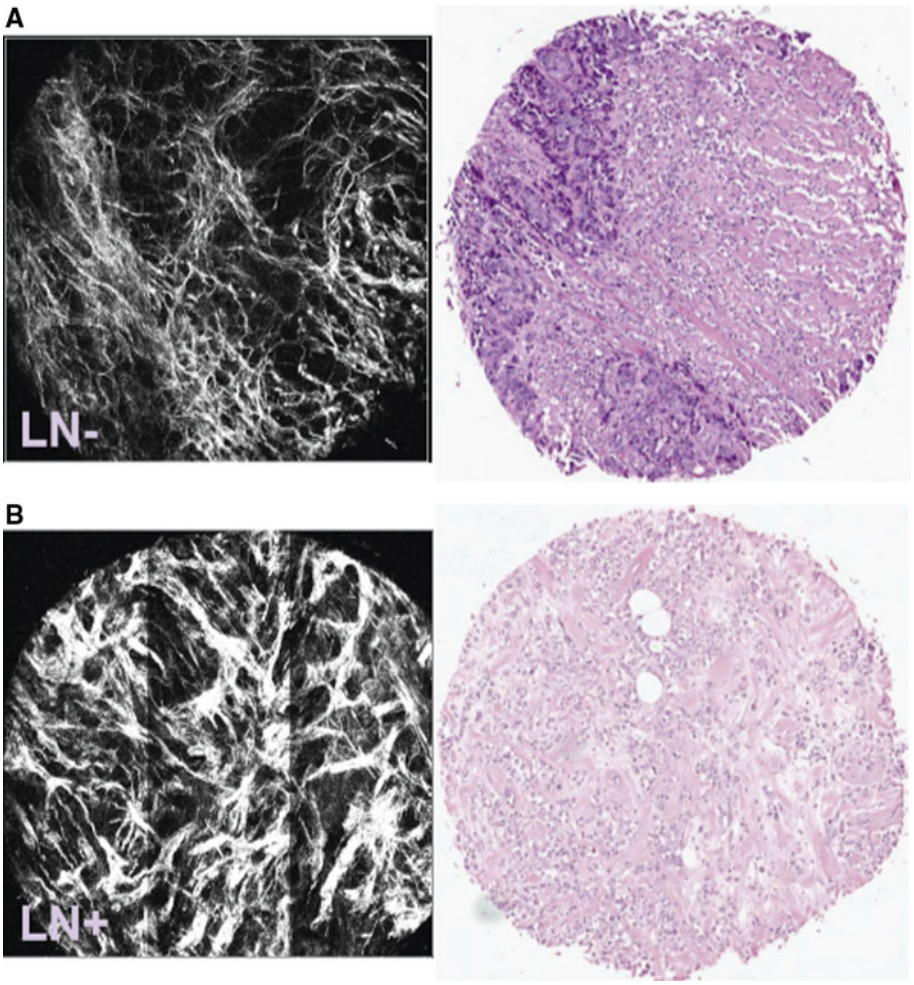


Figure 1: SHG (left) and corresponding hematoxylin and eosin (right) images from representative primary breast tumor biopsies of (**a**) a LN- patient and (**b**) a LN + patient. SHG images were obtained with a Zeiss 710 benchtop multiphoton microscope in tile imaging mode to cover the entire biopsy with a diameter of 1 mm

LDL cholesterol in both genders, and also reduced serum free fatty acid (FFA) and abdominal fat in females compared to the casein or casein + ISF diets. Moreover, dietary SP lowered hepatic total cholesterol, triglycerides and FFA, and prevented the accumulation of hepatic lipid droplet (HLD) compared to a casein diet in the males. Dietary SPC significantly elevated the plasma free thyroxine (T3) in both genders and total T3 in females compared to the casein diet. The SPC with depletion of α′ and A1–3 or α′ and A1–5 subunits increased total T3 in males and reduced plasma enzymatic activities of creatine kinase and lactate dehydrogenase compared to casein or casein + ISF diet. Overall, our results suggest that dietary soy proteins are responsible for the reduction in HLD formation, liver lipids, abdominal fat, liver weight and increased plasma total T3. However, neither the α′ subunit of β-conglycinin nor the A1–5 subunits of glycinin are essential for the hypolipidemic properties of soy proteins.

Analysis of free and peptide-bound amino acids using high-performance liquid chromatography

Sichao Jia[1], Sudath Dahanayaka[1], Zhenlong Wu[2], Zhaolai Dai[2], Ying Yang[2], Junjun Wang[2], and Guoyao Wu[1,2]

[1]Department of Animal Science, Texas A&M University, College Station, TX 77843, USA; and [2]State Key Laboratory of Animal Nutrition, China Agricultural University, Beijing, China 100193

Studies of the biochemistry, nutrition, pharmacology, and physiology of amino acids require reliable methods for their analysis. At present, the analytical methods that involve high-performance liquid chromatography (HPLC) include precolumn derivatization with 4-chloro-7-nitrobenzofurazan, 9-fluorenyl methylchloroformate, phenylisothiocyanate, naphthalene-2,3-dicarboxaldehyde, 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate, and *o*-phthaldialdehyde (OPA). In our laboratories, we have extensively used the OPA method to analyze free and peptide-bound amino acids in animal tissues and foods, as well as isolated cells and their culture medium. OPA reacts with primary amino acids (e.g., citrulline, arginine, and methylarginines) in the presence of 2-mercaptoethanol or 3-mercaptopropionic acid to form a highly fluorescent adduct. Proline does not react with OPA, and the reaction of cysteine or cystine with OPA is very limited. However, OPA readily reacts with: (1) 4-amino-1-butanol, which is produced from the oxidation of proline in the presence of chloramine-T and sodium borohydride at 60 °C; or (2) *S*-carboxymethyl-cysteine, which is formed from cysteine in the presence of iodoacetic acid. Fluorescence is monitored at excitation and emission wavelengths of 340 and 455 nm, respectively. Detection limits are 5 nM for amino acids. This method offers the following advantages: (1) simple procedures for the preparation of samples, reagents, and mobile phase solutions; (2) rapid formation of OPA derivatives and their efficient separation at room temperature; (3) high sensitivity of detection at pmol levels; (4) easy automation on the HPLC apparatus; (5) few interfering side reactions; (6) a stable chromatography baseline and accurate integration of peak areas; and (7) rapid regeneration of guard

and analytical columns. Thus, the OPA method provides a useful tool to determine amino acids in all biological samples.

S-Sulfocysteine and taurine: involvement in neuronal excitotoxicity and protection in molybdenum cofactor deficiency

Avadh Kumar, Guenter Schwarz, and Abdel Ali Belaidi

Institute of Biochemistry, Department of Chemistry and Center for Molecular Medicine, University of Cologne, 50674 Cologne, Germany

Molybdenum cofactor deficiency (MoCD) is a rare inherited metabolic disorder characterized by a massive and progressive neurological damage leading to death in early childhood. MoCD symptoms mainly rely on the loss of sulfite oxidase activity, which is crucial in detoxifying endogenously generated sulfite into sulfate. MoCD patients are diagnosed with microcephaly, profound mental retardation, spasticity and progressive neurodegeneration und usually die in the neonatal period of life. At biochemical level, sulfite, thiosulfate, *S*-sulfocysteine, and taurine are highly accumulated, while cystine and sulfate levels are below normal range. Until today, the molecular mechanism underlying the neuropathology and neurodegeneration in MoCD is poorly undertsood. Early studies suggested, in addition to the general cytotoxic function of sulfite, *S*-sulfocysteine as a neuroexcitotoxic metabolite that binds to NMDA receptors due to its structural similarity to glutamate. In this study, we first used cultured cortical neurons and investigated the toxicity of different MoCD-related metabolites within the pathway of cysteine catabolism. More specifically, we investigated the action of *S*-sulfocysteine on glutamate receptors using specific receptor blockers for NMDA and AMPA receptor subtypes. In addition, we evaluated the action of taurine in cultured neurons as many neuroprotective functions were attributed to this metabolite. Second, we used tungstate treatment of mice as an experimental tool to induce MoCD, which was demonstrated by a reduction in liver sulfite oxidase activity. Subsequently we monitored the change in excretion levels of MoCD biomarkers in urine of healthy and tungstate-treated mice aiming to identify the chronological development of the disease biomarkers. The contribution of these biomarkers in disease progression and diagnosis will be discussed.

Cysteine catabolism to sulfate in humans: enzymatic properties and regulation

Sita Arjune, Guenter Schwarz, and Abdel Ali Belaidi

Institute of Biochemistry, Department of Chemistry and Center for Molecular Medicine, University of Cologne, 50674 Cologne, Germany

Cysteine plays an essential role in protein structure and folding and participates in multiple metabolic reactions due to its reactive sulfhydryl group. Altered cysteine levels have been associated with various neurodegenerative and autoimmune disorders. Thus, a tight regulation of intracellular cysteine levels is crucial for maintaining a balance between cellular needs and toxicity. In mammals, the major route of cysteine catabolism involves its oxidation to cysteine sulfinic acid (CSA) catalyzed by cysteine dioxygenase (CDO). We characterized human CDO by steady-state kinetics and investigated the functional impact of its cross-linked cysteine-tyrosine cofactor on enzymatic activity and stability. For the latter, we investigated CDO ubiquitination using structure-guided mutagenesis aiming to identify ubiquitinated lysine residues. In the course of cysteine catabolism, CSA is deaminated by glutamate-oxaloactate-transaminase (GOT) yielding the putative compound β-sulfinylpyruvate, which was proposed to spontaneously decompose into pyruvate and sulfite. We investigated this reaction using the cytosolic (cGOT) and mitochondrial (mGOT) isoform. Steady-state kinetics revealed a higher catalytic activity for mGOT in comparison to cGOT, suggesting that sulfite is primarily formed in mitochondria. This finding matches to the mitochondrial localization of sulfite oxidase (SO), the terminal enzyme in this pathway. Furthermore, we developed a coupled enzyme assay utilizing human GOT and SO, which confirmed that sulfite is generated directly from CSA through the action of GOT and that ß-sulfinylpyruvate spontaneously and non-enzymatically decomposes into pyruvate and sulfite. Functional aspects regarding the cellular distribution of intermediates of cysteine catabolism will be discussed.

Methionine limitation—a threat to health? Using Atlantic salmon as the model

Marit Espe[1], Synne M. Andersen[1], Elisabeth Holen[1], Jens-Erik Zerrahn[2], Ivar Rønnestad[3], Eva Veiseth-Kent[4], and Anders Aksnes[5]

[1]National Institute of Nutrition and Seafood Research (NIFES), Po Box 2029, 5817 Bergen, Norway;
[2]Evonik Degussa Int. AG, 7100 Vejle, Denmark;
[3]Department of Biology, University of Bergen, Norway;
[4]Nofima, PO Box 210, 1431 Ås, Norway;
[5]Ewos Innovation AS, 4335 Dirdal, Norway

Soy proteins are widely used in fish feed due to its abundance and relatively low price. However, soy proteins contain low concentration of methionine that may affect health status in fish as methionine is converted to *S*-adenosylmethionine (SAM) the main methyl donor in endogenous synthesis of phospholipids. In addition SAM acts as the aminopropyl donor during synthesis of polyamines after being decarboxylated. To assess whether methionine limitation affects phospholipids synthesis or polyamine turnover juvenile Atlantic salmon was fed soy protein diets with deficient or adequate levels of methionine for 8 weeks. Free methionine in muscle and plasma was lower in fish fed the un-supplemented diet while liver methionine was unaffected by treatment. SAM increased in liver of fish fed the methionine deficient diet, but liver cystathionine was lower indicative of a reduced transsulfuration. Liver putrescine increased and spermine decreased in fish fed the methionine deficient diet, while both muscle spermidine and spermine increased in fish fed deficient methionine diet. Expression of either SAM-decarboxylase or ornithine decarboxylase were affected by methionine limitation. Concomitantly protein accretion and growth was lower in fish fed the methionine limiting diet while plasma and muscle free lysine were higher. Liver phospholipids, free fatty acids and cholesterol were lower in fish fed the methionine deficient diet, while plasma phospholipids was higher. As the low methionine diet led to reduced liver phospholipids and protein accretion it may affect the health of the fish in the long run. This was supported by an increased liver TNFα expression.

Expression of aquaporins in the porcine uterine endometrium

Cui Zhu[1,2], Fuller W. Bazer[1], Gregory A. Johnson[3], Robert C. Burghardt[3], Houssein Assaad[1,4], Zongyong Jiang[2], and Guoyao Wu[1]

[1]Department of Animal Science, Texas A & M University, College Station, TX, USA 77843;

[2]Key Laboratory of Animal Nutrition and Feed (South China) of Ministry of Agriculture, Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Guangzhou 510642, China;
[3]Department of Veterinary Integrative Bioscience, Texas A&M University, College Station, TX, USA 77843; and
[4]Department of Statistics, Texas A&M University, College Station, TX, USA 77843

Aquaporin (AQP) water channels are a family of small integral plasma membrane proteins that primarily transport water across the plasma membrane. Some of them can also transport glycerol, urea and other solutes. Thirteen isoforms of AQPs (AQP 0-12) have been identified in mammals and classified into three subgroups: (1) classical aquaporins (AQP 0, 1, 2, 4, 5, 6 and 8); (2) aquaglyceroporins (AQP 3, 7, 9 and 10); and 3) superaquaporins (AQP 11 and 12). This study determined expression of mRNAs for the AQPs in the porcine uterine endometrium during gestation. Endometrial samples were collected from pregnant gilts at Days 9, 10, 12, 13, 14, 15, 20, 25, 30, 35, 40, 60 and 85 of gestation (n = 3/Day). AQPs 1, 3, 4, 5, 6, 7, 8, 9 and 11 mRNAs were detected in the uterine endometrium. Results of real-time PCR indicated that the abundance of AQPs 1, 4, 5, 6, 7 and 9 mRNAs was higher on Day 60 of gestation than for other days of gestation ($P < 0.05$). The mRNA levels for AQP 8 on Days 30, 40 and 85 of gestation were higher than for other days of gestation ($P < 0.05$). Expression of AQPs 3 and 11 mRNAs decreased with advancing stage of gestation ($P < 0.05$). Collectively, our results indicate that AQPs are differentially expressed in the porcine uterine endometrium during gestation. These changes in expression of the AQPs likely play a role in regulating water homeostasis and fluid balance in the conceptus during both the peri-implantation period and during later stages of fetal-placental development when dynamic changes in volumes of fluid in the allantoic and amniotic sacs occur, particularly between Days 20 and 20 of gestation and between Days 45 and 60 of gestation.

Can we fine-tune the efficiency of essential amino acids in the fight against muscle loss during aging and disease?

E. Lichar Dillon

Department of Internal Medicine, University of Texas Medical Branch, Galveston, TX, USA

Essential amino acids (EAAs) serve as potent signaling molecules in the regulation of skeletal muscle protein synthesis in healthy and diseased humans, functioning beyond the mere requirement for maintaining protein abundances. Skeletal muscle wasting conditions, ranging from gradual losses during sarcopenia of aging to the rapid losses seen in cancer cachexia, generally involve decreased sensitivity of the protein synthetic pathways to the presence of EAAs. This anabolic resistance to amino acids leads to an elevation in the acute requirement for EAA concentrations necessary to elicit the maximum anabolic responses that match those observed in healthy younger adult muscle. While studies have shown that the skeletal muscle of older adults and those with diseases such as cancer are anabolically responsive to protein and amino acids from meals, prolonged periods of systemic or local muscle inflammation and stress may greatly diminish the anabolic potential of nutritional interventions. Concomitant exercise regimens or hormonal replacement therapies are therefore frequently warranted to provide added or synergistic benefits to nutritional strategies. It is likely that multiple approaches (i.e. increase insulin sensitivity, enhance muscle perfusion, replace suppressed endogenous sex hormones, etc.) should be considered to fine tune the anabolic efficiency of essential amino acids toward improving chronic maintenance of muscle mass in aging and disease.

Amino acids and autophagy: integrative proteomics and functional analysis in the identification of regulatory proteins in amino acids-induced survival in cancer cells

Chien-An A. Hu[1], and Songqin Pan[2]

[1]Department of Biochemistry and Molecular Biology, University of New Mexico School of Medicine, Albuquerque, New Mexico 87131, USA, and Proteomics Center for Plant Cell Biology (CEPCEB);
[2]Department of Botany and Plant Sciences, University of California, Riverside, CA 92521, USA

In the past decade, our multi-disciplinary team has employed proteomics and functional strategies to identify and characterize regulatory proteins in oxidative stress-induced apoptosis and autophagy in cancer cells. Specifically, we have created various gene-inducible cells in p53-null background that were cell death-prone when overexpressed p53, PUMA, apolipoprotein L6 (ApoL6) or ApoL1. We have observed that proteins, such as stathmin (OP18) and ApoL6, are sensitive to the level of ROS and are involved in apoptosis, but not in autophagy-associated cell death. Reducing intracellular free radicals restored cellular homoeostasis and survival in cells overexpressing pro-apoptotic genes. Interestingly, we recently showed that some of the amino acids elicited inhibitory effects on PUMA- and ApoL6-induced apoptosis in p53-null colorectal cancer cells, which may explain why these amino acids in combination with chemotherapeutic drugs might fail treating cancer patients. In this conference, we will display and discuss some of our recent unpublished results to address issues in cancer translational research.

Tetrahydrobiopterin, Nitric Oxide, and Diabetes

Cynthia J. Meininger[1], and Guoyao Wu[1,2]

[1]Department of Medical Physiology, Texas A&M Health Science Center, Temple, TX, USA; and
[2]Department of Animal Science, Texas A&M University, College Station, TX, USA

A primary cause of morbidity and mortality in people with diabetes is cardiovascular disease. Vascular complications of diabetes are known to result from endothelial cell dysfunction. Not all of the cellular and biochemical mechanisms responsible for the vascular complications of diabetes have been elucidated but many studies suggest that nitric oxide (NO) bioavailability is diminished in diabetes and impaired NO synthesis can disturb vascular responses. Hyperglycemia-induced oxidative stress reduces the endogenous antioxidant pool in endothelial cells and is associated with decreased NO bioavailability, therefore resulting in endothelial dysfunction. We have shown that the inability of endothelial cells to synthesize NO in diabetes can be due to a deficiency in tetrahydrobiopterin (BH4), a critical cofactor required for the proper activity of the endothelial enzyme NO synthase (eNOS). Tetrahydrobiopterin is absolutely essential for the production of NO. Without BH4, NO cannot be synthesized. Worse yet, in the absence of BH4, eNOS transfers electrons to molecular oxygen forming superoxide radicals that can go on to form other damaging reactive oxygen species and deplete the cell of NO. The eNOS enzyme is constitutively expressed but dynamically regulated by a number of factors. Building our knowledge of this regulation is necessary to understand and modulate the bioavailability of NO, central to proper vascular

function. Using this knowledge, our research group is pursuing various strategies to increase NO bioavailability and maintain BH4 levels in order to reduce or prevent the vascular complications associated with diabetes and other diseases.

(Supported by AHA and NIH).

Effects of co-regulation of proline oxidase and succinate dehydrogenase on mitochondrial respiration

Chad N. Hancock, James M. Phang

Basic Research Laboratory, Metabolism and Cancer Susceptibility Section, Frederick National Lab for Cancer Research, Frederick, MD, USA

Proline oxidase (POX) is an inner mitochondrial membrane protein that oxidizes proline to generate superoxide as well as ATP. Work in our laboratory has documented that POX activity is highly regulated during genotoxic and metabolic stress. Inducers of POX include p53, and proline-dependent ROS play a role in p53- and mitochondrial-mediated apoptosis. Succinate dehydrogenase, also known as Complex II of the electron transport chain (ETC), oxidizes the TCA cycle metabolite succinate to fumarate as it reduces ubiquinone to ubiquinol, generating ATP through oxidative respiration. Complex II and the p53-DNA damage repair pathway are intimately linked. Mutations in Complex II have been shown to increase risk of breast and thyroid cancer through destabilization of p53, and tocopherol-succinate has been demonstrated to reduce radiation-induced apoptosis. Thus, the ETC and activities of p53 appear to be linked through Complex II. Because of the ability of POX to generate ATP from proline, we explored the relationship between POX and the ETC. We found that POX binds directly to ubiquinone, and that ubiquinone is an effective acceptor of proline-derived electrons. Increased POX catalytic activity due to ubiquinone required Complex III and Complex IV of the ETC, indicating that ATP generated by POX is through oxidative respiration by the ETC. In addition, experiments using the Seahorse XF24 analyzer confirmed that while POX can utilize proline as a short term source of electrons during nutrient stress conditions, long term expression of POX results in reactive oxygen species (ROS)-dependent suppression of respiration, and down-regulation of subunits of Complexes I-IV of the ETC, consistent with its role in mitochondrial-mediated apoptosis. We found that the POX and Complex II were linked functionally, and that the presence of succinate dramatically affects POX activity. Lineweaver–Burk analysis showed that succinate inhibits POX catalytic activity using anti-competitive inhibition, a rare mechanism of enzymatic inhibition. Two highly selective inhibitors of the Complex II holoenzyme, TTFA and carboxin, inhibit the ubiquinone-mediated activities of POX; in addition, POX co-immunoprecipitates with Complex II, indicating a physical interaction between these two enzymes. Interestingly, the addition of proline appears to stimulate oxidation of succinate by Complex II in vitro, and POX expression in the presence of succinate dramatically increases methylation of histone H3. Finally, addition of succinate to cell culture inhibits ROS generation by POX and inhibits the long-term effects of POX on respiration. Together, these data support a model in which POX is an integral contributor in oxidative respiration during nutrient stress, and in which POX influence on the ETC is regulated by level and duration of POX expression, as well as levels of proline relative to the TCA cycle metabolite, and Complex II substrate, succinate.

L-Arginine stimulates DNA synthesis and reduces LPS-induced DNA damage in porcine intestine epithelial cells

Bie Tan[1], Xia Xiong[1], Hao Xiao[1,2], Jing Wang[1,2], Guangran Li[1,2], Yulong Yin[1*], Zhenlong Wu,[3] and Guoyao Wu[1,3,4]

[1]Observation and Experiment Station of Animal Nutrition and Feed Science in South-Central China, Ministry of Agriculture; Hunan Provincial Engineering Research Center for Healthy Livestock and Poultry Production; Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences; Changsha, Hunan 410125, China;
[2]University of the Chinese Academy of Sciences; Beijing 10008, China;
[3]State Key Laboratory of Animal Nutrition, China Agricultural University, Beijing, China 100193;
[4]Department of Animal Science, Texas A&M University, College Station, TX 77843, USA

A protective effect of arginine against lipopolysaccharide (LPS)-induced enterocyte damage has been demonstrated in our previous work. This study was designed to investigate LPS-induced DNA damage and impairment of the cell cycle in intestinal cells. Intestinal porcine epithelial cells (IPEC-1) were cultured for 4 days in arginine-free Dulbecco's modified Eagle's-F12 Ham medium containing 10, 100 or 350 μM arginine and 0 or 20 ng/ml LPS. DNA synthesis and damage, cell cycle, as well as expression of cell-survival regulatory proteins were determined. Results from the comet assay and 5-ethynyl-2′-deoxyuridine (EdU) incorporation indicated that DNA damage was higher ($P < 0.05$) in the LPS-treated IPEC-1 compared with control cells. An increase in EdU incorporation was detected with increasing arginine concentrations in normal and LPS-treated cells ($P < 0.05$). Flow cytometry analysis showed that the percentage of cells in the Gl phase and apoptotic cells increased ($P < 0.05$) while the percentage of cells in the S phase decreased ($P < 0.05$) in LPS-treated cells. Addition of arginine increased ($P < 0.05$) the numbers of cells in the S phase. In support of the data on cell growth and apoptosis, treatment with LPS enhanced ($P < 0.05$) expression of Gadd45a but reduced ($P < 0.05$) protein levels for phosphorylated Akt and Bcl2 in IPEC-1. Addition of 100 or 350 μM arginine to culture media increased ($P < 0.05$) protein levels for phosphorylated PI3K and phosphorylated Akt and reduced ($P < 0.05$) Bcl2 protein levels in normal and LPS-challenged cells. These results demonstrate that: (1) the cytotoxic effect of LPS is attributable to DNA damage and apoptosis; and (2) these adverse effects of LPS can be ameliorated by elevating extracellular concentrations of arginine via stimulation of DNA synthesis and the Akt-Bcl2 signaling pathway.

Deprivation of L-glutamine induces autophagy in porcine intestinal epithelial cells

Yuhua Zhu, Tianjiao Zhou, Zhaolai Dai, Tiantian Li, Zhenlong Wu, Guoyao Wu, Junjun Wang

State Key Laboratory of Animal Nutrition, China Agricultural University, Beijing 100193, China

L-Glutamine (Gln) has been reported to be essential for intestinal growth and integrity. However, it is unknown whether its deprivation

can induce autophagy in intestinal epithelial cells. To address this issue, intestinal porcine epithelial cells (IPEC-1) were used. After a 2-day period in DMEM-F12, cells were transferred to a Gln-free basal medium that was supplemented with 5 % fetal bovine serum and with 0 or 0.5 mM Gln. Concentrations of amino acids (AA) in the basal culture medium were similar to those found in the plasma of young pigs. Cell numbers, abundance of autophagy-related protein LC3B, as well as AA composition in medium were determined during an 8-h period of culture at 37 °C and 95 % O_2/5 % CO_2. Data were analyzed by one-way analysis of variance. Results indicated that the cell numbers at 1, 2, 4 and 8 h after Gln deprivation were reduced by 10, 18, 30 and 35 %, respectively ($P < 0.05$). Expression of LC3B at the protein level after Gln deprivation was increased ($P < 0.05$) in a time-dependent manner, indicating that a short-term deficiency of Gln could readily induce autophagy in intestinal cells. Further, the AA profile analysis revealed alterations ($P < 0.05$) in concentrations of Asp, Glu, Arg, Leu, Asn, Thr, and Orn in the culture medium for the Gln-deprived group. Collectively, our findings support the notion that Gln deficiency induces autophagy and disturbed AA metabolism in intestinal epithelial cells during an 8-h period.

Metabolomic analysis reveals altered amino acid metabolism in weanling rats fed a lactosucrose-supplemented diet

Yinfei Lv[1], Zheng Ruan, Yuhui Yang[1], Yan Zhou[1], Min Jiang[1], Zeyuan Deng[1], Kang Yao[2,3], Yulong Yin[1, 2], and Guoyao Wu[3]

[1]State Key Laboratory of Food Science and Technology, College of Life Science and Food Engineering, Nanchang University, Nanchang 330047 China;
[2]Hunan Engineering and Research Center of Animal and Poultry Science, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China;
[3]Department of Animal Science, Faculty of Nutrition, Texas A&M University, College Station, TX 77843-2471, USA

Weaning stress is an important factor for the development of intestinal disorders in animal life, which can be affected by nutrients or metabolites. However, the pathophysiological mechanisms that link weaning stress and metabolites/metabolism are unknown. The purpose of this study was to investigate the effect of lactosucrose (LS) supplementation on the metabolites and metabolism of weanling rats. Rats were weaned at 21 days of age to a standard rodent diet and then received daily oral administration of either LS (5 mg/ml) 1 ml or saline (control) for 7 days. Short-chain fatty acids in feces and the histological morphology in jejunum and colon were respectively measured by gas chromatography and hematoxylin and eosin staining. Metabolites in blood and urine were analyzed nuclear magnetic resonance (NMR)-based metabolomics combined with multivariate statistics. Dietary lactosucrose supplementation significantly improved growth performance, villus height in jejunum and colon, and small intestinal index compared to control rats, and increased the concentration of acetate and propionate in the feces of rats. Metabolic effects of LS included significant increases in urinary excretion of β-hydroxybutyrate, alanine, acetate, α-ketoglutarate, fumaric acid, succinate, isoleucine, taurine, creatinine, hippurate, allantoin, and TMAO, with a concomitant decrease in urinary excretion of lactate. An increase in serum concentrations of β-hydroxybutyrate, choline glycerophosphate, 1-methylhistidine, hippurate, and formate were also observed. The results indicate that LS supplementation to weaned rats promotes the production of short chain fatty acids in intestinal lumen that are associated with energy generation, and also alters the catabolism of amino acids and the activity of the tricarboxylic acid cycle in the whole body.

Biochemical properties and molecular mechanisms of antibacterial actions of carboxy-terminal RWL-tagged antimicrobial peptides

N. Dong, X. Zhu, Y. F. Lv, Q. Q. Ma, A. S. Shan

Institute of Animal Nutrition, Northeast Agricultural University, Harbin, China

Antimicrobial peptides (AMPs) constitute a diverse class of naturally occurring or synthetic antimicrobial molecules that have potential for use in the treatment of drug-resistant infections. Several undesirable properties of AMPs, however, may ultimately hinder their development as antimicrobial agents. Thus, new synthetic strategies, including primarily the de novo design of AMPs, urgently need to be developed. In this study, we designed a series of peptides H-(RWL)n (H represents GLRPKYS; n = 1, 2, 3, 4, 5), and the sequence H-RWL was truncated from the C-terminal of AVBD-4. Results displayed that the antimicrobial activity of RWL-tagged peptides showed a quadratic function with their chain lengths. Hemolysis activity and cytotoxicity were increased as side chain extension, and GW13, GLRPKYS-$(RWL)_2$, displayed the highest therapeutic index. Synthetic lipid vesicles analysis showed the blue shifts of the peptides in two lipid systems correlated positively with their biological activities. The mechanism of action against bacteria was elucidated through combined studies of electron microscopy (SEM and TEM) and fluorescence assays, showing that the peptide possessed membrane-lytic activities against microbial cells. Collectively, Our results indicate that the tandem of characteristic polyamino acid RWL is a promising strategy for generating AMPs with great therapeutic value, and GW13 has considerable potential for future development as an antimicrobial agent.

Dietary supplementation with glycine decreases glutamine concentrations in the plasma of milk protein-fed piglets

Weiwei Wang, Bin Wang, Zhenlong Wu, Zhaolai Dai, Junjun Wang, and Guoyao Wu

State Key Laboratory of Animal Nutrition, China Agricultural University, Beijing, China 100193

Glycine has recently been classified as a conditionally essential and also a functional amino acid for young pigs. Because sow's milk contains a relatively low content of glycine, this study was conducted to determine whether dietary supplementation to piglets might increase concentrations of glycine in plasma. Young pigs at 11 days of age were removed from sows to a nursery facility and then allotted randomly to be fed a milk replacer diet supplemented with 0, 0.5, 1.0 or 2.0 % of glycine (on the basis of milk replacer powder) after a 3-day period of adaptation. There were 4 pigs per treatment group. Fresh liquid milk replacer (200 ml/kg body weight, 18.6 % dry matter) was provided orally to piglets six times daily (0800, 1200, 1600, 2000, 2400 and 0400 h). Jugular venous blood samples were collected for metabolite analysis at days 14, 21 and 28 of age. Data were analyzed by one-way analysis of variance and the Student–Newman–Keuls multiple comparison. Average daily food intake did not differ ($P > 0.05$) among the four groups of piglets. Between 14 and 28 days of age, dietary supplementation with 0.5, 1.0 and 2.0 % glycine dose-dependently increased ($P < 0.01$) plasma concentrations of glycine by 49, 67 and 126 %, respectively, and decreased ($P < 0.01$) plasma concentrations of glutamine by 8, 29 and 32 %, respectively, compared with control piglets. Glycine supplementation only moderately increased ($P < 0.05$) concentrations of serine in piglet plasma and had no effects on concentrations of other amino acids. Expression of the gene for a glutamine transporter (SLC7A9) or the rates of glutamine transport

(measured using Using Chambers) in the small intestine did not differ ($P > 0.05$) between control and glycine-supplemented pigs. Collectively, dietary supplementation with glycine decreases plasma glutamine concentration in milk protein-fed piglets likely through reducing the availability of ammonia as a result of enhanced protein synthesis and reduced oxidation of amino acids in the whole body. These findings have important implications for designing a new means to improve growth of young mammals (including piglets).

Concentrations of free and peptide-bound hydroxyproline in the sow's milk and piglet plasma

Weiwei Wang[1,2], Reza Rezaei[1], Zhenlong Wu[1], Zhaolai Dai[1], Junjun Wang[1], and Guoyao Wu[1,2]

[1]Department of Animal Science, Texas A&M University, College Station, TX, USA, 77843;
[2]State Key Laboratory of Animal Nutrition, China Agricultural University, Beijing, China 100193

We previously reported relatively high concentrations of small peptides containing proline and glycine in sow's milk. The present study was conducted to identify such small peptides in both sow's milk and piglet plasma. On days 7 and 21 of lactation, milk samples were obtained from six sows fed a corn- and soybean meal-based diet (containing 18 % crude protein) at 2 h after feeding, and blood samples were withdrawn from the jugular vein of piglets (1 piglet/sow) into heparinized tubes at 1 h after suckling. The piglets had a normal birth weight ranging from 1.35 to 1.45 kg. Small peptides in deproteinized milk and plasma were analyzed using high performance liquid chromatography. Data were statistically analyzed by the paired t-test. Results indicated that concentrations of glycine-proline-hydroxyproline were exceedingly high in both sow's milk and the piglet plasma, and values were greater at day 7 than at day 21 of lactation (Table 1). Based on its concentrations in sow's milk, milk intake by piglets (measured using the weigh-suckle-weigh technique), and their body weights, we estimated that the consumption of free and peptide-bound hydroxyproline (Hyp) by the neonates was much lower ($P < 0.05$) in 21-day-old than in 7-day-old pigs, leading to a lower concentration of Hyp in the plasma of the older piglets. Nutritional and physiological significance of glycine-proline-hydroxyproline in sow's milk and young pigs remains to be determined.

Use of interferon tau to reduce obesity in Zucker diabetic fatty rats

Carmen D. Tekwe[1], Fuller W. Bazer[2], Cynthia J. Meininger[1], and Guoyao Wu[1,2]

[1]School of Public Health and [2]College of Medicine, Texas A&M Health Science Center, College Station, TX, USA 77843;
[2]Department of Animal Science, Texas A&M University, College Station, TX 77843, USA

Several factors are contributing to the rise in obesity worldwide, including the rate of energy intake being far greater than energy expenditure, living a sedentary lifestyle, genetic predisposition, consumption of high fat diets, and inflammation. As a chronic syndrome, obesity is a risk factor for both insulin resistance and type II diabetes mellitus, with obesity-induced inflammation playing a major role in insulin resistance and the development of diabetes. Obesity is often accompanied by inflammation in white adipose tissue (WAT) and liver due to increases in inflammatory cells. Inflammation has also been linked to oxidative stress leading to obesity and insulin resistance. Ovine interferon tau (IFNT) was discovered for its regulatory role in the reproductive cycle of sheep. IFNT possesses stable acid properties as well as antiviral, antiproliferative and immunomodulatory activities. IFNT is a member of the type-I interferon family but, unlike interferons alpha and beta, is considered non-toxic even at high concentrations. Therefore, IFNT can be administered intravenously to animals. Using the male Zucker diabetic fatty (ZDF) rats (an animal model of type-II diabetes mellitus), we have reported that rates of glucose and oleate oxidation in liver, brown adipose tissue, and abdominal adipose tissue, leucine catabolism in skeletal muscle, and lipolysis in white and brown adipose tissues are greater for rats treated with 8 μg IFNT/kg BW/day (provided via drinking water) in comparison with control rats. Treatment with 8 μg IFNT/kg BW/day increases heat production, reduces BW gain and adiposity, ameliorates fatty liver syndrome, delays the onset of diabetes, and decreases concentrations of glucose, free fatty acids, triacylglycerol, cholesterol, and branched-chain amino acids in plasma, compared to control rats. Oral administration of 8 μg IFNT/kg BW/day ameliorates oxidative stress in skeletal muscle, liver and adipose tissue, as indicated by decreased ratios of oxidized glutathione to reduced glutathione and increased concentrations of the antioxidant tetrahydrobiopterin. These results indicate that IFNT stimulates oxidation of energy substrates and reduces obesity in ZDF rats and may have broad important implications for preventing and treating obesity-related diseases in mammals.

Effects of antimicrobial peptide on growth performance and survival of weaned pigs

H. S. Yang[1], Z. K. Liu[2], Li Li[3], X. Xiong[1], G. Y. Wu[4], and Y. L. Yin[1]

[1]Observation and Experiment Station of Animal Nutrition and Feed Science in South-Central China, Ministry of Agriculture; Hunan Provincial Engineering Research Center for Healthy Livestock and Poultry Production; Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences; Changsha, Hunan 410125, China;
[2]Veterinary Faculty, Hunan Agricultural University, Changsha, Hunan, 410128, China;
[3]Guangzhou Glam Biotechnology Co., Ltd. Guangzhou Science City Kexue Road 99, 510530, Guangzhou, China; and
[4]Department of Animal Science, Texas A&M University, College Station, TX 77843, USA

Table 1 Concentrations of Hyp in sow's milk and in piglet plasma, and Hyp intake by piglets

Age of pigs (mean weight)	Milk intake (ml/kg BW/d)	Hyp in Sow's milk		Total Hyp intake from milk by piglets (mmol/kg BW/d)	Hyp in piglet plasma	
		Free Hyp (μmol/l)	Peptide-bound Hyp∓ (μmol/l)		Free Hyp (μmol/l)	Peptide-bound Hyp∓ (μmol/l)
7 days (2.5 kg)	312 ± 20	80 ± 5	8,995 ± 428	2.86 ± 0.15	109 ± 8	2,764 ± 121
21 days (5.7 kg)	184 ± 13*	54 ± 3*	6,301 ± 344*	1.14 ± 0.09*	77 ± 5*	2,108 ± 103*

Values are mean ± SEM, n = 6. Milk and plasma samples were analyzed for free and peptide-bound hydroxyproline (Hyp). ∓ Peptide-bound Hyp is in the form of glycine-proline-hydroxyproline. * $P < 0.05$ vs. 7-day-old pigs, as analyzed by the paired t-test

Weaned pigs often face postweaning challenges, including diarrhea, impaired growth, and low feed intake. Antimicrobial peptides (AMPs) are small gene-encoded peptides that show a broad range of activity against bacteria, fungi, and mycobacteria. Thus, AMPs have potential values in improving growth performance and health of weaned pigs. A total of 2,250 pigs from 5 swine farms (450 pigs each) were weaned at 28 days of age (7.98 ± 0.23 kg average BW) and randomly assigned to one of the three treatments during a 32-d feeding experiment: (1) a basal diet (control); (2) 2 g/kg of AMPs (AMP-2); and (3) 3 g/kg of AMPs (AMP-3). Each treatment had 15 replicated pens with 10 pigs per pen on each farm. The AMPs used in the present study were a mixture of lactoferrin, cecropin, defensin, and plectasin. Pigs had free access to feed and drinking water at all times throughout the experimental period. Pigs in AMP-2 and AMP-3 groups had greater average daily gain (ADG) than pigs in the control group. ADFI tended to be greater in pigs fed 2 g/kg of AMPs than pigs fed the control diet. Pigs fed 2 or 3 g/kg AMPs also tended to have increased G:F compared with pigs fed the control diet. The survival rates were increased in AMP-2 and AMP-3 groups compared with the control group. These results indicate that dietary supplementation with AMPs improves growth performance and survival of weaned piglets.

Arginine and secreted phosphoprotein 1 act synergistically to stimulate MTORC1/MTORC2 cell signaling and cytoskeletal organization for proliferation, migration and adhesion of ovine trophectoderm cells

Xiaoqiu Wang[1], Kathrin Dunlap[1], M. Carey Satterfield[1], Robert C. Burghardt[2], Greg A. Johnson[2], Guoyao Wu[1], and Fuller W. Bazer[1]

Departments of Animal Science[1] and Veterinary Integrative Biosciences[2], Texas A&M University, College Station TX, 77843, USA

Histotroph is required for survival and development of conceptuses (embryo and extra-embryonic membranes) throughout gestation, but particularly during the peri-implantation period of pregnancy in mammals. L-Arginine (Arg), a nutritionally essential amino acid for the fetus, is required for elongation of the conceptus, whereas secreted phosphoprotein 1 (SPP1), the most abundant extracellular matrix (ECM) protein in the uterine lumen, induces cell–cell and cell–ECM interactions, and mediates attachment and migration of ovine trophectoderm (oTr) cells. However, it remains to be elucidated how Arg and/or SPP1 activate signal transduction pathways to reorganize the cytoskeleton of trophectoderm cells undergoing elongation and implantation. This study tested the hypothesis that Arg and SPP1 act synergistically to activate cytoskeletal reorganization for proliferation, adhesion and migration of oTr cells via the MTORC1/MTORC2 cell signaling pathways. Our established oTr cell line was subjected to serum and insulin starvation for 24 h followed by arginine starvation for an additional 6 h, and then treatment with either: (1) basal medium (BM, Arg-free plus 5 % FBS); (2) Arg (0.2 mM Arg in BM); (3) recombinant SPP1 (10 μg/ml rSPP1 in BM); or (4) Arg + rSPP1 (0.2 mM Arg plus 10 μg/ml rSPP1 in BM), for either 12, 24 or 48 h. Cell proliferation, cell migration and adhesion assays, as well as immunofluorescence analyses of key cellular proteins were performed. Results of this study demonstrated that Arg and rSPP1 act synergistically on oTr cells to: (1) stimulate ($P < 0.05$) proliferation, migration and adhesion of oTr cells; (2) activate PDK1/Akt/MTORC1 (MTOR/Raptor) and MTORC2 (MTOR/Rictor) signaling pathways; (3) release inhibition of MTOR by phosphorylating TSC2; and (4) induce cytoskeletal reorganization (i.e., cytokeratin, α-tubulin, F-actin, integrin β3 and talin) to effect changes in morphology. Collectively, these results indicate that, Arg and SPP1 act synergistically to stimulate oTr cell proliferation primarily through the MTORC1 signaling pathway, whereas Arg and SPP1 likely induce migration and adhesion via the MTORC2 signaling pathway. Further, our results strongly suggest that Arg acts primarily to drive signal transduction, whereas SPP1 mediates cell–cell and cell–ECM interactions to regulate changes in cytoskeletal organization. We conclude that the cell signaling cascades and cytoskeletal reorganization required for elongation, survival and development of ovine conceptuses during the peri-implantation period of pregnancy are optimized through the complementary effects of a functional amino acid (Arg) and an ECM protein (SPP1).

(This research was supported by Agriculture and Food Research Initiative Competitive Grant no. 2011-67015-20028 from the USDA National Institute of Food and Agriculture.)

Intestinal nitrogen metabolism of non-essential amino acids in rats

Hidehiro Nakamura

Institute for Innovation, Ajinomoto. Co., Inc., 1-1 Suzuki-cho, Kawasaki, Japan 210-8681

Although requirements for non-essential amino acids (NEAAs) have not been determined in either animals or humans, they are required for growth of young animals presumably as nitrogen (N) sources; feeding of NEAA-free diet in rats has been reported to suppress the growth markedly. Further, available evidence shows that the growth-promoting effects of NEAAs differ from each other, suggesting differences in their N-efficacies. However, there seem few studies comparing their N-metabolisms in the body. The small intestines are not only the route of dietary nutrients but also play pivotal roles in metabolizing dietary amino acids (AAs). Previous studies have shown that significant amount of carbon (C) skeletons of dietary AAs were utilized for intestinal energy production and for the synthesis of other AAs such as alanine and citrulline. Despite of the quantitative impact of the small intestines in the C-metabolisms of dietary AAs, there is only limited information for their N-metabolisms in the gut. Therefore, we investigated N-metabolisms of dietary NEAAs in the guts using their ^{15}N-labels. Rats were given hourly meals containing each [^{15}N]NEAA (glutamate, glutamine, proline, alanine, aspartate, serine or glycine) to investigate their intestinal N-fates quantitatively. The results indicated varieties of their N-fates in the small intestine and N-distributions of individual dietary NEAAs to circulating AAs. The presentation will compare nutritional significances of individual dietary NEAAs as N-sources for the synthesis of other AAs and will discuss quantitative and qualitative importance of dietary glutamate.

Monosodium glutamate supplementation to the diet for lactating sows enhances growth performance and survival of suckling piglets

Reza Rezaei[1], Sichao Jia[1], Houssein Assaad[1,2], Ana San Gabriel[3], Yoshiyuki Fujishima[3], Hisayuki Uneyama[3], and Guoyao Wu[1,2]

[1]Department of Animal Science and
[2]Department of Statistics, Texas A&M University, College Station, TX, USA 77843;
[3]Ajinomoto Inc., 15-1, Kyobashi 1-chome, Chuo-ku, Tokyo, Japan 104-8315

We have previously reported that milk production by sows cannot provide sufficient amino acids to support maximal growth and performance of 7- to 28-day-old suckling piglets. In the current study, we examined the efficacy of supplementing monosodium L-glutamate (MSG) to the diet of lactating sows on growth performance and survival of suckling piglets. Twenty seven multiparous sows (Landrace × Large White) were assigned randomly into one of the three groups: (1) control (basal diet), (2) basal diet + 1 % MSG; and (3) basal diet + 2 % MSG. Diets were made isonitrogenous by addition of appropriate amounts of L-alanine, and also included appropriate amounts of NaCl and cornstarch in the diets for the control and the 1 % MSG groups to balance for sodium and energy intakes, respectively. Lactating sows had free access to the drinking water and their respective diets. The number of live-born piglets was normalized to 9 per sow at Day 0 of lactation. On Days 3, 14 and 28 of lactation, body weight and milk consumption of piglets were measured and blood samples obtained from sows and piglets at 2 h after feeding. Data were analyzed by 1-way ANOVA. Feed intake of sows did not differ significantly ($P > 0.05$) among the three groups of sows. However, concentrations of glutamine, glutamate, aspartate, arginine and citrulline were significantly higher ($P < 0.05$) in the plasma of MSG-supplemented sows and their piglets, compared to the control group. Concentrations of free and peptide-bound amino acids in sow's milk were also increased ($P < 0.05$) by MSG supplementation. At the end of the experiment, piglets in the 2 % MSG group were significantly heavier ($P < 0.05$) than those in other groups. This effect was associated with increased milk intake by piglets of the MSG-supplemented sows during 28 days of lactation. The preweaning survival rates of piglets were higher ($P < 0.05$) in the MSG groups than in the control group. Collectively, these results indicate that supplementing up to 2 % MSG to the diet of lactating sows is safe and beneficially enhances their lactation performance and the growth of suckling piglets.

(Supported by Ajinomoto Inc. and Texas AgriLife Research).

Cysteinyl-glycine reduces mucosal pro-inflammatory cytokine responses in piglet models of intestinal injury

Janet A. Brunton, Matthew G. Nosworthy, and Robert F. Bertolo

Department of Biochemistry, Memorial University of Newfoundland, St. John's NL, Canada A1B 3X9

PepT1 is a small intestinal (SI) di/tripeptide transporter also capable of transporting bacterial peptides. We measured SI adaptation and mucosal cytokine response in SI-resected piglets fed dipeptide-containing diets and found no benefits to SI morphology in spite of reduced TNF-α and IFN-. Subsequently, in piglets with parenteral nutrition (PN)-induced SI atrophy, we performed an in situ SI perfusion study to assess the effects of a pro-inflammatory peptide (fMLP) on cytokine responses when co-perfused with cysteinyl-glycine (CG). CG competes for PepT1-mediated uptake and has anti-inflammatory potential. Pigs (n = 6, 10 d) received PN for 4 d to induce SI atrophy; littermate controls (n = 6) were sow-fed (SF). Five 10 cm loops of ileum were isolated and perfused for 3 h with one of: (1) free cysteine + glycine (5 mM each) (cys + gly); (2) 5 mM CG; (3) 10 μM fMLP; (4) cys + gly + 10 μM fMLP; and (5) CG + 10 μM fMLP. In PN and SF, fMLP perfusion induced mucosal TNF-α and IFN- responses ($p < 0.001$), while IFN- was higher in PN than in SF pigs ($p < 0.01$). CG lowered the IFN-response in both SF and PN ($p < 0.05$), but neither responded significantly to free cys + gly. CG lowered TNF-α in both groups ($p < 0.001$); in SF the response to CG was greater than with cys + gly ($p < 0.001$). CG, but not cys + gly, also lowered the mucosal MPO response in the PN-treated piglets ($p < 0.01$). CG was more effective than free cys + gly at attenuating a bacterial peptide-induced inflammatory response, particularly in the injured SI.

Analysis of energy expenditure in diet-induced obese rats with abnormal metabolism of amino acids

Houssein Assaad[1,2,3], Kang Yao[1], Fuller W. Bazer[1], Lan Zhou[2], Raymond J. Carroll[2], Cynthia J. Meininger[4], and Guoyao Wu[1,3,4]

[1]Department of Animal Science,
[2]Department of Statistics, and
[3]Faculty of Nutrition, Texas A&M University, and
[4]Department of Medical Physiology, Texas A&M Health Science Center, College Station, TX, USA

Obesity is a major risk factor for type II diabetes, metabolic syndrome, some types of cancer, and premature death in humans. This disease is mainly caused by a greater energy intake than energy expenditure. A hallmark of obesity is an abnormal metabolic profile of amino acids in plasma as a result of alterations in their whole-body metabolism. It is not known whether obesity results primarily from direct conversion of dietary fat into body fat or reduced energy expenditure in mammals. The major goal of this study was to study how energy expenditure may affect development of diet-induced obesity in a rodent model. Male Sprague–Dawley rats (28 days of age) were assigned randomly to either a low-fat (LF) or high-fat (HF) diet (n = 16/diet). Body weights and food intakes of rats were determined weekly. In Week 9 of the study, when rats were 13 weeks of age, 24-h energy expenditure was measured by placing them individually in a computer-controlled Oxymas open circuit calorimeter. This provided information about diurnal changes in whole-body energy metabolism as indicated by the volume of oxygen consumption, volume of carbon dioxide production, respiratory quotient, and heat production. Dietary intakes of energy, protein, vitamins, and minerals per kg body weight did not differ ($P > 0.05$) between rats fed LF and HF diets. A regression model was constructed to accurately predict weight gain in adult animals based on diet, initial body weight, the principal component scores of the volume of oxygen consumption and heat production ($R^2 = 0.96$; $P < 0.01$). At the end of the 9-week experimental period, HF-fed rats weighed more ($P < 0.01$) than LF-fed rats. However, rates of 24-h heat production per kg body weight did not differ ($P > 0.05$) between the two groups of rats. We conclude that direct conversion of dietary fat into body fat, rather than changes in whole-body energy expenditure, contributes primarily to the development of obesity in HF-fed rats.

(Supported by NIH and AHA.)

New insights into sulfur amino acid function in gut health and disease

Douglas Burrin, Barbara Stoll, Nancy Benight, and Caroline Bauchart-Thevret

Baylor College of Medicine, Houston, TX, USA

The gastrointestinal tract (GIT) is a metabolically significant site of sulfur amino acids (SAAs) metabolism in the body. Aside from their role in protein synthesis, methionine and cysteine are involved in many biological functions and diseases. Methionine (MET) is an indispensable amino acid and is transmethylated to homocysteine via *S*-adenosylmethionine (SAM), the principal biological methyl donor in mammalian cells and a precursor for polyamine synthesis. We have examined the role of SAA metabolism in GIT health and disease. Our studies in young pigs showed that the whole-body methionine transmethylation and remethylation rates were higher during duodenal [^{13}C]-MET than intravenous [$^{2}H_3$]-MET infusion. Thus, transmethylation and transsulfuration in the GIT represented 27 % and 23 % of whole-body fluxes, respectively. Additional studies show how disruption of methionine cycle activity and dietary supplementation with methionine metabolites affects the susceptibility to colitis in mice. We found that mice fed vitamin B_{12}/B_6 deficient diets are protected against colitis with reduced inflammation and tissue injury. We also found that B-vitamin deficiency suppressed inflammatory gene expression in association with altered MET cycle activity and indices of methylation status. We also showed that supplementation with the MET cycle metabolite, methylthioadenosine (MTA), prevented inflammation during colitis in mice. These results suggest that MTA also is protective against experimental colitis and reduced tissue injury and expression of multiple inflammatory genes. The presentation will discuss the evidence of sulfur amino acid metabolism in GIT and consequences of MET cycle activity in health and disease.

Molecular cloning and characterization of porcine calcium-sensing receptor (CaSR)

Chengbo Yang[1,2], Tiejun Huang[2], Li Wu[1], Tiejun Li[1], Guoyao Wu[3] and Yulong Yin[1]

[1]Institute of Subtropical Agriculture, the Chinese Academy of Sciences, Yuanda Road, Changsha, Hunan 410125, China;
[2]Lucta (Guangzhou) Flavor, Co., Ltd, Guangzhou, Guangdong 510730, China;
[3]Department of Animal Science, Texas A&M University, College Station, TX, USA 77843

The calcium-sensing receptor (CaSR) is a G-protein coupled receptor which plays a central role in extracellular calcium homeostasis and nutrient sensing in mammals. Recent research has indicated that CaSR agonist (amino acids, peptides, GSH) can enhance sweet, salty, and umami tastes known as "*kokumi taste*". The calcium-sensing receptor (CaSR) has been cloned and characterized in human and mouse. However, localization, distribution, and function of CaSR have not been described in pig. The objective of this study was to clone and characterize the CaSR in pig. We determined a 3.3-kB full cDNA sequence (Genbank: JX913858.1) of CaSR which showed 91, 93, 88 and 87 % homology with the human, bovine, mouse and rat sequences, respectively. The CaSR protein sequence with 1,089 amino acid residues showed 94, 95, 92 and 92 % homology with human, bovine, mouse and rat sequences, respectively. Tissue distribution analyses showed that pig CaSR. Tissue distribution analyses showed that pig CaSR was expressed taste cells, intestine, kidney, lung, liver and heart. Results indicated that CaSR is much conserved among different species and widely expressed in the different tissues. The functions and agonist screening of pig CaSR will be further investigated with bioinformatics tools, cell-reporter assay and animal trials which will be very helpful for developing new feed flavor and functional feed additives to improve gut health, nutrient utilization efficiency, and animal wellbeing.

Mechanism underlying the role of taurine as an antioxidant and a cytoprotective agent

Chian Ju Jong[1], Takashi Ito[2], Junichi Azuma[2], Stephen Schaffer[1]

[1]University of South Alabama, Department of Pharmacology, College of Medicine, Mobile, AL-36688, USA; and
[2]Hyogo University of Health Sciences, School of Pharmacy, Kobe, Japan

Taurine, which has a high concentration in the heart, is known for its antioxidant action. However, the mechanism underlying its oxidant prevention remains unclear as taurine is not a classical free radical scavenger. We proposed that taurine prevents superoxide production by the mitochondria via a mechanism related to the formation of a taurine-modified uridine of mitochondrial $tRNA^{Leu(UUR)}$. By decreasing its post-transcriptional wobble modification, taurine deficiency reduces the levels of respiratory chain subunits (ND2, ND6). This effect diminishes respiratory chain complex activity and oxygen consumption. The reduction in electron flux promotes the diversion of electrons to oxygen, producing superoxide. Indeed, taurine-depleted hearts demonstrated evidence of oxidative stress as exemplified by decreased aconitase activity and the glutathione redox ratio. Taurine deficiency also triggers cell death, as shown by an increase in caspase 3 immunoreactivity and percentage of apoptotic cells. Because Tiron prevents apoptosis in taurine-deficient cardiomyocytes, cell death is attributable to oxidative stress. In conclusion, this study demonstrates that taurine regulates respiratory chain function, mitochondrial oxidative stress and apoptosis, most likely via a post-transcriptional modification of the wobble base of $tRNA^{Leu(UUR)}$.

The impact of tissue taurine deficiency on senescence and longevity; a study in taurine transporter knockout mouse

Takashi Ito[1], Stephen W. Schaffer[2], Junichi Azuma[1]

[1]Hyogo University of Health Sciences, School of Pharmacy, Kobe, Japan; and
[2]University of South Alabama, Department of Pharmacology, College of Medicine, Mobile, AL-36688, USA

Since taurine has a variety of biological actions which protect the cell from pathological and physiological stresses, it is a candidate for an anti-aging agent. Our previous studies using taurine transporter knockout (TauTKO) mouse have demonstrated that taurine depletion causes to decrease in exercise capacity concomitant with skeletal muscle defects, including muscle atrophy. Moreover, TauTKO mouse exhibits aging-associated disorders in heart and liver. In the present study, we investigated the influence of taurine depletion to lifespan and tissue senescence. The survival rate was shorter in TauTKO mouse than WT mouse. Furthermore, senescence markers, such as cyclin-dependent kinase inhibitor p16INK4a, are increased in old TauTKO tissues than WT tissues. Concomitantly, several aging-associated phenotypes appear early in TauTKO mouse compared to WT mouse. In conclusion, tissue taurine deficiency may accelerate tissue senescence and ultimately reduced lifespan.

Differential regulation of the hippocampal taurine transporter protein in rat brain: mechanisms contributing to neuronal volume regulation during cytotoxic brain edema

Amanda N. Freeman, and James E. Olson

Wright State University School of Medicine, Dayton, OH, USA

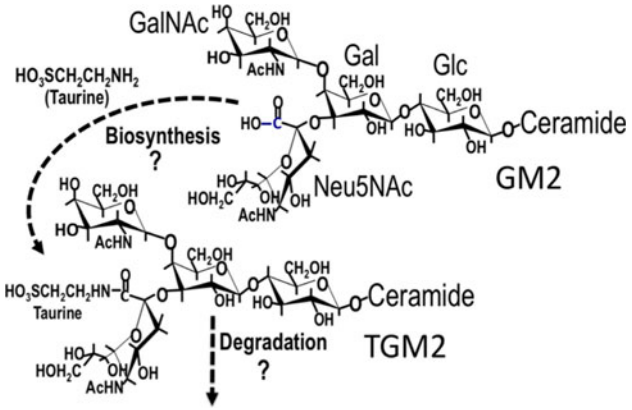
In cytotoxic edema, net efflux of taurine from neurons and accumulation by astrocytes contributes to neuronal volume regulation and astrocytic swelling. Taurine is accumulated in both cell types by a sodium- and chloride-dependent 72–75 kDa protein transporter, TauT. TauT functional activity decreases in osmotically swollen neurons but is unaltered in swollen astrocytes, in vitro. This swelling-induced downregulation of neuronal TauT activity is blocked with the tyrosine kinase (TK) inhibitor, genistein. In contrast, PKC activation has no effect on neuronal TauT, but inhibits astrocytic TauT. Thus, we hypothesize that neuronal TauT activity is regulated by a TK signaling pathway whereas astroglial TauT activity is regulated by serine/threonine kinases. This differential regulation contributes to neuronal volume regulation and astrocytic swelling via taurine redistribution during cytotoxic brain edema. Primary neuronal and astrocytic cultures from rat hippocampus were incubated under iso- or hypo-osmotic conditions in the presence or absence of activators or inhibitors of TK or PKC. Subcellular TauT localization was measured after 30 min using cell fractionation, cell surface biotinylation and western blot analyses. Phosphorylation was measured after 30 min using immunoprecipitation and western blot analyses with phosphoprotein-specific antibodies. We found neuronal and astroglial TauT primarily localized to cytosolic and membrane/particulate fractions in isoosmotic conditions. However, cell surface biotinylation of TauT decreased in swollen neurons while phosphorylation of tyrosine residues increased. In contrast, phosphorylation of serine and threonine on neuronal TauT was unchanged. Surface biotinylation decreased and phosphorylation of serine and threonine residues on astrocytic TauT increased upon treatment with 1 μM PMA. Cell surface biotinylation and phosphorylation of TauT in astrocytes were unaffected by cell swelling. The results suggest the signal for neuronal TauT translocation from the cell membrane during hypoosmotic cell swelling involves tyrosine phosphorylation. Membrane localization of astroglial TauT remains unchanged during hypoosmotic cell swelling. These changes may account for the observed reduction in functional TauT activity in swollen neurons and may contribute to neuronal volume regulation during cytotoxic edema.

Taurine-conjugated GM2 in Tay-Sachs brain

Yu-Teh Li, Su-Chen Li

Department of Biochemistry and Molecular Biology, Tulane University School of Medicine, New Orleans LA 70112, USA

Tay-Sachs disease (TSD) or GM2-gangliosidosis is a classical inborn lysosomal glycosphingolipid (GSL) storage disease of the central nervous system. The hallmark of this GSL storage disease is the massive cerebral accumulation of ganglioside GM2 due to the deficiency of either beta-hexosaminidase A or GM2 activator protein. Despite our clear understanding of the molecular and biochemical bases of TSD, very little is known about how the cerebral accumulation of GM2 leads to different disease progression and tissue dysfunction. To understand how the neural tissues of TS patients respond to and cope with the massive accumulation of GM2, we carried out a detailed analysis of GSLs in TS brain samples and detected a taurine-conjugated GM2 (TGM2) in which the carboxyl group of Neu5Ac was amidated by taurine (Li, Y.-T., Maskos, K., Chou, C.-W., Cole, R. B., and Li, S.-C., *J. Biol. Chem.* **278,** 35286, 2003). Using rabbit anti-TGM2 antibodies, TGM2 was only detected in TS brains but not in normal brains. Since taurine-conjugation is intimately associated with detoxification in higher animals to remove xenobiotics by increasing their aqueous solubility, we hypothesized that neural tissues may use taurine-conjugation as a vehicle for removing the excessive GM2 accumulated in TS brain. We would like to unveil the location and the mechanism by which TGM2 is synthesized and degraded in TS brain as shown in the following figure.



Neuroprotective functions of taurine

Howard Prentice, Payam Gharibani, Jigar Modi, Chunliu Pan, Zhiyuan Ma, Janet Menzie, Po-Chih Chen, Rui Tao, and Jang-Yen Wu

Florida Atlantic University, Charles E. Schmidt College of Medicine, Boca Raton, FL 33431, USA

Taurine is one of the most highly abundant amino acids in mammals and the molecule is enriched in electrically excitable tissues including brain and heart. Amongst its multiple functions, taurine has been shown to possess the properties of antioxidant, a trophic factor, a neurotransmitter/neuromodulator and a neuroprotectant. The neuroprotective mechanisms of taurine include decreasing endoplasmic reticulum (ER) stress, maintaining calcium homeostasis and preventing apoptosis. In neuronal cultures subjected to glutamate excitotoxicity or to hypoxia/re-oxygenation, activation of endoplasmic reticulum (ER) stress has been found to stimulate three key signaling pathways, the p-IRE-1, PERK and ATF6 pathways. We have demonstrated that in neuronal cultures subjected to glutamate or hypoxia/re-oxygenation exposure, ER stress can be reduced by taurine with a selective targeting of the p-IRE1 and ATF6 pathways. In an in vivo stroke model, the middle cerebral artery occlusion model, we have demonstrated a protective action of taurine as determined by a decrease in infarct size quantified from staining with triphenyltetrazolium chloride (TTC). The neuroprotection occurred in conjunction with a down-regulation of the ER stress protein markers Grp78, caspase-12, p-IRE-1 and cleaved ATF6 (the active form of ATF6). Downstream pro-death proteins CHOP and Bax were also dramatically decreased in the stroke model following taurine administration. In conclusion our findings indicate a protective action by taurine in neuronal cultures subjected to glutamate treatment or hypoxia/re-oxygenation as well as in the MCAO stroke model. In both the cell culture and the MCAO models taurine decreased signaling via the p-IRE1 and ATF6 pathways. Our findings point the way for future applications of

taurine to stroke therapy and help to elucidate the role of taurine in preventing ER stress in stroke.

Alteration of myofibrillar calcium sensitivity by taurine deficiency

Stephen Schaffer[1], K. C. Ramila[1], Takashi Ito[2], and Junichi Azuma[2]

[1]University of South Alabama, College of Medicine, Department of Pharmacology, Mobile, AL 36688, USA;
[2]Hyogo University of Health Sciences, School of Pharmacy, Kobe, Japan

Taurine is an abundant amino acid in the heart involved in the regulation of calcium transport, osmolality, oxidative stress and ATP generation. Taurine deficiency is associated with the development of a cardiomyopathy. Using chemically skinned fibers, Steele et al. (J Physiol 422:499–511, 1990) previously showed that elevations in taurine increased calcium sensitivity of the myofibrils. However, the mechanism underlying this effect was not examined. Moreover, it was unclear if taurine deficiency could also affect calcium sensitivity. To examine these questions, the calcium sensitivity of skinned myofibrillar proteins from wild type and taurine transporter knockout hearts was examined. Taurine deficiency was associated with a shift in the calcium dependence of the Ca^{2+}-dependent myosin ATPase to the right, indicative of reduced calcium sensitivity. The reduction in calcium sensitivity was associated with an elevation in the phosphorylation of the muscle protein, troponin I, whose phosphorylation is known to decrease the binding of calcium to troponin C and consequently decrease the activation of myosin ATPase activity. Taurine deficiency had no effect on the enzyme involved in the dephosphorylation of troponin I, but increased the activity of protein kinase C, which is involved in the phosphorylation of troponin I. In conclusion, the modulation of myosin ATPase activity and myofibrillar tension by taurine appears to involve changes in the phosphorylation state of troponin I resulting from taurine-linked activation of protein kinase C.

Evaluation of the role of taurine supplementation in the metabolic and antioxidant actions of metformin in an animal model of diabetes

C. A. Lau-Cam

Department of Pharmaceutical Sciences, St. John's University, College of Pharmacy and Health Professions, Jamaica, New York 11439

This study has investigated the merits of using taurine (TAU) as a supplement to the oral hypoglycemic drug metformin (MET) in the management of metabolic and oxidative changes associated with type 2 diabetes mellitus in an animal model. To this end, male Sprague–Dawley rats, assigned to groups of 6 and made diabetic with streptozotocin (60 mg/kg, i.p.), were treated from day 15 to 56 with a daily dose of (a) physiological saline (2 ml, p.o., the diabetic group), (b) MET, TAU or MET-TAU (2.4 mmol/kg, i.p., the treatment groups) or (c) insulin (INS, 4 U/kg, s.c., the reference group). Naive rats served as controls. Body weight gains and tail vein blood glucose levels were monitored on a weekly basis for 8 weeks. Blood and kidney samples, collected on day 57, were processed to obtain plasma, red blood cells (RBCs) and kidney homogenates suitable for the analysis of biochemical indices of altered glucose and lipid metabolism and of oxidative stress. Diabetes was found to lower the body weights and plasma INS and to raise the blood glucose, blood glycated hemoglobin and plasma cholesterol and triglycerides levels to a significant extent ($p \leq 0.01$ vs. control values). In addition the plasma malondialdehyde was elevated but the reduced to oxidized glutathione ratio and activities of catalase, glutathione peroxidase and superoxide dismutase were drastically decreased ($p < 0.001$ vs. controls). The analysis of RBC samples revealed extensive hemolysis, increased membrane cholesterol to phospholipids ratio, increased malondialdehyde formation, and a loss of antioxidant enzyme activities. A similar trend in the values of malondialdehyde and of antioxidant enzymes was verified in kidney samples. Although both MET and TAU normalized the growth rate and attenuated all of the aforementioned alterations, their potencies differed according to the sample and parameter examined. In RBCs the magnitudes of the results for MET were, for the most part, not significantly different, from those for TAU. However, MET was more effective than TAU in influencing the changes in plasma glucose, INS and glycated hemoglobin but less effective in counteracting oxidative stress. In the kidney, TAU was more protective than MET in lowering the decrease in redox status and the losses in enzymatic defenses caused by diabetes even though they were equipotent in minimizing lipid peroxidation. On the other hand, a supplementation with TAU enhanced the beneficial actions of MET against diabetes-induced alterations of the plasma and RBC membrane lipids and of the redox status and antioxidant enzymes in RBCs and the kidney. Finding the INS was not as effective as TAU in counteracting changes in plasma triglycerides and in indices of oxidative stress would signify that changes in plasma lipids and indices of oxidative imbalance occur independently of hyperglycemia and hypoinsulinemia. On this basis, TAU could be of value as an adjunct to the treatment of type 2 diabetes with a hypoglycemic agent such as MET.

Knockout of the *TauT* gene predisposes C57BL/6 mice to STZ-induced diabetic nephropathy

Xiaobin Han[1], Andrea B. Patters[1], Takashi Ito[2], Junichi Azuma[2], Stephen Shaffer[3], and Russell W. Chesney[1]

[1]Department of Pediatrics, University of Tennessee Health Science Center, and the Children's Foundation Research Center at Le Bonheur Children's Medical Center, 50 N. Dunlap, Memphis, TN, 38103, USA;
[2]Hyogo University of Health Sciences, School of Pharmacy, Kobe, Japan; and
[3]University of South Alabama, Department of Pharmacology, College of Medicine, Mobile, AL-36688, USA

Diabetic nephropathy (DN) is the leading cause of end-stage renal disease (ESRD). Despite tremendous efforts, scientists have yet to identify an ideal animal model that can reproduce the characteristics of human DN. In this study, we hypothesize that the taurine transporter gene (*TauT*), which serves a protective role in the kidney, is a critical risk factor for DN development in patients with diabetes mellitus (DM). This hypothesis was tested in vivo in *TauT* heterozygous deletion ($TauT^{+/-}$) and *TauT* homozygous knockout ($TauT^{-/-}$) in C57BL/6 background mice. We have discovered that genetic alterations of the *TauT* gene have a substantial effect on the susceptibility to diabetic kidney disease development in both $TauT^{+/-}$ and $TauT^{-/-}$ models of diabetes. These animals developed histological changes that included glomerulosclerosis, nodular lesions, arteriosclerosis, arteriolar dilation and tubulointerstitial fibrosis. Immunohistochemical staining of molecular markers of smooth muscle actin (SMA), CD34, Ki67, and collagen IV confirmed these observations. Thus, our results are the first to demonstrate that both homozygous and heterozygous *TauT* gene deletions predispose C57BL/6 mice to develop end-stage

diabetic kidney disease, which closely replicates the pathological features of diabetic nephropathy in diabetic patients.

Protective effect of taurine on the up-regulated expressions of retinoid X receptor genes in brains of mice exposed to arsenic

Fengyuan Piao[1], Fanyin Cheng[2]

[1]Department of Occupational and Environmental Health, Dalian Medical University, Dalian, Liaoning 116044, People's Republic of China;
[2]Department of Health Administration, Dalian Medical University, Dalian, Liaoning 116044, People's Republic of China

This study aimed at evaluating protective effect of taurine on the down-regulated expressions of retinoid X receptor (RXR) genes in brains of mice exposed to arsenic (As). The SPF mice were randomly divided into As exposure group, protective group and control group. The As exposure group was administered with 4 ppm As_2O_3 through drinking water for 60 days. The protective group was treated with both 4 ppm As_2O_3 and 150 mg/kg taurine. The control group was given with drinking water alone. The gene expressions of RXRα and RXRβ in the mice brains of the three groups were analyzed by real time PCR. Their protein expressions were examined by Western blot and immunohistochemistry. Our results showed that the gene expressions of RXRα and RXRβ were up-regulated in cerebrum of mice exposed to As. The expression of their proteins in the cerebrum significantly increased in the group exposed to As compared to the control group. However, the up-regulated expressions of RXRα and RXRβ genes or their proteins were significantly rescued in the group co-administered with taurine as antioxidant. These results indicated that taurine may have the protective effect on the up-regulated expressions of RXR genes in brains of mice exposed to As.

Taurine improves neonatal survival in cysteine sulfinic acid decarboxylase knockout mice

Eunkyue Park[1], Seung Yong Park[2], Bo Sook Kim[3], In Soo Cho[4], Carl Dobkin[5], Euisun Jung[1], Chuanhua Wang[6], and Georgia Schuller-Levis[1]

Laboratories of Cellular Immunology[1] and Physiology[6], Department of Developmental Neurobiology, and [5]Laboratory of Molecular Genetics, Department of Human Genetics, New York State Institute for Basic Research in Developmental Disabilities, Staten Island NY 10314 USA,
[2]College of Veterinary Medicine, Konkuk University, Seoul, 143-701 Korea,
[3]Seoul Grand Zoo, Gwacheon, Gyunggido, 427-702 Korea,
[4]National Veterinary Research and Quarantine Service, Anyang, Gyunggido, 430-824 Korea

Taurine deficiency has pleitropic effects on development that lead to brain abnormalities and severely impact the reproductive and immune systems. These effects were initially identified in studies of the taurine deficient cat but this model has serious limitations. Since cysteine sulfinic acid decarboxylase (CSAD) is a rate limiting enzyme for taurine biosynthesis in the mouse, we engineered a CSAD gene knock-out (KO) mouse. This model makes it possible to investigate the physiological roles of taurine and to focus on its role in the pathogenesis of developmental disabilities and birth defects as well as to investigate possible treatments. The knock out of the CSAD gene and its activity was verified by Southern, northern and western blotting; homozygous ($CSAD^{-/-}$) and heterozygous ($CSAD^{+/-}$) animals were identified by polymerase chain reaction. High performance liquid chromatography indicated an 83 % decrease of taurine concentration in blood plasma of $CSAD^{-/-}$ animals. Because initial observations focused our interest on reproduction, we compared litter size and perinatal survival of different CSAD genotypes. Although F1 $CSAD^{-/-}$ and F2 $CSAD^{-/-}$ survived and appeared healthy, the offspring (F3 $CSAD^{-/-}$) from the second generation of $CSAD^{-/-}$ (F2 $CSAD^{-/-}$) died within 24 h of birth. Taurine concentrations in the brain and liver of $CSAD^{-/-}$ animals were very low at birth. The threshold levels of taurine for survival are undetermined. The addition of 0.05 % taurine to the drinking water restored the wild type (WT) taurine concentration in the brain and increased the survival rates of F3 $CSAD^{-/-}$from 15 % to 92 %. We examined the expression of CSAD, cysteine dioxygenase (CDO), cysteamine dioxygenase (ADO), taurine transporter (TauT) and antioxidant genes using RT^2 qPCR assay. The RNA expression levels of CDO, ADO and TauT were not different in $CSAD^{-/-}$ or in $CSAD^{-/-}$animals treated with taurine. CSAD RNA was not expressed in CSAD KO, confirming the deletion of this gene. In addition, RNA expression of some antioxidant enzymes including peroxireductase 2 and 3 as well as glutathione peroxidase 1 was not significantly different in CSAD compared to WT. However, glutathione peroxidase 3 was increased significantly in $CSAD^{-/-}$ and restored to wild-type level in taurine-treated $CSAD^{-/-}$ animals. Since newborn F3 $CSAD^{-/-}$ often lacked milk spots, which are a predictor of survival in newborn mice, we examined expression of lactoferrin and the prolactin receptor and found that their expression was decreased significantly in $CSAD^{-/-}$ animals. Prolactin receptor expression was restored using taurine treatment, but lactoferrin was not. These data indicate that CSAD is an effective target for lowering taurine levels and that wild-type taurine concentration can be restored in the brains of CSAD KO mice when they are treated with taurine in their drinking water. Taurine treatment dramatically increased survival rates of F3 CSAD mice, suggesting that this is a good model for studying both the effects of taurine deficiency and its treatment by taurine supplementation.

Cancer's dependence on serine biosynthesis and adequate nutrient availability

Richard Possemato, Kivanc Birsoy, and David M. Sabatini

Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142, USA;
Howard Hughes Medical Institute and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA;
Broad Institute of Harvard and MIT, Seven Cambridge Center, Cambridge, MA 02142, USA; and
The David H. Koch Institute for Integrative Cancer Research at MIT, 77 Massachusetts Avenue, Cambridge, MA 02139, USA

Cancer cells exhibit an altered metabolism driving macromolecular biosynthesis for rapid cell growth and proliferation, and exist in a microenvironment with lower levels of specific nutrients. Here, we developed methods for identifying novel cancer targets via negative selection RNAi screening in solid tumours or under defined nutrient states. Using this in vivo screening method, we identified genes required for tumourigenesis including phosphoglycerate dehydrogenase (*PHGDH*), which catalyzes the first step in the serine biosynthesis pathway. Breast cancer cells with high PHGDH expression have elevations in serine synthesis flux, and suppression of PHGDH in these cell lines causes a strong decrease in cell

proliferation and a reduction in serine synthesis. Inhibition of PHGDH causes a drop in the levels of alpha-ketoglutarate, another output of the pathway and a TCA cycle intermediate, as the serine synthesis pathway contributes approximately 50 % of the total anaplerotic flux of glutamine into the TCA cycle in PHGDH-amplified cells. These results reveal that certain breast cancers are dependent upon increased serine pathway flux caused by PHGDH over-expression and demonstrate the utility of in vivo negative selection RNAi screens for finding potential anticancer targets. Subsequently, we have developed cell culture methods enabling us to maintain specific key nutrients at chronically low levels. In combination with a cell line DNA-barcoding system that we developed, we can perform competition assays to rapidly identify cell lines sensitive to limitation of specific nutrients found to be present at low levels in tumors. Cell lines grown in nutrient limited conditions exhibit differential sensitivity to inhibitors of specific metabolic pathways. These results underscore the importance of studying cancer metabolism and antimetabolite treatments under tumor-relevant conditions.

Maternal glutamine supplementation mitigates negative fetal developmental effects from prenatal alcohol exposure

Shannon E. Washburn[a], Onkar B. Sawant[a], Guoyao Wu[b]

[a]Department of Veterinary Physiology and Pharmacology and Michael E. DeBakey Institute, and
[b]Department of Animal Science, Texas A&M University, College Station, TX, USA, 77843

The failure of education to significantly reduce the incidence of Fetal Alcohol Syndrome has made it important to understand the mechanisms in order to develop preventative or ameliorative strategies. Prenatal alcohol exposure is known to be associated with altered fetal blood flow, oxidative stress and growth restriction that can persist into adolescence. Low birth weight is also associated with altered development and programming which can have lifelong consequences. Glutamine has been linked with fetal nitrogen and carbon metabolism, synthesis of the cellular anti-oxidant glutathione, and apoptosis suppression. It is a precursor for the synthesis of other amino acids, endothelial-dependent relaxation, and increases in protein synthesis and is used clinically as a nutrient supplement in low birth weight infants. We hypothesized that chronic third trimester-equivalent prenatal alcohol exposure would decrease the bioavailability of amino acids, hamper fetal body growth, increase fetal cerebellar oxidative stress, and alter fetal blood flow; a corollary hypothesis was that maternal L-glutamine supplementation may improve these negative developmental effects of prenatal alcohol exposure. Pregnant sheep were randomly assigned to 4 groups: saline control, glutamine (GLN) control, alcohol or alcohol + GLN. Alcohol or saline infusions were given intravenously (IV) over 1 h from gestation day (GD) 109 to 132 or from GD 99 to 121, 3 consecutive days per week to mimic a weekend binge drinking pattern. 100 mg/kg of GLN dose was administered IV as a 4.5 %w/v aqueous bolus three times a day. Third trimester equivalent prenatal alcohol exposure reduced fetal weight and height and also increased the ponderal index compared to the control group; these parameters were significantly improved in the alcohol + GLN group compared to the alcohol group. Prenatal alcohol exposure decreased fetal plasma concentrations of arginine, aspartate, glutamine, taurine and tyrosine; maternal glutamine supplementation improved the bioavailability of amino acids in both the maternal and fetal compartments. We observed a decrease in oxidized glutathione in maternal hemocytes and the fetal cerebellum for the alcohol + GLN group compared to the alcohol group. Fetal cerebellar blood flow was significantly increased in the alcohol group, hence increasing the cerebellar alcohol delivery. This alcohol-induced increase in fetal cerebellar blood flow was mitigated in the alcohol + GLN group. In summary, maternal glutamine supplementation improved amino acid bioavailability, fetal growth and endogenous antioxidant levels in alcohol exposed fetuses. Thus, glutamine shows great potential as a therapeutic or intervention strategy for the negative effects of prenatal alcohol exposure.

Effect of dietary supplementation with samgiomarome on growth performance, diarrhea score and serum concentrations of amino acids in weanling pigs

Gang Liu[1], Yordan Martínez Aguilar[1,2], Wenkai Ren[1], Shuai Chen[1], Tiejun Li[1], Guyao Wu, and Yulong Yin[1]

[1]Scientific Observing and Experimental Station of Animal Nutrition and Feed Science in South-Central, Ministry of Agriculture, Hunan Provincial Engineering Research Center of Healthy Livestock and Poultry, Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, Hunan, China, 410125;
[2]Study Center of Animal Production, Faculty of Veterinary Medicine, University of Granma, Bayamo, Carretera Manzanillo 17 km, P.O. box 21, Granma, Cuba, 85100.

Sanguinarine is of great practical and research interest because of its anti- microbial and anti-inflammatory responses in experimental animals. An experiment was conducted to determine the growth performance, diarrhea rate, and serum amino acids concentrations receiving a sanguinarine supplement. A total of 96 weanling pigs were randomly assigned to three dietary treatments, with 5 replicates per treatment and 8 piglets per replicate. All pigs received a basal diet similar in composition and nutrients, treatments were a control (no additive), antibiotic (200 mg/kg colistin), and sanguinarine (40 mg/kg). Blood samples were obtained from five piglets selected randomly from each treatment on days 7, 14 and 28. Compared with the control group, dietary sanguinarine did not affect ADFI and ADG, whereas colistin group decreased average daily feed intake by 13.1 % and increased average daily gain by 36 %. Dietary sanguinarine and colistin group decreased diarrhea rate by 78.6 % and 57.1 %, respectively. Addition of sanguinarine to the diet increased serum concentrations of Leu, Lys and Arg on days 7 and 14, increased Thr, Ala, Lys and Arg on days 28. These findings indicate that sanguinarine regulate amino acids metabolism and beneficially increasing the entry of dietary amino acids into the systemic circulation and could be a suitable alternative to antibiotics.

[This study was partly supported by Hunan provincial Science & Technology Department program (13JJ2034,2012WK4021), The national science and technology support program (2012BAC 17B0102), and Knowledge Innovation Program of the Chinese Academy of Sciences (KSCX2-EW-G-16)].

Importance of tryptophan along the non-polar helix face of an antimicrobial amphipathic peptide for improving killing efficacy against *Pseudomonas aeruginosa*

X. Zhu, and A. S. Shan

Institute of Animal Nutrition, Northeast Agricultural University, Harbin 150030, China

A series of threonine-substituted mutants (T9K, T9I, T9F, and T9W) were designed from N-terminal of PMAP-36, and biological activity and membrane-peptide interactions of the peptides were also analyzed. Circular dichroism results demonstrated that T9K resulted in decreased helical content in SDS (bacterial membrane-mimicking environment) compared with other mutants (T9I, T9F, and T9W), whereas all mutants showed similar helical structure in TFE (mammalian membrane-mimicking environment). Biological assays demonstrated that all mutants showed no toxicity to erythrocytes and maintained comparable activity against *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. Importantly and interestingly, T9W mutation showed a strong bactericidal activity (2 μM) against *Pseudomonas aeruginosa* than other mutants (above 128 μM). NPN and $diSC_3$-5 assays demonstrated that T9W effectively depolarized the outer and inner membrane of *Pseudomonas aeruginosa*, respectively, and the major effect of T9W was on the integrity of the cytoplasmic membrane studied by the ONPG method. Moreover, the addition of salts (NaCl, KCl, NH_4Cl, $MgCl_2$, and $FeCl_3$) at physiological concentrations did not compromise the bactericidal activity of T9W against *Pseudomonas aeruginosa*. We highlight a significant role of tryptophan on the non-polar face of an amphipathic helical peptide in terms of antimicrobial activity against *Pseudomonas aeruginosa*, augmenting the arsenal of strategies to rationally design antimicrobial peptides.

Molecular cloning, distribution, and ontogenetic expression of the amino acid transreceptor SNAT2 mRNA in the small intestine of piglets

Guangran Li[1, 2], Bie Tan[1*], Jing Wang[1, 2], Xia Xiong[1], Yulong Yin[1*], Zhenlong Wu[3], and Guoyao Wu[3,4]

[1]Observation and Experiment Station of Animal Nutrition and Feed Science in South-Central China, Ministry of Agriculture; Hunan Provincial Engineering Research Center for Healthy Livestock and Poultry Production; Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences; Changsha, Hunan 410125, China;
[2]University of the Chinese academy of sciences; Beijing 10008, China;
[3]State Key Laboratory of Animal Nutrition, China Agricultural University, Beijing, China 100193;
[4]Department of Animal Science, Texas A&M University, College Station, TX 77843, USA

The sodium-coupled neutral amino acid transporter 2 (SNAT2) may play dual transport/receptor functions and be transduced to signaling pathways through processes of the coupled transport. SNAT2 is well documented in eukaryotes and some mammalian systems but have yet to be identified in pigs. This study aims to clone the gene encoding the porcine SNAT2 (SLC38A2) and investigate its distribution and ontogenetic expression in the small intestine of piglets. The 1,521-bp porcine full cDNA sequence of the SLC38A2 (KC769999) from the small intestine of Duroc × Landrace × Large Yorkshire piglets was cloned. The open reading frame of this cDNA encodes 506 deduced amino acid residues that showed 90, 84 and 84 % homology with known human, rat and mouse gene sequences, respectively. SLC38A2 mRNA levels were evaluated in the small intestine of piglets during postnatal development and the first week post-weaning (weaned at 14 days of age) using real-time RT-PCR. SLC38A2 mRNA was detected in duodenum, jejunum and ileum. The jejunum had the highest SLC38A2 mRNA abundance compared with that of the duodenum and ileum. SLC38A2 mRNA levels in the duodenum and jejunum were increased progressively from day 1 to day 14 and then decreased from day 14 to day 21, whereas the gene expression in the ileum decreased from 7 day of age. Within the first week post-weaning, a sharp decline in SLC38A2 mRNA abundance in the duodenum and jejunum was observed on the first day after weaning and then decreased steadily with increasing age. Studies with Intestinal porcine epithelial cells (IPEC-1) in vitro showed that amino acids starvation and supplementation with 2 mM glutamate, arginine, proline, leucine or 0.5 mM putrescine enhanced, but supplementation with 2 mM glutamine or alanine reduced, SLC38A2 mRNA levels in porcine enterocytes. These results provide a basic tool for further studies on the biological function of SNAT2 and the mechanism of amino acid sensing and signal transduction induced by SNAT2 in the porcine small intestine. Such findings have broad important implications for amino acid and protein nutrition in humans and other animals (including piglets).

Methionine deficiency in culture medium promotes apoptosis in intestinal epithelial cells infected with enterotoxigenic *Escherichia coli*

Yulong Tang[1], Weikai Ren[1], Fengna Li[1], Gang Liu[1], Bie Tan[1], Guoyao Wu[2], Charles M. Nyachoti[3] and Yulong YIN[1]

[1]Key Laboratory of Agroecology and Processing of Subtropical Region, Institute of Subtropical Agriculture, Research Center for Healthy Breeding of Livestock and Poultry, Hunan Engineering and Research Center for Animal and Poultry Science, Scientific Observing and Experimental Station of Animal Nutrition and Feed Science in South-Central China, The Chinese Academy of Science, Ministry of Agriculture, 410125 Changsha City, Hunan, People's Republic of China;
[2]Department of Animal Science, Texas A&M University, College Station, TX 77843, USA;
[3]Department of Animal Science, University of Manitoba, Winnipeg, MB, Canada R3T 2N2

Infections by enterotoxigenic Escherichia coli (ETEC) result in large economic losses in the swine industry worldwide. Adhesion of ETEC to the intestinal epithelial cells is an initial step in the pathogenesis of the disease. Methionine plays a role in a variety of biologically important reactions. Therefore, the goal of this study was to identify a role for methionine in protecting intestinal mucosal cells from ETEC-induced infection. Intestinal porcine epithelial cells (IPEC-1) were cultured in medium with or without 0.12 mM L-methionine. Methionine deprivation in the medium reduced the proliferation of IPEC-1 cells. Cells incubated with the methionine-deprived media exhibited increased adhesion (3.1 vs. 8.6 %, $P < 0.05$) and cytotoxicity (6.3 vs. 16.3 % in 1 h and 13.6 vs. 34.3 % in 2 h) to ETEC. To further understand the cell injury, we determined if methionine deprivation could induce apoptosis in IPEC-1 cells when infected with ETEC. Both Hoechst–PI staining and TUNEL assays revealed apoptotic responses. Results of the western blot analysis showed that methionine deprivation increased the expression of cleaved capase-3 and decreased the expression of Bcl-2, which was ameliorated by the presence of 0.12 mM methionine. We suggest that methionine plays a protective role against ETEC infection in the small intestine by decreasing adhesion of the pathogen to intestinal epithelial cells and attenuating the rate of their apoptosis.

Stimulation of pentose cycle activity in porcine trophectoderm cells by L-glutamine, but not L-arginine

Gang Lin[1, 2], Defa Li[1], Junjun Wang[1], Fuller W. Bazer[2], Guoyao Wu[1, 2]

[1]State Key Laboratory of Animal Nutrition, China Agricultural University, Beijing, China 100193,
[2]Department of Animal Science, Texas A&M University, College Station, TX 77843, USA

The pentose phosphate pathway (pentose cycle) is an alternative route for glucose metabolism via glycolysis and for generation of NADPH and pentose sugars essential for biosynthesis in rapidly proliferating tissues. Pig blastocysts undergo rapid expansion, differentiation and development, which requires a large amount of ribose and NADPH during the peri-implantation period of pregnancy. At present, little is known about the activity of pentose cycle in trophectoderm cells that become chorion cells of the mature placenta. The objective of the present study was to determine whether the pentose cycle is active in trophectoderm cells and whether functional amino acids (arginine and glutamine) can regulate this metabolic pathway. Porcine trophectoderm (pTr) cells prepared from pig blastocysts on Day 12 of pregnancy were used in this study. Cells were incubated at 37 °C for 1, 2 and 3 h in our customized DMEM medium containing 5 mM glucose. The activity of the pentose cycle was estimated using D-[1-^{14}C]glucose and D-[6-^{14}C]glucose. To determine effects of arginine and glutamine on the pentose cycle, cells were cultured at 37 °C for 2 h in the same customized medium containing different concentrations of L-arginine (0, 0.2 and 0.5 mM) and L-glutamine (0, 0.5 and 1 mM) within physiological ranges. A large portion of glucose taken up by pTr cells was metabolized via the pentose cycle. Pentose cycle was highly active in pTr2 cells and the rates of glucose metabolism via this pathway increased linearly with time (i.e., 14.9, 21.9 and 29.9 nmol/10^6 cells for 1, 2 and 3 h incubation, respectively; mean values for 6 independent experiments). ^{14}C-glucose was incorporated into glycoprotein in a time-dependent manner. Arginine had no effect ($P > 0.05$) on pentose cycle activity, and no utilization of extracellular L-arginine by pTr cells was detected within a 2-h period of incubation. However, L-glutamine (0.5 and 1 mM) increased ($P < 0.05$) the production of $^{14}CO_2$ from [1-^{14}C]glucose, but had no effect on production of $^{14}CO_2$ from [6-^{14}C]glucose. Thus, 0.5 and 1 mM L-glutamine stimulated pentose cycle activity by 17 and 31 %, respectively, as compared to the 0 mM L-glutamine treatment ($P < 0.05$). Rates of utilization of glutamine, as well as production of glutamate, aspartate and alanine per 10^6 cells increased ($P < 0.05$) with increasing extracellular concentrations of glutamine from 0 to 1 mM. These results provide the first line of evidence for high activity of the pentose cycle in pTr cells and for an important role of L-glutamine in enhancing this metabolic pathway in the pTr cells.

A novel role for arginine in enhancing neonatal thermogenesis

M. Carey Satterfield, Sorin M. Greff, Guoyao Wu

Departments of Animal Science, Texas A&M University, College Station, TX 77843, USA

Neonatal thermoregulation is an essential physiological process, primarily mediated by non-shivering thermogenesis in brown adipose tissue (BAT). We recently reported that maternal arginine supplementation increased fetal peri-renal BAT mass by nearly 50 % in sheep. Therefore, we tested the hypothesis that increased fetal BAT will enhance neonatal thermogenesis in response to cold. Thirty-one multiparous ewes were assigned to receive either intravenous injections of L-arginine (27 mg/kg bodyweight; n = 17) or sterile saline (n = 14) thrice daily from Days 75 to 125 of gestation. Following parturition lambs were removed from their mothers, placed in a thermoneutral environment, and fed artificial colostrum per body weight. At 4 h of age, lambs were challenged at 0 °C for 2 h. At 6 h of age all singletons and one lamb of each twin pair was sacrificed. The remaining twin lamb was challenged again at 22 h of age for an additional 2 h prior to necropsy. Rectal temperatures were higher for the duration of both cold challenges in lambs from arginine-treated ewes ($P < 0.05$). There was no difference ($P > 0.10$) in BAT weight between treatments. Expression of mRNAs for *PGC1A*, *NRF1*, *NRF2*, *PPARG*, *B3AR*, *ARG2*, *RPS6KA1*, *EIF4EBP1*, and *ODC1* were not affected by treatment ($P > 0.10$) but increased ($P < 0.05$) with age. Concentrations of serine and glycine were higher ($P < 0.05$) in lambs from arginine-treated mothers than controls. Results indicate that maternal arginine treatment increases neonatal thermogenesis after birth. These data are a first step in improving neonatal survival and well-being.

(Supported by USDA-NRI 2009-35206-05211).

Imaging cancer cell surface markers with cystine knot peptides

Richard H. Kimura

Canary Center for Early Cancer Detection, Department of Radiology, School of Medicine, Stanford University, Palo Alto, CA USA

Cyclotides and Knottins are peptides (~3–4 kDa) that have cysteine knot structural motifs at their cores. Many cysteine knots are very stable in serum, and remain intact beyond 24 h. Over the past few years, we have studied several cysteine knots engineered to bind molecular targets of cancer in preclinical mouse models using positron emission tomography. We are currently translating a cystine knot peptide that binds integrin avb6, a biomarker of human pancreatic cancer. The cystine knot motif occurs in peptides found in diverse plants, insects, and marine life. The cystine knot is a stabilizing structure defined 3 disulfide bonds that are intertwined to form a molecular knot, while necessarily forming surface exposed loops. These loops can fit into molecular crevices of proteins such as cell surface receptors overexpressed in cancer. Using yeast surface display technology, rational biocombinatorial library design, and pharmacokinetic engineering methods we have produced highly selective and very sensitive PET radiotracers for target-specific imaging of cell surface cancer markers. The cystine knot PET radiotracers demonstrate high tumor uptake and rapid clearance from non-target tissues, thus resulting in excellent tumor-to-normal tissue contrast. Collectively, the biophysical and in vivo characteristics of cystine knots make them well suited for cancer detection in human subjects.

Regulation of endogenous hydrogen sulfide (H_2S) in the kidney

Jerzy Bełtowski[1*], Grażyna Wójcicka[1], Anna Jamroz-Wiśniewska[2]

[1]Department of Pathophysiology, Medical University, Lublin, Poland; [2]Department of Neurology, Medical University, Lublin, Poland

Hydrogen sulfide (H_2S) is a new endogenous mediator synthesized from L-cysteine by either cystathionine β-synthase (CBS) or cystathionine γ-lyase (CSE) and enzymatically oxidized in mitochondria by sulfide:quinone oxidoreductase (SQR). H_2S regulates renal hemodynamics and tubular transport, and exogenous H_2S has been demonstrated to be either protective or detrimental in various experimental nephropathies. However, the regulation of H_2S level in the kidney was not studied so far. We measured H_2S concentration in renal interstitial fluid obtained from the renal cortex and medulla of anesthetized rats by microdialysis technique using H_2S-specific sensor. We found that although CBS and CSE expression and

activities were higher in the cortex, H_2S concentration was about fivefold greater in the renal medulla and was inversely correlated with medullary pO_2. Infusion of SQR inhibitor, stigmatellin, to the renal artery or transient ischemia induced by renal artery banding increased H_2S concentration. Similarly, H_2S concentration in renal interstitial fluid was higher in rats treated with rosuvastatin which inhibits 3-hydroxy-3-methylglutarylcoenzyme A reductase and decreases coenzyme Q concentration. Infusion of either furosemide or ouabain which inhibit tubular Na^+ reabsorption and reduce O_2 consumption decreased H_2S in the renal medulla. We conclude that: (1) mitochondrial H_2S oxidation plays an important role in the regulation of intrarenal H_2S, (2) H_2S concentration is higher in the renal medulla most likely due to physiological hypoxia in this tissue, (3) nephroprotective effect of statins demonstrated in many studies may be mediated by elevation of H_2S.

N-Ethyl-L-glutamine enhances the synthesis of nitric oxide by endothelial cells

Ying Yang[1,2], Zhenlong Wu[2], Katherine Kelly[3], Cynthia J. Meininger[3], and Guoyao Wu[1-3]

[1]Department of Animal Science, Texas A&M University, College Station, TX 77843, USA;
[2]State Key Laboratory of Animal Nutrition, China Agricultural University, Beijing, China 100193;
[3]Department of Medical Physiology, Texas A&M Health Science Center, College Station, TX, USA 77843

Results of recent studies indicate that enhanced production of glucosamine from glutamine and fructose-6-phosphate in endothelial cells (EC) results in reduced synthesis of nitric oxide (NO) from L-arginine by NO synthase (NOS), leading to impaired relaxation of blood vessels. Glutamine:fructose-6-phosphate amidotransferase (GFAT) is the first and rate-controlling enzyme in glucosamine synthesis. Expression of GFAT is markedly elevated in EC of obese subjects. Studies involving in vitro cell cultures have shown that two known inhibitors of GFAT, namely 6-diazo-5-oxo-L-norleucine and azaserine, can increase NO synthesis by EC in the presence of glutamine and glucose. Although these results are promising, highly toxic effects of 6-diazo-5-oxo-L-norleucine and azaserine (chemically synthesized substances) in vivo limit their usefulness for improving NO generation in humans. We hypothesized that *N*-ethyl-L-glutamine (also known as theanine), an analogue of L-glutamine, may increase NO synthesis by EC. *N*-Ethyl-L-glutamine is an amino acid naturally occurring in certain plants (particularly teas) and has been recognized by the United States Food and Drug Administration as a GRAS (Generally Recognized as Safe) dietary supplement for use in food and beverage products. In our study, bovine venular EC were cultured for 2 days in Dulbecco's modified Eagle's medium containing 0.2 mM L-arginine and 0, 10, 25, 50, 100 or 200 μM *N*-ethyl-L-glutamine. We found that addition of *N*-ethyl-L-glutamine to culture medium increased pentose cycle activity, the intracellular availability of NADH and tetrahydrobiopterin, and NO synthesis in EC, while reducing the production of superoxide anion and lactate by EC, in a dose-dependent manner ($P < 0.05$; $n = 8$). In EC extracts, *N*-ethyl-L-glutamine competitively inhibited ($P < 0.05$) GFAT activity with a K_i value of 62 ± 3.8 μM (mean ± SEM, $n = 8$). We suggest that increasing extracellular concentrations of *N*-ethyl-L-glutamine (e.g., through dietary supplementation) may provide a potentially novel strategy for preventing and treating cardiovascular disease in obese subjects.

Preparation and culture of porcine mammary epithelial cells

Reza Rezaei[1], Sudath Dahanayaka[1], Weston W. Porter[2], Robert C. Burghardt[2], Gregory A. Johnson[2], Yanan Tian[3], Fuller W. Bazer[1], and Guoyao Wu[1]

[1]Departments of Animal Science,
[2]Veterinary Integrative Biosciences, and Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX 77843, USA

Mammary epithelial cells (MEC) are the functional units of the mammary gland during lactation. There are well-established MEC cell lines, such as bovine MAC-T, as well as mouse and human cell lines. Primary mammary epithelial cell lines can be a useful model to understand how metabolism, growth and differentiation of mammary cells are regulated by amino acids. We recently found that dietary supplementation with either branched-chain amino acids or glutamate enhanced milk production by sows; therefore, development of a porcine mammary cell line to elucidate the underlying mechanisms was highly desirable. Porcine mammary epithelial cells (PMEC) were isolated from mammary glands of a 9-month-old nonpregnant and nonlactating female pig through the following sequential steps: (1) placing 10 g minced mammary tissue in 10 ml Ham-F12 medium containing collagenase (10 mg/ml); (2) incubating the tissue on a rocker at 37 °C for 14 h; (3) removing the floating cells and cell debris from the tube; (4) washing the cell pellet (i.e., PMEC) three times with 10 ml wash buffer [DMEM-F12 containing 5 % fetal calf serum (FCS) and antifungal and antibiotics from Invitrogen]; (5) re-suspending the cell pellet in 1 ml of 0.25 % trypsin-1 mM EDTA (prepared in phosphate-buffered saline); (6) washing the cell pellet three times with 5 ml wash buffer; (7) passing the cells in solution through a 70–100 μm filter into a 50-ml conical tube and washing the filter with 10 ml wash buffer to collect the effluent. Cells were then cultured at 37 °C in an atmosphere of 5 % CO_2 in air in 10 ml Ham-F12 medium containing 5 μg/ml insulin, 1 μg/ml hydrocortisone, 5 ng/ml EGF, 50 μg/ml gentamycin, 5 % FCS, and antifungal and antibiotics from Invitrogen. At 70 % confluence, cells were trypsinized, harvested, and stored in liquid nitrogen. Primary PMEC expressed the specific epithelial cell marker cytokeratin-18 but not vimentin, as determined by immunohistochemistry. These cells also expressed the mammary gland-specific gene for beta-casein and synthesized and released the beta-casein protein into culture medium, based on results from RT-PCR and western blot analyses involving porcine beta-casein primer and porcine beta-casein antibody. Growth of PMEC was stimulated by 0.2–2 mM L-leucine, as well as a mixture of 0.2–2 mM L-leucine, 0.2–2 mM valine and 0.2–2 mM L-isoleucine. Compared with the absence of prolactin, addition of 0.2–2 μg/ml prolactin to culture medium for 3 days stimulated production of beta-casein by primary PMEC in a dose-dependent manner. Thus, we successfully developed new primary PMEC for future studies of cellular and molecular regulation of milk synthesis in porcine mammary epithelial cells.

Major urinary proteins are targeted for *N*-homocysteinylation in mice

Jacek Wróblewski[1], Joanna Perła-Kaján[1], Hieronim Jakubowski[1,2,3]

[1]Poznań University of Life Sciences, Poznan, Poland;
[2]Institute of Bioorganic Chemistry, Poznan, Poland,
[3]New Jersey Medical School, Newark, NJ, USA

Major Urinary Proteins (MUPs) play an important role in sexual signaling by binding volatile pheromones and prolonging persistence of urinary scent marks. Female mice tend to avoid senesced, older males that are unable to produce sexual signals. Factors affecting MUPs expression are largely unknown. Here, we examined how elevated homocysteine (Hcy) affects MUPs expression in the mouse. We used cystathionine β-synthase (Cbs)-deficient mouse model, C57BL/6J *Tg-I278T* $Cbs^{-/-}$. MUPs and urinary creatinine levels in $Cbs^{-/-}$ mice ($n = 7$) and $Cbs^{+/+}$ littermates ($n = 5$) were assayed using standard methods. Patterns of MUPs expression were analyzed using SDS-PAGE. Urinary homocysteine (Hcy), Hcy-thiolactone, and *N*-Hcy-protein levels were measured using HPLC-based assays. Site-specific *N*-homocysteinylation of MUPs lysine residues was analyzed using LC–MS/MS. Urinary Hcy, Hcy-thiolactone, and *N*-Hcy-protein levels were extremely elevated in $Cbs^{-/-}$ mice, compared to $Cbs^{+/+}$ animals. $Cbs^{-/-}$ male mice had significantly lower urinary MUP (protein/creatinine ratio 0.38 ± 0.13 in $Cbs^{-/-}$ mice *vs*. 0.93 ± 0.08 in $Cbs^{+/+}$ animals, $p = 0.004$). Patterns of MUPs separated on SDS-PAGE gels differed between $Cbs^{-/-}$ and $Cbs^{+/+}$ mice. LC–MS/MS analysis of tryptic digests of MUPs from male and female $Cbs^{-/-}$ mice identified a MUP11 peptide, ^{95}AG(N-Hcy-K)YSVTYDGFNTF^{108}, containing Hcy *N*-linked to lysine residue K^{97}. We conclude that hyperhomocysteinemia increases *N*-homocysteinylation and decreases expression of MUPs in mice. These findings identify MUPs as a new target for *N*-homocysteinylation in vivo and suggest that hyperhomocysteinemic males are impaired in their ability to produce scent marks, and thus may be less preferred by their female mates.

Effect of increasing methionine supplementation on dietary requirement for taurine in a model insectivore, the Giant Anteater (*Myrmecophaga tridactyla*)

Sally A. Nofs[a,1], Ellen S. Dierenfeld[b], and Robert Backus[c]

[a]Nashville Zoo at Grassmere, 3777 Nolensville Pike, Nashville, TN USA 37211;
[b]University of Missouri, Division of Animal Sciences, 920 East Campus Drive, Columbia, MO USA 65211;
[c]University of Missouri, Department of Veterinary Medicine and Surgery, 900 East Campus Drive, Columbia, MO USA 65211;
[1]Current Address: Baylor College of Medicine, Department of Molecular Virology and Microbiology, Houston, TX 77030, USA

For some species, such as obligate carnivores including the domestic cat, tissue taurine (TAU) needs cannot be met by synthesis from dietary protein or sulfur amino acid (SAA) precursors such as methionine (MET), although those metabolic pathways suffice in other species (i.e. dogs). A dietary need for preformed TAU in giant anteaters (*Myrmecophaga tridactyla*) is suggested due to reported cases of cardiac insufficiency with severe bilateral biventricular dilative cardiomyopathy associated with feeding diets low in taurine relative to diets formulated for cats, a species for which dietary taurine requirement is proven. The study objective was to determine whether canids or felids are a more appropriate physiologic model for TAU metabolism in this insectivorous species. With a non-invasive urine floor-sampling technique, urinary TAU excretion, normalized to creatinine, was evaluated in 3 trials on 4–6 captive adult anteaters fed a diet containing varying levels of added TAU or MET. Excretion of TAU equilibrated with change in dietary taurine within 2 wks, appeared regulated at dietary taurine above 0.21 % from a break-point analysis, and linearly increased with dietary MET increase from 0.64 to 1.04 %. Results indicated that giant anteaters regulate TAU excretion, substantially synthesize TAU from MET, and that canids may be a suitable nutritional model for dietary husbandry recommendations. This study provides baseline information for a unique species, and a novel methodology for pursuing TAU and SAA investigations in other insectivores.

Antibacterial properties of immobilized antimicrobial peptides

C. Z. Cai[1], C. M. Santos[1], Y. Li[1], A. Kumar[1], G. T. Qin[1], A. Lopez[1], S. Kolar[2], and A. M. McDermott[2]

[1]Department of Chemistry and
[2]College of Optometry, University of Houston, Houston, TX 77204, USA

Antimicrobial peptides (AMP) are the first line of defense by most organisms. To study the surface presentation of AMP on their antibacterial properties, we immobilized N_3-EG_{12}-IG-25, a truncated version of human antimicrobial peptide LL-37 with an oligo(ethylene glycol) (OEG) linker terminated with an azido group, onto a variety of alkynyl- or carboxylic acid-terminated solid substrates and liposomes. The immobilization was performed covalently via Cu-catalyzed alkyne-azide cycloaddition ("click" reaction) or amidation, or non-covalently by physisorption, at various densities. The efficacy of these surfaces against *Pseudomonas aeruginosa* (PA01) is strongly dependent upon the surface presentation of the AMP, suggesting a cooperative effect of the immobilized AMP for disrupting the bacterial cell membranes.

Functional characterization of promoter polymorphisms of the human GRIN2B glutamate receptor gene associated with altered memory in older adults

Mingkuan Lin[1], Yang Jiang[2], Gregory Jicha[3,4], Richard Kryscio[4,5], Frederick Schmitt[3,4], Robert H. Lipsky[1,6]

[1]Department of Molecular Neuroscience, the Krasnow Institute for Advanced Study, George Mason University, Fairfax, Virginia, USA;
[2]Department of Behavioral Science,
[3]Department of Neurology,
[4]Sanders-Brown Center on Aging, and
[5]Department of Statistics, University of Kentucky, Lexington, Kentucky, USA;
[6]Department of Neurosciences, Inova Health System, Inova Fairfax Hospital, Falls Church, VA, USA

The *N*-methyl-D-aspartate (NMDA) receptor class of glutamate receptors is critical to activity-dependent changes in synaptic transmission underlying learning and memory. Of particular interest is the GluN2B receptor (encoded by the *GRIN2B* gene) which is highly expressed in the hippocampus and forebrain. Animal studies showed that changes in GluN2B protein levels have profound effects on memory function. However, the influence of genetic polymorphisms affecting expression of the *GRIN2B* gene is not well understood. Our goal is to functionally characterize genetic variants that are predicted to control expression of the human *GRIN2B* gene. Previously, we described one of these DNA sequence variants, a single nucleotide polymorphism (SNP) in an association study that suggested that it may represent a biomarker for mild cognitive impairment (MCI). We

tested the hypothesis that SNPs can modify transcription factor binding to the human *GRIN2B* gene promoter, altering its transcriptional activity. One SNP, which creates an Elk-1 transcription factor binding site, is responsible for allele-specific changes in human *GRIN2B* gene expression under basal and activity-dependent conditions using a firefly luciferase reporter gene assay with transfected cells. For undifferentiated and differentiated human SH-SY5Y cells and differentiated mouse N2A cells, the *GRIN2B* A allele firefly luciferase reporter (the A allele creates an Elk-1 binding site) had 2.2-fold higher levels of basal expression relative to the G allele. Other SNPs in the *GRIN2B* promoter did not produce allele-specific changes in luciferase activity. The expression data were normalized for transfection efficiency using *Renilla* luciferase activity and then corrected for firefly luciferase activity using the same allele-specific expression plasmid but in the opposite orientation. For activity-dependent conditions, differentiated N2A cells were transfected with each of the Elk-1 binding site variants and subsequently treated for 4 h with increasing concentrations of NMDA. Six hours later, luciferase activity was measured. A dose-dependent increase in luciferase activity from 2–5 fold was seen for the A allele. N2A cells transfected with the G allele luciferase reporter plasmid did not respond to NMDA treatment. Taken together, our results are the first functional evidence at the cellular level to support a role for a *GRIN2B* gene variant associated with human memory performance.

Aminotransferases catalyze conversion of organoselenium and organosulfur amino acids to chemopreventive metabolites

Arthur J. L. Cooper, John T. Pinto

Department of Biochemistry and Molecular Biology, New York Medical College, Valhalla, NY, USA

Epidemiological findings show that chalcogen-containing amino acids reduce cancer risks. Aminotransferases catalyze β-elimination reactions and transamination reactions with (a) several cysteine *S*-conjugates [$RSCH_2CH(NH_3^+)CO^-$] including *S*-allyl-L-cysteine (SAC, R=CH_2=$CHCH_2$–), *S*-allylmercapto-L-cysteine (SAMC, R=CH_2 =$CHCH_2S$–), and (b) selenocysteine *Se*-conjugates [$RSeCH_2CH(NH_3^+)CO^-$] including *Se*-methyl-L-selenocysteine (MSC). Cystathionine γ-lyase also catalyzes β-elimination reactions as well as a γ-elimination with selenomethionine (SM). β-Elimination of cysteine *S*-conjugates yields pyruvate, ammonium and RSH (from SAC) or RSSH (from SAMC). β-Elimination from MSC yields pyruvate, ammonium and methylselenol (CH_3SeH). γ-Elimination from SM yields α-ketobutyrate, ammonium and CH_3SeH. CH_3SeH is highly reactive and undergoes intracellular oxidation by glutathione disulfide or with protein-disulfides to dimethyldiselenide or methylseleninic acid. These chalcogen elimination metabolites modify redox-sensitive signal proteins at their cysteinyl domains and affect proliferation and/or apoptotic responses. In the presence of α-keto acid co-substrates, aminotransferases convert chalcogen amino acids into the corresponding sulfo- or selenoketo acids. Sulfoketo acids inhibit DNA methyltransferases and histone deacetylases and show epigenetic control of cancer progression. MSC and SM are transaminated by glutamine aminotransferases to β-methylselenopyruvate (MSP) and α-keto-γ-methylselenobutyrate (KMSB), respectively. Both MSP and KMSB increase *p21* promoter activity and acetylation of histones thereby regulating critical proteins that control cancer cell growth. Allium plants are rich sources of chalcogen amino acids which modulate cancer cells and offer promise as potential for cancer prevention and control.

Beneficial effects of growth hormone on cognitive function

Fred Nyberg

Department of Pharmaceutical Biosciences, Uppsala University, P.O. Box 591, 751 24 Uppsala, Sweden

Studies carried out during the past two decades have demonstrated a profound role of growth hormone (GH) and its mediator insulin-like growth factor-I (IGF-1) on the central nervous system (CNS). GH replacement therapy has been shown to produce several beneficial effects on certain CNS functions, including memory, mental alertness, and working capacity. In GH deficient children significant improvements in many behavioral disabilities related to the CNS have been observed. Also in experimental animals GH and IGF-1 are shown to promote cognitive capabilities. Moreover, studies exploring the mechanisms underlying these effects are shown to involve glutamate transmission through the NMDA receptor complex. Earlier studies have shown that chronic opiates and even alcoholism may inhibit cell growth and trigger apoptosis, which leads to cognitive impairments in both humans and other mammals. We have demonstrated that GH may reverse opiate-induced apoptosis in cells derived from prenatal mouse hippocampus. This presentation describes our recent studies on the ability of GH to counteract cognitive disabilities induced by drugs and other conditions affecting the brain. It also describes a recent study on a patient with opioid-induced cognitive disabilities responding to GH treatment as confirmed by cognitive tests as well as MRI analysis. The possibility of using GH replacement therapy for other CNS-related disorders will be discussed.

Translational molecular imaging with PET using peptide-based probes

Andreas Kjaer

Department of Clinical Physiology, Nuclear Medicine & PET and Cluster for Molecular Imaging, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

The change in paradigm towards individualized, tailored therapy has led to a need for diagnosing at the molecular level. Most of the molecular biology methods used today need tissue sampling for *in vitro* analysis. In contrast, molecular imaging allows for non-invasive studies at the molecular level in living, intact organisms. With PET it is possible to label an array of compounds with radioactive isotopes. This method can be used for non-invasive visualization of tumor specific receptors and tissue characteristics such as ability to metastasize. Especially within cancer biology the technique is expected to lead to a break-through in diagnosing and treatment. Among the different techniques for molecular imaging, the nuclear medicine based technologies have the greatest potential for *translational* use since methods developed in animal models may directly be transferred and used in humans. Furthermore, PET has a high sensitivity and allows for quantification. Labeling of peptides seems particularly promising for development of new PET probes in cancer. We will present examples of development of probes targeting the somatostatin receptor type 2, over-expressed in neuroendocrine tumors as well as probes targeting uPAR for visualization of the invasive phenotype. In addition, our first-in-man studies of ^{64}Cu-DOTATATE will be discussed.

Molecular Imaging Probes Based Upon Bombesin Peptide.

Charles J. Smith[1,2], Prasant K. Nanda[1,2], Lixin Ma[1,2], Tamila Stott Reynolds[3], Timothy J. Hoffman[1,2], Nicole E. Bernskoetter[2], and Zongrun Jiang[1,2]

[1]The Harry S. Truman Memorial Veterans' Hospital, Columbia, MO USA. [2]The University of Missouri School of Medicine, Columbia, MO USA. [3]The University of Missouri School of Veterinary Medicine, Columbia, MO USA

Molecular imaging agents are used to visualize, characterize, and measure specific biological processes that occur at the molecular and cellular levels in humans and other living systems. Agents of this type are oftentimes comprised of small molecules, amino acids, peptides, or larger molecular weight proteins conjugated to a radioligand, nanoparticle, and/or fluorescent/magnetic resonance imaging (MRI) probe. Molecular imaging agents are "chemically tailored" to exhibit very high affinity for cellular biomarkers or cell-surface receptors that tend to be expressed in very high numbers. The procedure is considered to be non-invasive and requires clinical administration of the cell-targeting agent followed by acquisition and quantification of signal by single photon emission computed tomography (SPECT), positron emission tomography (PET), magnetic resonance imaging, fluorescence imaging, or ultrasound. The ability to design novel molecular imaging agents having high affinity for the gastrin releasing peptide receptor (GRPR) has been the central focus of our research investigations for many years. These agents are based upon bombesin (BBN) peptide and exhibit very high-affinity for targeting the GRPR subtype. All of the new agents of which we describe have provided opportunities to tailor receptor-specific uptake, optimize localization in cancerous tissues, and minimize uptake in normal tissues to produce high-quality, high-contrast images (SPECT, PET, MRI, and fluorescence) for early diagnosis and staging of primary and metastatic human prostate cancer.

Radiolabeled Alpha-Melanocyte Stimulating Hormone Peptides for Melanoma Imaging

Yubin Miao[1,2,3]

[1]College of Pharmacy,
[2]Cancer Research and Treatment Center,
[3]Department of Dermatology, University of New Mexico, Albuquerque, NM 87131, USA

Malignant melanoma is the most lethal form of skin cancer with an increasing incidence. It is predicted that 76,690 cases will be newly diagnosed and 9,480 fatalities will occur in the US in 2013. Unfortunately, no curative treatment is available for metastatic melanoma. Early diagnosis followed by prompt surgical removal of primary melanoma lesions are patients' best hope for cures and prolonged survivals. Therefore, it is highly desirable to develop receptor-targeting imaging probes for melanoma detection using single photon emission computed tomography (SPECT) and positron emission tomography (PET). Melanocortin-1 (MC1) receptor is a distinct molecular target for developing melanoma imaging probes due to its over-expression on both human and murine melanoma cells. Meanwhile, alpha-melanocyte stimulating hormone (α-MSH) peptides can bind the MC1 receptors with nanomolar binding affinities. Through peptide-receptor interaction, the α-MSH peptides can serve as effective delivery vehicles to specifically target diagnostic radionuclides to melanoma cells for imaging of melanoma. This presentation will highlight the advance of novel radiolabeled MC1 receptor-targeting α-MSH peptides for melanoma imaging. The novel radiolabeled α-MSH peptides highlighted in this presentation will provide opportunities for early detection of primary and metastatic melanoma.

Target-Specific Molecular Imaging with Cystine Knot Peptides

Kimura RH, and Gambhir SS

Molecular Imaging Program at Stanford and Bio-X Program, Canary Center at Stanford for Cancer Early Detection, University

Cyclotides and Knottins are peptides (~3-4kDa) that have cysteine knot structural motifs at their cores. Many cysteine knots are very stable in serum, and remain intact beyond 24 hrs. Over the past few years, we have studied several cysteine knots engineered to bind molecular targets of cancer in preclinical mouse models using positron emission tomography. We are currently translating a cystine knot peptide that binds integrin avb6, a biomarker of human pancreatic cancer. The cystine knot motif stabilizes many naturally occurring peptides found in plants, insects, and marine life. The cystine knot is a stabilizing structure defined 3 disulfide bonds that wrap around each other to form a molecular knot, while necessarily forming surface exposed loops. These loops can fit into molecular crevices of proteins such as the integrin receptors. Using yeast surface display technology, simple library design, and FACS, we have developed pharmacokinetic engineering methods to produce highly selective and very sensitive PET radiotracers for target-specific imaging of cell surface cancer markers.

Affibody proteins for multimodality molecular imaging and therapy of cancer

Zhen Cheng

Molecular Imaging Program at Stanford and Bio-X Program, Canary Center at Stanford for Cancer Early Detection, Stanford University, 1201 Welch Road, Lucas Center, P095, Stanford, CA 94305-5484

Affibody proteins represent a new type of protein scaffold and have attracted lots of research interests recently, especially in the field of cancer molecular imaging and therapy. Affibodies are based on the Z-domain scaffold derived from one of the IgG-binding domains of staphylococcal protein A. They are an engineered nonimmunoglobulin small protein scaffold with only 58-amino acid residues (~7 kDa), a three-helix bundle structure, and can be either biologically produced or chemically synthesized. The Affibody molecule libraries can be easily constructed by randomization of 13 amino acid residues in helices 1 and 2 of the three-helix bundle protein, and Affibody binders with high affinities and specificities against a variety of desired targets, for examples: the epidermal growth factor receptor (EGFR), human epidermal growth factor receptor type 2 (HER2), human serum albumin (HSA), etc. have thus been quickly identified and selected using phage-display libraries technology and affinity maturation. Many of these Affibody binders have been labeled with a variety of radionuclides, fluorescent dyes and nanoparticles for targeted cancer imaging and therapy in pre-clinical studies. Importantly, an anti-HER2 Affibody labeled with ^{68}Ga or ^{111}In has been successfully and safely used to image breast cancer patients. All these works highlight that Affibodies are a promising new class of cancer targeting agents. In this presentation, we will provide an overview on our recent research progress on developing Affibodies for targeted cancer imaging and therapy.

Expression and functions of osteopontin and integrins in nutrient transport by placentae

Greg A. Johnson[1], Fuller W. Bazer[2] and Guoyao Wu[2]

[1]Department of Veterinary Integrative Biosciences and
[2]Department of Animal Science, Texas A&M University,
College Station TX, 77843

Osteopontin (OPN) and its integrin receptors are major constituents of the uterine-placental interface where they mediate attachment and migration of placental epithelia during implantation and placentation. However, OPN and integrins are highly expressed by many epithelia that do not exhibit these properties, suggesting other roles for secreted OPN. OPN is a non-classical ECM or 'matricellular protein'. It modulates cell function due to its ability to associate with, act as a reservoir for, and alter the function of various bioactive molecules in the pericellular matrix. In the present study, Day 60 chorioallantoic membranes from pigs were placed in Ussing chambers and the transepithelial voltage was measured as an index of ion flux across the placenta. Addition of Day 60 placenta-conditioned medium (PCM) doubled the transepithelial voltage, indicating that a molecule(s) released from the pig placenta increases ion transport across the chorioallantois. We tested the hypothesis that OPN activates ion transporters in pig placenta. Recombinant rat OPN increased ion transport similar to PCM. To identify OPN as the factor in PCM responsible for increasing ion transport, we removed OPN from PCM by immunoprecipitation. OPN-depleted PCM did not stimulate transplacental ion transport. To determine if integrins mediate OPN actions on ion transport, we used recombinant rat OPN with a mutated RGD sequence that does not bind integrins (OPN-RAD). Addition of OPN-RAD ablated the increase in ion transport. Finally, we have evidence that an OPN-based nutrient transport system is present in the pig chorioallantois. We have identified strong β3 integrin protein expression in pig chorion (particularly in areolae), suggesting expression of the αvβ3 receptor for OPN. In addition, we have localized mRNA for the glucose transporters SLC2A1 and SLC2A2, and the arginine transporter SLC7A3 in chorion. We propose a here-to-fore unknown role for OPN and integrins at the uterine-placental interface of pregnancy that is stimulation of ion transport. Our working hypothesis is that OPN is synthesized and secreted from uterine epithelia, binds to integrins on the chorionic epithelium and alters the magnitude of and/or cellular localization of ion transporters and/or the activity of those transporters to drive movement of glucose, amino acids and other nutrients across the chorion to the placental vasculature for transfer to the embryo/fetus. These results provide new insight into why OPN is highly expressed at sites of active nutrient transport in a variety of placentae including the uterine-placental interface of species with epitheliochorial and synepitheliochorial placentae, uterine decidua of rodents, and cytotrophoblasts of human chorionic villi. Further, these results may have broad implications for understanding ion transport across other epithelia known to express abundant OPN including the epithelia of mammary glands, kidney tubules, and intestinal villi in which an understanding of the role(s) of OPN continues to perplex the scientific community.

Application of Survival Analysis Methodology to the Quantitative Analysis of LC-MS Proteomics Data

Carmen D. Tekwe[1], Alan R. Dabney[2], Raymond J. Carroll[2]

[1]Department of Epidemiology and Biostatistics, School of Rural Public Heath, Texas A&M Health Science Center; and
[2]Department of Statistics, Texas A&M University,
College Station, TX, USA, 77843

Protein abundance in quantitative proteomics is often based on observed spectral features derived from LC-MS experiments. LC-MS data frequently have large proportions of missing peak intensities due to censoring mechanisms on low-abundance spectral features. Recognizing that the observed peak intensities detected with the LC-MS method are all positive, skewed and often left-censored, we propose using survival methodology to carry out differential expression analysis of proteins. Various standard statistical techniques including non-parametric tests such as the Kolmogorov-Smirnov and Wilcoxon-Mann-Whitney rank sum tests, as well as the parametric survival model, accelerated failure time model with the Lognormal, Weibull and Loglogistic distributions were used to detect any differentially expressed proteins. The statistical operating characteristics of each method are explored using both real and simulated data set.

The heterogeneous hepatoprotective properties of taurine

Teruo Miyazaki

Joint Research Center, Tokyo Medical University Ibaraki Medical Center, Ami, Japan.

Liver is the organ that biosynthesizes taurine from sulfur-contained amino acid; methionine and cystein, and conjugates it with bile acids. Because of the subcellular structure of liver acinus and heterogeneous distribution of each specific enzyme, many metabolism are different depending on the regions of hepatic lobule. The hepatic lobule is histologically divided into three regions: the periportal (PP) region around the portal vein, the pericentral (PC) region around the central vein, and the intermediate region. In the hepatic lobule, taurine content, biosynthesis ability, and exogeneous transport are the predominant in the PC region than in the PP region. In the depletion models of hepatic taurine level, serious liver damages were observed in the PC region. Many previous studies showed the protective effect of taurine against hepatic damages in the PC region induced by xenobiotcs including Ethanol, acetaminophen, carbon tetrachloride, and thioacetamide. On the other hand, the hepatoprotective effect of taurine against the xenobiotc-induced damages in the PP region has not been confirmed. The xenobiotics that injury the PC region are mainly catabolized by NADPH-dependent cytochrome P450 2E1 (CYP2E1) that is also predominantly expressed in the PC region. In the process of detoxication by the CYP2E1, free radicals occur, and consequently, the free radicals would injury hepatic cells. Taurine seems to act as a free radical scavenger for the CYP2E1-related liver diseases with predominant damages in the PC region.

Taurine and brain excitability

Abdeslem El Idrissi

College of Staten Island, City University of New York, Staten Island, NY 10314

The goal of this study is to characterize neuronal plasticity of the GABAergic system induced by chronic supplementation of taurine. We have shown previously that chronic supplementation of taurine in drinking water induces a state of brain excitability characterized by increased susceptibility to kainic acid (KA)-induced seizures. This reduction in seizure threshold was demonstrated by a decreased

latency for the onset of clonic seizures, an increased incidence and duration of tonic-clonic seizures, increased neuronal death in the CA3 region of the hippocampus and a higher post-seizure mortality rate of the animals. KA causes severe convulsions in mice and has been used as a rodent model for human temporal lobe epilepsy. Using this paradigm of taurine treatment to induced hyper-excitability, we examined changes that occur in the GABAergic system as a possible compensatory mechanism for the increased excitability. We found that taurine-fed mice have elevated brain levels of glutamate and GABA. This increase in neurotransmitter levels was accompanied by an increase in the expression of GABA synthesizing enzyme, glutamic acid decarboxylase (GAD). Furthermore, taurine-fed mice have reduced expression of $GABA_A$ receptors in the hippocampus. It should be noted however, that the excitability induced by taurine is compatible with normal brain function and sub-threshold to induce spontaneous seizures. In this model seizure KA injections are used to induce seizures and the threshold for seizure induction is used to determine the excitability levels. Although taurine-fed mice are hyper-excitable and potentially seizure prone, this state of hyper excitability may is beneficial for brain function. We found that supplementation of taurine to old mice seems to ameliorate the age-dependent decline in cognitive functions.

Neuroprotective role of taurine during aging

Abdeslem El Idrissi

College of Staten Island, City University of New York, Staten Island, NY 10314

Aging of the brain is characterized by several neurochemical modifications involving structural proteins, neurotransmitters, neuropeptides and related receptors. Alterations of neurochemical indices of synaptic function are indicators of age-related impairment of central functions, such as locomotion, memory and sensory performances. Several studies demonstrate that GABA receptors, glutamic acid decarboxylase (GAD 65 & 67), and different subpopulations of GABAergic neurons are markedly decreased in experimental animal brains during aging. Additionally, levels of several neuropeptides co-expressed with GAD decrease during aging. Thus, the age-related decline in cognitive functions could be attributable, at least in part, to decrements in GABA inhibitory neurotransmission. In this study we show that chronic supplementation of taurine to aged mice significantly ameliorated the age-dependent decline in memory acquisition and retention. We could also show that concomitant with the amelioration in cognitive function, taurine caused significant alterations in the GABAergic and somatonergic system. These changes include 1) increased levels of the neurotransmitters GABA and glutamate, 2) increased expression of both isoforms of GAD and the neuropeptide somatostatin, 3) decreased hippocampal expression of the beta (β) 3 subunits of the $GABA_A$ receptor, 4) an increase in the number of somatostatin-positive neurons, 5) an increase in the amplitude and duration of population spikes recorded from CA1 in response to Schaefer collateral stimulation and 6) enhanced paired pulse facilitation in the hippocampus. These specific alterations of the inhibitory system caused by taurine treatment oppose those naturally aging brain, suggesting a protective role of taurine in this process. An increased understanding of age-related neurochemical changes in the GABAergic system will be important in elucidating the underpinnings of the functional changes of aging. Taurine treatment might help forestall the age-related decline in cognitive functions through interaction with the GABAergic system.

Synthesis of 5-trifluoromethyl-L-proline from L-glutamic acid

Stéphanie Ortial,[1,2] Zohra Benfodda,[1,2] David Bénimélis[1,2] and Patrick Meffre[1,2]

[1]Institut des Biomolécules Max Mousseron-UMR 5247 -CNRS-Universités Montpellier 1 et 2, Place E. Bataillon, 34095 Montpellier Cedex 5, France;
[2]UNIVERSITE DE NIMES, Laboratoire de ChimieBioOrganique-LCBO, Site des Carmes, Place Gabriel Péri, 30000 Nîmes, France

The conformation of peptides is known to influence their biological properties. The increased rigidity of the peptidomimetics containing constrained amino acids has been correlated with an enhanced activity toward biological targets. The introduction of cyclic amino acids such as proline into peptides helps controlling the *cis*/*trans* isomerization of the amide bound, leading to a modification of its global conformation. On the other hand, there is a growing interest in fluorinated amino acids because of their various biomedical applications. The presence of fluorine atoms in molecular and supramolecular structures confers them different physical and biological properties compared to the natural ones, increasing protein-ligand interactions. Therefore, our group decided to focus on the preparation of trifluoromethyl proline as a constrained fluorinated amino acid that can be included into peptides to study structure-activity relationships. If a large literature about 2-, 3- or 4-trifluoromethyl prolines exist, the preparation of 5-trifluoromethyl proline has attracted less attention so far. Recently, the synthesis of 5-trifluoromethyl-1,3-oxazolidines as pseudoprolines has been reported and the *cis*-5-trifluoromethyl proline has been synthesized through the reduction of a pyrrole derivative as a mixture of enantiomers. We were then interested to attempt the synthesis of the enantiopure 5-trifluoromethyl-L-proline following a more straightforward and selective strategy. The preparation of both *cis* and *trans* diastereisomers through a 6 to 8-steps linear sequence from L-glutamic acid will be discussed.

Total solid-phase synthesis of Jagaricin, a novel natural macrocyclic lipodepsipeptide from the mushroom pathogen *Janthinobacterium agaricidamnosum*

Chuanguang Qin, Huifang Liu, Bo Lian, Yuanyan Shen

Department of Applied Chemistry, School of Natural and Applied Sciences, Northwestern Polytechnical University, Xi'an, 710072

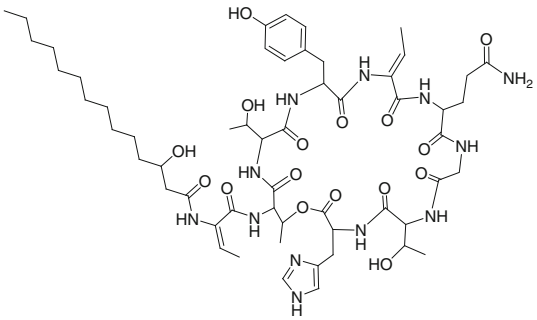


Figure 1. Structure of Jagaricin

The development of combinatorial chemistry and the synthesis of a wide variety of nonpolymeric substrates on resin have greatly increased the interest of researchers in the practical advantages of solid-phase synthesis. Since the pioneering work of Merrifield et al., this technique has become standard for the preparation of certain families of natural oligomers, such as nucleotides, oligosaccharides, and peptides. However, no general solid-phase approach equivalent to that applied to peptides has been established for the preparation of macrocyclic lipodepsipeptides, structurally related classes of compounds that are present in terrestrial and marine sources. Macrocyclic lipodepsipeptides are oligomers that are similar to cyclopeptides but in which some of the amino acids are replaced by hydroxy acids, so that amide and ester bonds are present along the main chain. The most representative examples of macrocyclic lipodepsipeptides such as the fungal antibiotic valinomycin and the families of daptomycins, kahalalides, taumycins, apratoxins, arenastatins, etc are isolated from natural sources. More recently, Hertweck et al. isolated a novel macrocyclic lipodepsipeptide named jagaricin from the mushroom pathogen *Janthinobacterium agaricidamnosum,* and fully elucidated the structure of jagaricin as N-β-hydroxymyristylated (HMA) Dhb-cyclo(Thr-Thr-Tyr-Dhb-Gln-Gly-Thr-His) (Dhb, dehydrobutyrine, see Figure 1). Based on our previous work on total solid-phase synthesis of cyclic peptides and depipeptides, we report here a novel chemical approach to the synthesis of Jagaricin analogues using on-resin head-to-tail cyclization by using Dmab group as a temporary α-COOH protecting group during total solid-phase synthesis with Fmoc chemistry. This synthetic method for the natural products will not only expedite the elucidation of the structure-activity relationships, but also significantly facilitate the optimization of their therapeutic index for containment of microbial resistance.

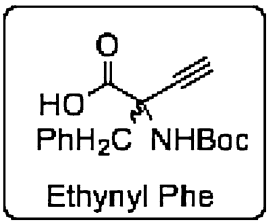
[This work was financially sponsored by the National Natural Science Foundation of China (31001012, 31101304) and the Natural Science Basic Research Plan in Shaanxi Province of China (2012JZ2002)].

Synthesis of acetylenic analogue of phenylalanine

Zohra Benfodda,[1,2] Stéphanie Ortial,[1,2] David Bénimélis,[1,2] Valérie, Rolland,[1] and Patrick Meffre[1,2]

[1]Institut des Biomolécules Max Mousseron-UMR 5247 -CNRS-Universités Montpellier 1 et 2, Place E. Bataillon, 34095 Montpellier Cedex 5, France
[2]UNIVERSITE DE NIMES, Laboratoire de ChimieBioOrganique-LCBO, Site des Carmes, Place Gabriel Péri, 30000 Nîmes

α -alkyl α-amino acids constitute an interesting class of non proteinogenic amino acids and are used to build new peptidic sequences with enhanced properties in various physiologically active peptides and proteins. The additional alkyl substituent could prevent the free rotation of the residue's side chain leading to unique folding when incorporated into peptides. Peptides containing quaternary α-amino acids also tend to have increased hydrophobicity, as well as an increased stability toward both chemical and metabolic decompositions. Unsaturated α-amino acids have turned out to be especially important building blocks for these studies due to the diverse reactivities of the multiple bonds and their ability to introduce biologically active functionalities. β,γ-unsaturated amino acid derivatives have received crucial attention since they are important enzyme inhibitors. α-vinyl glycine is known to inhibit pyridoxal phosphate dependant enzymes in particular decarboxylase. Moreover, α-ethynyl amino acids are described such as potential suicide inhibitors of glutamic acid decarboxylase in particular ethynyl glycine is a well-known natural antibiotic and a suicide substrate for alanine racemase. Given this background, we herein report the synthesis of DL-ethynyl phenylalanine starting from DL-benzylserine in 9 steps.



Proline-centered *cis-trans* isomerization in peptides and proteins, behaviors *in vivo* and *in vitro*

Chunxiao Wang[a,b,*], Wenhui Wu[a,b]

[a]Branch of Marine Biopharmaceutical, College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China; and,
[b]Institute of Marine Sciences, Shanghai Ocean University, Shanghai 201306, China

Proline is unique among 20 protein-forming amino acids in that the amine nitrogen is bound to not one but two alkyl groups, thus making it a secondary amine. It is actually an imino acid. When incorporated into proteins or peptides, it forms a tertiary amide. Despite having a completely aliphatic side chain, proline is usually solvent-exposed, since it is commonly found in beta turns, aiding in their formation. Whereas most peptide bonds overwhelmingly adopt the *trans* isomer, the *cis* and *trans* isomers of the X-Pro peptide bond both experience steric clashes with the neighboring substitution and are nearly equal energetically. Therefore, both isomers coexist, albeit proline residues are exclusively synthesized in the ribosome as *trans* isomer form. *In vivo*, *cis-trans* proline isomerization is such a slow process that it can impede the progress of protein folding by trapping one or more proline residues crucial for folding in the non-native isomer. It is especially true when the native protein requires the *cis* conformation. All organisms possess prolyl isomerase enzymes to catalyze this isomerization. *In vitro*, with prolyl isomerase enzymes not available, peptides and proteins possessing proline residues may behave chromatographically abnormal. We discovered that a double peak may appear despite the fact that the homogeneous state of the sample has been confirmed. For a small peptide, in which a conformational change is much easier, a "slowly exchanging conformational state" may exist, and gives an appearance that the sample can never be purified to a state of chromatographic peak homogeneity, i.e., the double peak keeps appearing. This phenomenon deserves to be noticed in analysis of a peptide or protein sample, no matter it is naturally made, artificially made, or the hydrolysis fragments.

Branched-chain amino acids up-regulate β-defensins expression and affect intestinal immune defence of weaned piglets

Man Ren, Shihai Zhang, Xiangfang Zeng and Shiyan Qiao

State Key Laboratry of Animal Nutrition, China Agricultural University, Beijing 100193, China

Branched-chain amino acids (BCAAs) are necessary to the immune function, but it is unknown about the impact of BCAAs on the ability of intestinal innate immune system. The projective of this study is to

determine whether supplementing of BCAAs in protein restricted diet affects intestinal barrier function and manage post-weaning gut disorders. In the study, three treatments, the control treatment (CON), the negative diet (NE) and the branched-chain amino acids diet (BCAA) were designed to offer to 108 crossbred weaned piglets (7.96 ± 0.26 kg). After 14 d trail period, the plasma amino acids concentration, intestinal morphology, immunoglobulin proteins and defensins of intestine were tested. In results, the plasma urea concentration of pigs was declined significantly in the BCAA group than other two groups (P =0.001), and compared with the CON group, the supplementation of crystalline amino acids significantly increased plasma concentration of these amino acids. A significant lower on villus height (P =0.003) and the villus height:crypt depth ratio (P =0.009) was observed in the duodenum. Pigs offered the BCAA diet had an increased villus height in the duodenum compared with pigs offered the NE diets. Pigs offered different diets had the same epithelial goblet cell number in whole intestine. The intra-epithelial lymphocytes (IELs) number of NE group was observed significantly more than that of CON and BCAA groups in the duodenum (P <0.001), the jejunum (P =0.007) or the ileum (P =0.07). Reducing dietary protein affects intestinal immunoglobulins concentration, that sIgA (P =0.03), IgA (P =0.04) and IgM (P =0.08) in the jejunum or IgA (P =0.01) and IgG (P =0.08) in ileum of the NE group was significant lower than that of CON group. Branched-chain amino acids supplementation significantly improved expression of the immunoglobulins (above mentioned) in jejunum and in ileum. In duodenum and mesenteric lymph node, no significant effect was found on mRNA expression of pBD-2, pBD-114 and pBD-129 relative to either β-actin or GADPH. The NE and CON groups had a similar mRNA expression of all β-defensins in small intestine, however, supplementation of branched-chain amino acids significantly increased (2-10 times) the expression of four β-defensins (pBD-1, pBD-2, pBD-114 and pBD-129) in jejunum and ileum, respectively, compared with the CON and NE group. In conclusion, the supplementation of branched-chain amino acids was necessary in protein restricted diet to maintain the gut growth and branched-chain amino acids improved intestinal immune defense function through the increasing of expression of immunoglobulin and defensins in the jejunum and ileum of weaned piglets.

Effects of arginine and N-carbamylglutamic acid on protein synthesis in skeletal muscle and mRNA levels for intestinal basic amino acid transporters in sheep

Jin-Xia Li, Hai-Zhou Sun, Cun-Fa Zhao, Hong-Li Zhao, Dan Sang, Sheng-li Li, Chun-Hua Zhang

Inner Mongolia Academy of Agriculture and Animal Husbandry, Hohhot, China

It has been shown that, as a functional amino acid, arginine (Arg) may play a role in regulating animal protein synthesis and promoting intestinal health. The objective of this study was to determine effects of dietary supplementation with rumen-protected (RP) arginine and NCG on protein synthesis in skeletal muscle and mRNA levels for intestinal basic amino acid transporter in sheep. Fifteen sheep (body weight = 25 ± 3.39 kg; 5 month-old) were assigned randomly into five groups, representing supplementation with 0 g/d RP Arg or 0 g/d RP-NCG (control group 1), 1.5 g/d RP-Arg (group 2), 2.0 g/d RP-Arg (group 3), 0.15 g/d RP-NCG (group 4), or 0.20 g/d RP-NCG (group 5) to a corn-and soybean meal-based diet. After 45 d period of feeding, the sheep were slaughtered. Sheep were injected with a flooding-dose of L-[ring-D5] phenylalanine, then longissimus dorsi muscle tissue samples were collected at 90 min post-injection to measure fractional protein synthesis rates (FSR) and absolute protein synthesis rates by GC-MS. The duodenum and jejunum tissues samples was selected to analyze mRNA levels for solute carrier (SLC3A1, SLC7A9, SLC3A2 and SLC7A1) by real-time fluorescent quantitative PCR, these SLC encode basic amino acid transporters rBAT, b0,+AT, 4F2hc and CAT-1 respectively. Results indicated that the FSR in the longissimus dorsi muscle of the treatment groups were significantly higher than that of the control group ($P < 0.05$). Group 2 is significantly higher than group 3, group 5 is significantly higher than group 4 (P <0.05). Furthermore, all treatment groups had greater rates of absolute protein synthesis in longissimus dorsi muscle compared with the control group ($P < 0.05$). In addition, the mRNA levels for SLC3A1, SLC7A9, SLC3A2 and SLC7A1 in the duodenum and jejunum of the treatment groups were significantly higher than those in the control group ($P < 0.05$). Sheep in the 1.5 g/d RP-Arg and 0.20 g/d RP-NCG groups exhibited better responses than other treatment groups. Thus, increasing Arg availability in the plasma may enhance growth of young sheep.

The relationship between excitatory amino acid transporter 3 and the growth of small intestine in chick embryos

Xiang-Guang Li · Xiu-qi Wang

College of Animal Science, South China Agricultural University/ Guangdong Provincial Key Laboratory of Agro-Animal Genomics and Molecular Breeding/ Key Laboratory of Chicken Genetics, Breeding and Reproduction, Ministry of Agriculture, Guangzhou 510642, P. R. China

Dietary amino acids, especially glutamate, are the major fuels for the intestinal mucosa. Functional properties of special glutamate transporters, present in the epithelium of the small intestine, strongly influence glutamate absorption capacity. However, the correlation of glutamate transporters and growth of the small intestine has not been well documented. Therefore, studies were conducted to investigate the underlying relationship between excitatory amino acid transporter 3 (EAAT3) and growth of the small intestine in chick embryos. Gene expression of EAAT3 in the small intestine increased during embryo development and was affected by embryonic day. Moreover, the correlation coefficients of EAAT3 gene expression to weights of the small intestine in WENS Yellow Feather Chicken (WYFC) embryos or White Plymouth Rock Chicken (WPRC) embryos were 0.95 and 0.92, respectively. It can be inferred that EAAT3 can be used as an indicator of small-intestine growth. In addition, the effect of *in ovo* feeding L-trans pyrrolidine-2, 4-dicarboxylic acid (PDC), a potent competitive inhibitor of glutamate uptake, on growth of the small intestine was studied. Weights of the small intestine, excluded from other organs, in chicks from the 0.225 mg/egg PDC injection group were decreased on day of hatch (DOH). The gene expression of EAAT3 in the small intestine and the concentration of free glutamate in serum were not changed by PDC injection. The inhibition of small-intestine growth by PDC did not appear to directly depend on EAAT3 gene expression, but maybe on the transport efficiency of glutamate.