SESSION 7 : Intestinal iron transport

Role of HIF-2 in intestinal iron absorption in physiopathological processes

Maria Mastrogiannaki, Pavle Matak, Stéphanie Delga, Sophie Vaulont, Carole Peyssonnaux

Cochin Institute, INSERM U1016, CNRS UMR 8104, Université Paris Descartes, 24 rue du Faubourg Saint Jacques, 75014 PARIS.

Maintenance of iron homeostasis is critical, as iron deficiency can lead to anemia while iron overload is toxic and detrimental to human health. Since humans do not possess a regulated iron excretion pathway, iron homeostasis must be tightly regulated at the level of intestinal absorption. Hypoxia Inducible Factors (HIFs), HIF-1 and HIF-2, are central mediators of cellular adaptation to hypoxia. We hypothesized that the HIF transcription factors, stabilized in the hypoxic epithelial layers of the intestine, could play a central role in maintaining iron balance in the body by regulating iron absorption. Divalent metal transporter 1 (DMT1), duodenal cytochrome b (Dcytb), and ferroportin (FPN1), are the major genes involved in iron uptake and export and have only recently been characterized, but the molecular mechanisms of their basal, iron-dependant or hypoxic regulation are still largely unknown.We generated mice with targeted deletion of *HIF-1*αor*HIF-2*α specifically in the intestinal epithelium including the proximal part of the duodenum, which is the main site of dietary iron uptake. Weshowed a specific regulation of murine *DMT1*, *Dcytb* and *FPN* mRNA levels *in vivo* byHIF-2α, and not by HIF-1α.Diminished *DMT1* expression due to disrupted HIF-2 signaling in the duodenum correlated with a decrease in serum and liver iron. Our findings suggest that HIF-2αis a central local iron sensor and iron absorption regulator in the duodenum.

We next reasoned that HIF−2 could mediate iron hyper−absorption in conditions characterized by pathological iron loading, observed in Hereditary hemochromatosis (HH), a highly prevalent genetic disorder due to hepcidin deficiency. We intercrossed hepcidin knockout mice (which display parenchymal iron overload) with intestinal HIF−2 alpha KO mice. Interestingly, the double knockout present a decreased iron accumulation in the liver and pancreas. Our data suggest that hepcidin deficiency induces HIF−2 alpha stabilization in the duodenum, which in turn contributes to the chronic iron accumulation by up−regulation of iron absorption. This study may point out a role of HIF−2 in the dysregulation of iron absorption and chronic iron accumulation in patients with hemochromatosis and other iron−overload related disorders. Modulation of duodenal HIF−2 alpha protein levels or transcriptional activity may therefore represent an interesting therapeutic/preventive approach for these patients.

Genes Nutr (2012) 6 (Suppl 1):S45–S47
DOI 10.1007/s12263-011-0274-2

Intestinal brush-border Na^+/H^+ exchanger NHE3 is required for iron homeostasis in the mouse

Ali Shawki[1], Robert Kim[1], Sarah R Anthony[1], Patrick B Knight[1], Rusty Baik[1], Emily M Bradford[2], Gary E Shull[2], and Bryan Mackenzie[1]

[1]Molecular & Cellular Physiology, [2]Molecular Genetics, Biochemistry & Microbiology, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0576, USA

Introduction: Divalent metal-ion transporter-1 (DMT1) is critical for intestinal iron absorption. DMT1 is energized by the H^+ electrochemical potential gradient but the provenance of the H^+ required to drive apical iron uptake is not known.

Aim: To assess the roles of gastric acid and the brush-border acidic microclimate in aiding intestinal iron transport.

Methods: We have examined iron homeostasis and intestinal iron handling in mouse models lacking the α-subunit of the gastric H^+/K^+-ATPase (gHKA) or the brush-border Na^+/H^+ exchangers NHE3 and NHE2.

Results: We found that liver nonheme iron stores (a preferred indicator of chronic iron status) in gHKA-null mice were no different from wildtype mice whether fed normal or low-iron diets (6 weeks). In NHE2-null mice liver nonheme iron stores were depleted by 30–80% compared with wildtype mice but no hematological changes were observed. Liver nonheme iron stores were severely depleted in NHE3-null mice, by 70–90% compared with wildtype mice. In preliminary experiments in which we fed mice ^{59}Fe by oral gavage, lower amounts of ^{59}Fe appeared in blood (30 min – 4 h) and in enterocytes, liver and spleen (harvested at 4 h) of NHE3-null mice compared with their wildtype littermates, suggesting that loss of NHE3 results in defective apical intestinal iron uptake.

Conclusions: Our data indicate that intestinal Na^+/H^+ exchangers NHE2 and NHE3—but not the gastric H^+/K^+-ATPase—are required to maintain normal iron stores in the mouse, and raise the possibility that the acidic microclimate is critical for DMT1-mediated iron uptake at the intestinal brush border.

Measuring enteric neuronal activity with multi-electrode arrays

Lisa Würner, Martin Diener

Institute for Veterinary Physiology and Biochemistry, Justus-Liebig-University Giessen, Germany

Introduction: The measurement of neuronal activity with multielectrode arrays (MEAs) is a useful means to investigate excitability in a network of neurons. The neurons can be cultured directly on the MEAs, which are glass chips with e.g. 60 electrodes on their surface. This allows the measurement of action potentials extracellularly and the modulation of them by the application of substances, such as bradykinin. This kinin plays an important role in the genesis of inflammatory bowel diseases. It binds to two receptors, the constitutively expressed receptor B2 and the inducible receptor B1, which is found only under inflammatory conditions.

The aim of this study was to investigate the role of bradykinin and its two receptors in the excitability of myenteric neurons, under healthy and inflamed conditions.

Methods: The MEAs we used were glass chips with 60 titanium nitride electrodes. Myenteric neurons were enzymatically isolated from newborn rats and plated on poly-L-lysin/laminin coated MEAs. After an incubation period of one to three days the action potentials were measured. The addition of bradykinin and bradykinin-receptor agonists and antagonists was made with a pipette. The measurement took place over a period of 12 minutes after the addition of the substance and the frequency as ”spikes per 30 seconds” was calculated.

Results: Bradykinin led to a biphasic increase in the frequency of action potentials. The addition of B1-, as well as B2-receptor-agonists stimulated the neurons, whereas B1- and B2-blockers, when applied individually, resulted in a partial inhibition of the effect of bradykinin. After the combined application of a B1- and a B2-blocker, the stimulation induced by bradykinin was totally abolished. Immunhistochemical staining at different time points during the incubation showed an increasing signal of the B1-receptor with the duration of the culture, whereas the signal of the B2-receptor was stable over time.

In conclusion, the inflammatory mediator bradykinin leads to a biphasic stimulation of myenteric neurons, with the first peak probably due to a stimulation of mechanosensitive neurons. The stimulation is mediated by B1-, as well as B2-receptors. It is likely that the expression of the B1-receptor is driven by the procedure of cell culture. In future experiments the effect of bradykinin on inflamed tissue will be investigated.