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Short Communication

Brain energy metabolism during hypoglycaemia in healthy and Type 1 diabetic subjects

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Abstract

Aims/hypothesis. This study aimed to examine brain energy metabolism during moderate insulin-induced hypoglycaemia in Type 1 diabetic patients and healthy volunteers.
Methods. Type 1 diabetic patients (mean diabetes duration 13±2.5 years; HbA_1c 6.8±0.3%) and matched controls were studied before, during (0–120 min) and after (120–240 min) hypoglycaemic (~3.0 mmol/l) hyperinsulinaemic (1.5 $mU \cdot kg^{-1} \cdot min^{-1}$) clamp tests. Brain energy metabolism was assessed by in vivo ^{31}P nuclear magnetic resonance spectroscopy of the occipital lobe (3 Tesla, 10-cm surface coil).
Results. During hypoglycaemia, the diabetic patients showed blunted endocrine counter-regulation. Throughout the study, the phosphocreatine:γ-ATP ratios were lower in the diabetic patients (baseline: controls 3.08±0.29 vs diabetic patients 2.65±0.43, $p<0.01$; hypoglycaemia: 2.97±0.38 vs 2.60±0.35, $p<0.05$; recovery: 3.01±0.28 vs 2.60±0.35, $p<0.01$). Intracellular pH increased in both groups, being higher in diabetic patients (7.096±0.010 vs. 7.107±0.015, $p<0.04$), whereas intracellular magnesium concentrations decreased in both groups (controls: 377±33 vs 321±39; diabetic patients: 388±47 vs 336±68 μmol/l; $p<0.05$).
Conclusions/interpretation. Despite a lower cerebral phosphocreatine:γ-ATP ratio in Type 1 diabetic patients at baseline, this ratio does not change in control or diabetic patients during modest hypoglycaemia. However, both groups exhibit subtle changes in intracellular pH and intracellular magnesium concentrations.

Keywords Cerebral metabolism · Hypoglycaemia counter-regulation · Insulin antagonists · Near-normoglycaemic insulin therapy · Phosphate compounds · Phosphorus-31 nuclear magnetic resonance spectroscopy · Type 1 diabetes mellitus

Introduction

Although hypoglycaemia is frequently observed in Type 1 diabetic patients, our knowledge of human brain energy metabolism during hypoglycaemia is limited in these patients. Animal studies have revealed that during severe hypoglycaemia, the concentration of key metabolites such as phosphocreatine (PCr) and ATP decrease [1]. In contrast, moderate hypoglycaemia as encountered frequently in Type 1 diabetic patients does not change intracerebral ATP or PCr concentrations, at least in non-diabetic subjects [2]. A short-term decrease of blood glucose down to approximately 1.4 mmol/l does not affect cerebral metabolism of phosphate compounds and intracellular pH

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Abbreviations: $[Mg^{2+}]_i$, intracellular magnesium · NMR, nuclear magnetic resonance · PCr, phosphocreatine · pH_i, intracellular pH · P_i, intracellular inorganic phosphate

(pH_i) [2]. The effect of hypoglycaemia on brain energy metabolism has not yet been reported for Type 1 diabetic patients.

By combining non-invasive in vivo ^{31}P nuclear magnetic resonance (NMR) spectroscopy with hyperinsulinaemic hypoglycaemic clamp tests, the present study aims to examine cerebral energy metabolism during moderate, insulin-induced hypoglycaemia, and to compare the results of Type 1 diabetic patients with those of healthy subjects.

Subjects and methods

Subjects. Male Type 1 diabetic patients (n=6; age 28±2 years; BMI 22.6±0.7 kg/m^2; mean diabetes duration 13±2.5 years) with good metabolic control (HbA_1c 6.8±0.3%), and healthy subjects matched for age and BMI (control; n=6; age 26±1 years; BMI 24.2±0.7 kg/m^2; HbA_1c 5.0±0.1%) were studied. Full written informed consent was obtained prior to each study and the study protocol was approved by the local ethics committee. The Type 1 diabetic patients were undertaking normoglycaemic ("functional") insulin therapy, which relies on the separate substitution of prandial and basal insulin with multiple daily insulin injections. Patients were instructed to avoid hypoglycaemia (blood glucose <3.4 mmol/l) by meticulous adherence to their pre-set algorithms and by avoiding unusual strenuous exercise. By reviewing the blood glucose profiles (at least five measurements per day) it was ensured that the patients were only included if they had no history of severe hypoglycaemic episodes for at least 4 weeks before the study. None of the patients had experienced hypoglycaemic coma.

The diabetic patients were instructed to omit NPH or Zn insulin and to correct their blood glucose with regular insulin for 24 hours before the study. At 00.00 hours on the study day, the patients were admitted to the metabolic ward, and catheters were inserted into antecubital veins of both arms for blood sampling and glucose/insulin infusions. During the night, plasma glucose concentrations were kept between 4.4 and 6.7 mmol/l with a variable intravenous insulin infusion. Healthy subjects were admitted at 08.00 hours on the study day, and catheters were inserted as described above. At 08.45 hours (0 min), a hyperinsulinaemic (1.5 mU·kg^{-1}·min^{-1}) hypoglycaemic (~3.0 mmol/l) clamp test was started. At 10.45 hours (120 min), the insulin infusion was stopped and glucose was infused to increase plasma glucose to above 5.6 mmol/l and to maintain this concentration for the following 120 min. Plasma glucose was monitored every 5 minutes between 0 and 240 min. ^{31}P NMR spectroscopy of the brain was performed before the clamp test (–45 to 0 min), during hypoglycaemia (80 to 120 min) and during the subsequent recovery period (200 to 240 min). Blood samples for measurement of hormones and metabolites were drawn before and after each NMR spectroscopic measurement.

Analytical methods. Plasma glucose concentrations were measured using glucose oxidase (Glucose Analyzer II; Beckman, Fullerton, Calif., USA). HbA_1c, insulin, C-peptide, glucagon, growth hormone, cortisol and epinephrine were quantified as described previously [3].

In vivo 31P NMR spectroscopy. For NMR spectroscopy, patients lay in a 3-Tesla spectrometer (80 cm Medspec-DBX; Bruker Medical, Ettlingen, Germany) in the supine position, with their heads resting on a surface coil 10 cm in diameter. ^{31}P NMR spectra were obtained by a pulse-and-acquire method (repetition time 5 seconds) from the occipital lobe of the brain, since this location allowed comfortable placement of the coil. To localise the signal from the region of interest and suppress the signal coming from superficial tissues and the skull, the flip angle of 250-µs rectangular excitation pulses was set to about 180° in the coil plane. Peak positions and intensities were read manually from the spectra using the software supplied by Bruker. The chemical shift of the peak of intracellular inorganic phosphate (P_i) relative to PCr was used to calculate pH_i, while intracellular magnesium ($[Mg^{2+}]_i$) was calculated from the difference of chemical shifts of the α-ATP and β-ATP peaks [4].

Statistical analysis. All data are presented as means ± SD. Paired t tests or repeated measurement ANOVA followed by post hoc testing (Bonferroni's t test) were used for statistical comparisons within different groups. Statistical comparisons between control subjects and Type 1 diabetic patients were performed using the unpaired Student's t test. Differences were considered statistically significant at a p value of less than 0.05.

Results

At baseline, plasma concentrations of glucagon, cortisol, growth hormone and epinephrine (Table 1) were not different in Type 1 diabetic patients from those in healthy subjects. During the hyperinsulinaemic hypoglycaemic clamp test, plasma glucose decreased to approximately 3.0 mmol/l within 40 min and there was no difference between the groups (mean glucose concentration 30–120 min: control 3.1±0.02 vs diabetic patients 2.9±0.09 mmol/l; NS). While plasma insulin concentrations were higher in diabetic patients at baseline (p<0.01), no difference was observed during and after hypoglycaemia. During hypoglycaemia, the peak concentrations of counter-regulatory hormones were markedly reduced in diabetic patients compared to those in healthy controls (p<0.001 for all hormones).

The ^{31}P NMR spectroscopy revealed a decreased PCr:γ-ATP ratio at baseline (p<0.01; Fig. 1) but normal pH_i and $[Mg^{2+}]_i$ in diabetic patients. During hypoglycaemia, pH_i increased in both groups (p<0.05) and was higher in diabetic patients (p<0.05), while $[Mg^{2+}]_i$ decreased in both groups (p<0.05), there being no difference between the two values. The PCr:γ-ATP ratio remained lower in diabetic patients throughout the experiment (Table 1).

Discussion

We report for the first time the ^{31}P NMR spectroscopy measurement of energy-rich phosphates, pH_i and $[Mg^{2+}]_i$ during hypoglycaemia in the occipital lobe of Type 1 diabetic patients and a matched control group.

Table 1. Plasma concentrations of glucose and counter-regulatory hormones, and intracerebral parameters before, during and after insulin-induced hypoglycaemia in six healthy and six Type 1 diabetic volunteers

		Baseline	Hypoglycaemia	Recovery
Glucose (mmol/l)	C	5.5±0.2	3.1±0.02[b]	5.4±0.2[c]
	D	6.2±0.2[d]	2.9±0.09[b]	6.1±0.2[c,d]
Counter-regulatory hormones				
Insulin (pmol/l)	C	36±16	652±177[b]	45±21[c]
	D	212±191[e]	578±95[b]	60±35.4[c]
Glucagon (ng/l)	C	77±15	158±44[b]	65±10[c]
	D	65±13	63±22[e]	62±13
Cortisol (nmol/l)	C	441±111	662±55[b]	386±111[c]
	D	414±83	442±111[e]	276±83
Growth hormone (µg/l)	C	1.9±2.9	40.9±13.1[b]	7.4±5.1[c]
	D	0.8±0.8	19.1±13.1[b,e]	5.3±6.4[c]
Epinephrine (pg/ml)	C	278±109	5163±3493[b]	284±115[c]
	D	158±76	1512±1544[b,e]	224±115[c]
Intracerebral metabolites (^{31}P NMR spectroscopy)				
PCr:γ-ATP	C	3.08±0.29	2.97±0.38	3.01±0.28
	D	2.65±0.43[e]	2.60±0.35[d]	2.60±0.35[e]
pH_i	C	7.088±0.010	7.096±0.010[a]	7.081±0.016[c]
	D	7.088±0.010	7.107±0.015[b,d]	7.083±0.010[c]
$[Mg^{2+}]_i$ (µmol/l)	C	377±33	321±39[a]	380±62[c]
	D	388±47	336±68[a]	391±51

Data are means ± SD. C, control subjects; D, Type 1 diabetic patients. [a] $p<0.05$ vs basal; [b] $p<0.01$ vs basal; [c] $p<0.01$ vs during hypoglycaemia; [d] $p<0.05$ vs control; [e] $p<0.01$ vs control

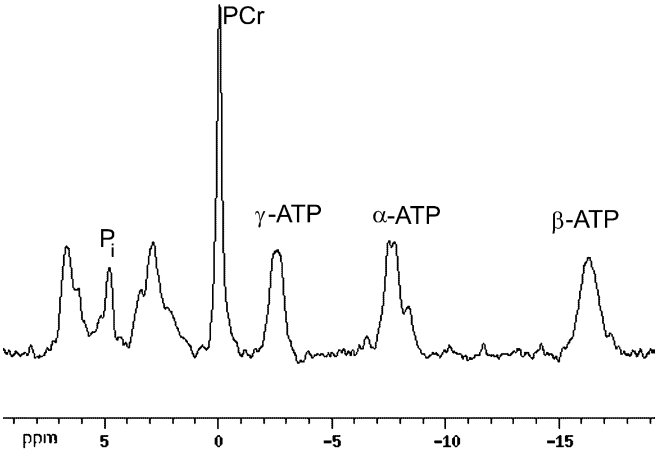

Fig. 1. A typical ^{31}P spectrum measured in the occipital lobe of a volunteer. The signal of phosphocreatine is taken as the chemical shift reference (0 parts per million [ppm]). P_i, intracellular inorganic phosphate

The major finding of our study is a lower PCr:γ-ATP ratio in diabetic patients, which we observed throughout the experiment. Chronically decreased PCr:γ-ATP ratios have been previously found in patients suffering from brain tumours, migraine syndromes and mitochondrial dysfunction [5]. A possible explanation for reduced PCr:γ-ATP ratios is therefore a diabetes-induced reduction of creatine kinase activity [6] or mitochondrial transcription [7]. Previous studies using ^{1}H NMR spectroscopy have revealed a reduced *N*-acetyl:creatine ratio and an increase in myo-inositol and choline metabolites in Type 1 diabetic patients [8]. Taken together, these studies support the contention that brain metabolism could be chronically altered in patients with Type 1 diabetes.

In our study, the PCr:γ-ATP ratio remained unchanged in both healthy volunteers and Type 1 diabetic patients. Our findings are consistent with a previous study reporting no changes in energy-rich phosphate compounds during short-term hypoglycaemia in healthy subjects [2]. PCr serves as a rapidly available energy store, and the PCr:γ-ATP ratio is acutely reduced during metabolic insults such as severe hypoglycaemia or hypoxia [1]. The stable PCr:γ-ATP ratio reported could be due to the relatively mild degree of hypoglycaemia feasible in human experimental research, since animal studies as well as in vitro experiments show that plasma glucose has to be lowered to well below 1.4 mmol/l to disrupt brain energy metabolism and induce a decrease in PCr [1, 9]. However, we did find at least indirect evidence to suggest that subtle changes in energy metabolism take place during hypoglycaemia as outlined below.

Firstly, $[Mg^{2+}]_i$ decreased in both groups. Such a decrease has been previously described in patients with defective mitochondrial respiration or ischaemic brain injury. It was proposed that reduced $[Mg^{2+}]_i$ could be a physiological response to impaired energy metabolism, since the amount of free energy released by ATP hydrolysis is increased and the equilibrium constant of the creatine kinase reaction is reduced [10].

Secondly, we observed an increase in pH_i during hypoglycaemia. While both increased and decreased pH_i have been reported in animal studies, no change in pH_i has been observed in healthy subjects during hypoglycaemia [2]. Performing NMR spectroscopy at 3 Tesla enabled us to obtain spectra with higher resolution and to detect a small but statistically significant increase. With regard to energy metabolism, an increase in pH_i would help to facilitate glycolysis, since protons are powerful inhibitors of phosphofructokinase [11].

The limitation of our study is that non-localised ^{31}P NMR spectroscopy does not allow absolute quantification of metabolites, and therefore inter-individual comparison of the concentrations of phosphorus-containing metabolites is not possible. However, the ratios of metabolites can be compared, and the time course can be followed under dynamic conditions, as done in this study. Thus, we were able to demonstrate alterations of energy-rich phosphate ratios in Type 1 diabetic patients compared with matched controls.

To ensure similar plasma glucose concentrations in the two groups at the beginning of the clamp test, we applied an overnight insulin infusion to the diabetic patients. Therefore, plasma insulin concentrations were higher in these patients at baseline, but similar during and after hypoglycaemia. An acute effect on the observed pattern of energy-rich compounds seems unlikely, since insulin substitution always results in peripheral hyperinsulinaemia, and since brain glucose uptake is independent of plasma insulin concentrations.

In conclusion, we found decreased cerebral PCr:γ-ATP ratios in Type 1 diabetic patients at baseline. Clinical hypoglycaemia did not affect the ratio of these energy-rich phosphates in non-diabetic or diabetic subjects. However, changes observed in pH_i and $[Mg^{2+}]_i$ do suggest subtle changes in energy metabolism.

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References

1. Prichard JW, Alger JR, Behar KL, Petroff OA, Shulman RG (1983) Cerebral metabolic studies in vivo by ^{31}P NMR. Proc Natl Acad Sci USA 80:2748–2751
2. Hilsted J, Jensen KE, Thomsen C, Larsen S, Henriksen O (1998) Maintenence of high-energy brain phosphorous compounds during insulin-induced hypoglycemia in men. Diabetes 37:760–762
3. Bischof MG, Bernroider E, Krssak M et al. (2002) Hepatic glycogen metabolism in type 1 diabetes after long- term near normoglycemia. Diabetes 51:49–54
4. Jelicks LA, Gupta R (1992) ^{31}P-NMR of high energy phosphates in perfused rat heart during metabolic acidosis. Am J Physiol 253:H903–H909
5. Vion-Dury J, Meyerhoff DJ, Cozzone PJ, Weiner MW (1994) What might be the impact on neurology of the analysis of brain metabolism by in vivo magnetic resonance spectroscopy? J Neurol 241:354–371
6. Zhao X, Bassirat M, Zeinab K, Helme RD (1999) Effects of diabetes on creatine kinase activity in streptozotocin-diabetic rats. Chin Med J 112:1028–1031
7. Kristal BS, Koopmans SJ, Jackson CT, Ikeno Y, Park BJ, Yu BP (1997) Oxidant-mediated repression of mitochondrial transcription in diabetic rats. Free Radic Biol Med 22: 813–822
8. Kreis R, Ross BD (1992) Cerebral metabolic disturbances in patients with subacute and chronic diabetes mellitus: detection with proton MR spectroscopy. Radiology 184:123–130
9. Alves PM, Fonseca LL, Peixoto CC, Almeida AC, Carrondo MJT, Santos H (2000) NMR studies on energy metabolism of immobilized primary neurons and astrocytes during hypoxia, ischemia and hypoglycemia. NMR Biomed 13:438–448
10. Lodi R, Iotti S, Cortelli P et al. (2001) Deficient energy metabolism is associated with low free magnesium in the brains of patients with migraine and cluster headache. Brain Res Bull 54:437–441
11. Erecinska M, Deas J, Silver IA (1995) The effect of pH on glycolysis and phosphofructokinase activity in cultured cells and synaptosomes. J Neurochem 65:2765–2772