Differential energetic response of brain vs. skeletal muscle upon glycemic variations in healthy humans

Kerstin M. Oltmanns,1,4 Uwe H. Melchert,2 Harald G. Scholand-Engler,2 Maria C. Howitz,3 Bernd Schultes,1 Ulrich Schweiger,1 Fritz Hohagen,1 Jan Born,1 Achim Peters,3 and Luc Pellerin5

Departments of Psychiatry and Psychotherapy,1 Neuroradiology,2 Internal Medicine 1,3 and Neuroendocrinology,4 University of Luebeck, Luebeck, Germany; and 5Department of Physiology, University of Lausanne, Lausanne, Switzerland

Submitted 9 February 2007; accepted in final form 28 October 2007

Oltmanns KM, Melchert UH, Scholand-Engler HG, Howitz MC, Schultes B, Schweiger U, Hohagen F, Born J, Peters A, Pellerin L. Differential energetic response of brain vs. skeletal muscle upon glycemic variations in healthy humans. Am J Physiol Regul Integr Comp Physiol 294: R12–R16, 2008. First published October 31, 2007; doi:10.1152/ajpregu.00093.2007.—The brain regulates all metabolic processes within the organism, and therefore, its energy supply is preserved even during fasting. However, the underlying mechanism is unknown. Here, it is shown, using 31P-magnetic resonance spectroscopy that during short periods of hypoglycemia and hyperglycemia, the brain can rapidly increase its high-energy phosphate content, whereas there is no change in skeletal muscle. We investigated the key metabolites of high-energy phosphate metabolism as rapidly available energy stores by 31P MRS in brain and skeletal muscle of 17 healthy men. Measurements were performed at baseline and during dextrose or insulin-induced hyperglycemia and hypoglycemia. During hyperglycemia, phosphocreatine (PCr) concentrations increased significantly in the brain (P = 0.013), while there was a similar trend in the hypoglycemic condition (P = 0.055). Skeletal muscle content remained constant in both conditions (P > 0.1). ANOVA analyses comparing changes from baseline to the respective glycemic plateau in brain (up to +15%) vs. muscle (up to −4%) revealed clear divergent effects in both conditions (P < 0.05). These effects were reflected by PCR/Pi ratio (P < 0.05). Total ATP concentrations revealed the observed divergency only during hyperglycemia (P = 0.018). These data suggest that the brain, in contrast to peripheral organs, can activate some specific mechanisms to modulate its energy status during variations in glucose supply. A disturbance of these mechanisms may have far-reaching implications for metabolic dysregulation associated with obesity or diabetes mellitus.

31P magnetic resonance spectroscopy; cerebral energy metabolism; healthy men; adenosine 5’-triphosphate

THE BRAIN IS THE CENTRAL REGULATOR of the organism’s energy homeostasis (4, 14). This regulation is based on neural sensors of afferent inputs signaling from peripheral organs, as well as efferent pathways controlling the function of peripheral organs (15). Because of this superordinate hierarchical position of the brain within the organism, sufficient energetic supply of the brain is of the highest priority. Therefore, the brain provides itself with energy sources, mainly glucose under physiological conditions, from the circulating blood by a mechanism termed “energy on demand” (6). During intense cycling, that is, conditions of highly increased muscular energy demand, brain extraction of glucose is even increased by 55% (2). On the basis of the hierarchically superordinate position of the brain, we hypothesized that under conditions of varying glucose supply, the brain favors adjustment of its own energy content over supply to peripheral organs (11). To test this hypothesis, we compared the energy metabolism in brain and skeletal muscle by in vivo 31P MRS during short periods of dextrose or insulin-induced hyperglycemia and hypoglycemia in healthy lean men. Because bloodborne lactate may contribute to the energy supply of the brain (17), we monitored circulating lactate levels in parallel.

MATERIALS AND METHODS

Subjects. We included 17 healthy and lean Caucasian men (mean age of 25.4 ± 0.8 years) with a body mass index <25 kg/m² (23.7 ± 0.6 kg/m²) in the study. Exclusion criteria were chronic or acute physical and mental illness, alcohol or drug abuse, smoking, competitive sports, exceptional physical or psychological stress (e.g., final exams), and current medication of any kind. The study has been carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association and has been approved by the ethics committee of the University of Luebeck. Each volunteer gave written informed consent.

Study design and procedure. Each subject was examined in two different sessions at basal conditions and during a hyperglycemic (>10.0 mmol/l) or hypoglycemic (<2.2 mmol/l) state induced by dextrose infusion and insulin bolus application, respectively. The participants were instructed to abstain from alcohol, not to perform any kind of exhausting physical activity, and to go to bed no later than 11:00 PM on the day preceding the study. On the study day, subjects reported to the medical research unit after an overnight fast of at least 12 h. A cannula was inserted into a vein on the back of the hand and a second cannula into an antecubital vein of the contralateral arm. Baseline blood samples for determining plasma glucose were collected, and 31P-MRS of the brain (occipital lobe) and skeletal muscle (trapezius muscle) was performed. After the 1-h baseline period, dextrose (20%) was applied by infusion at a rate of 325 ml/h in the hyperglycemic condition. For induction of hypoglycemia an intravenous insulin bolus (H-insulin, Hoechst, Frankfurt, Germany) of 0.1
U/kg was administered. Blood glucose concentration was monitored at 5-min intervals during the entire experimental epoch (B-Glucose-Data-Management, HemoCue GmbH, Grossostheim, Germany). When blood glucose levels reached a level >10.0 mmol/l or <2.2 mmol/l respectively, we performed a second $^{31}$P-MRS of brain and skeletal muscle. Thereafter, dextrose infusion was stopped, and blood glucose normalized after hyperglycemia. At the end of the hypoglycemic session, blood glucose was normalized again by infusion of a 20% dextrose solution. Circulating lactate concentrations were measured at baseline, after having reached the respective target glucose level, and 5 and 10 min thereafter. Plasma lactate was assessed by photometric lactate oxidase method (lactic acid; Abbott D89–20, Abbott Laboratories, Abbott Park, IL) on the Aeroset Clinical Chemistry Analyzer (Abbott).

$^{31}$P-MRS. Each participant lay in a clinically used whole body 1.5-Tesla MRS (Magnetom Symphony, Siemens Medical, Erlangen, Germany) in a supine position, with his head and shoulder, respectively, rested on a transmit/receive surface coil of 8 cm in diameter. Subjects had to relax and keep their eyes open. The order of brain and muscle measurements was randomized across subjects.

Recording of spectra was carried out by 4 dummy excitations to reach a steady state of the magnetization followed by averaging 128 measurements (repetition time 1,500 ms, 1,024 data points, bandwidth 4 kHz). To localize the signal from the volume of interest (VOI) and suppress the signal coming from superficial tissues and the skull, the flip angle of rectangular excitation pulses was set to about 180° in the coil plane. To verify that the VOI was indeed localized over skeletal muscle and brain, we used scout images. Volume selection was accomplished by the limited penetration depth of the used surface coil to about 4 cm. Renouncement of the magnetic field gradients led to a sufficient signal-to-noise ratio in the chosen acquisition time of 3 min and 18 s.

The Magnetic Resonance User Interface was used for evaluation of spectra data (9). Spectral line positions and intensities were calculated using the AMARES (Advanced Method for Accurate, Robust, and Efficient Spectral Fitting) algorithm (20).

We mainly investigated the high-energy phosphate compounds ATP and phosphocreatine (PCr). PCr represents a high-energy reservoir linked to ATP in a bidirectional reaction in which ATP is formed by PCr and vice versa, catalyzed by the creatine-phosphokinase, at a 1:1 (PCr:ATP) molar ratio. The equilibrium for this reaction favors ATP formation so that energy demands in excess of the cells’ capacity for ATP synthesis are met initially through a shift in this equilibrium, whereby ATP concentrations are held constant through PCr hydrolysis. In addition to PCr and ATP, the ratio of PCr/P, is often used as an indicator of intracellular energy status since it does not require a further reference compound (5, 13).

Statistical analysis. Data analysis was performed using Superior Performing Software Systems (SPSS), version 11.5. Values are presented as means ± SE. Statistical analysis was based on paired Student’s t-test and ANOVA for repeated measurements, including the factors “tissue” (brain vs. skeletal muscle) and “time” (representing the basal and hyperglycemic/hypoglycemic conditions). High-energy phosphate concentrations at baseline and during glycemic variations were compared as well as “time by tissue” interaction. Since concentrations in skeletal muscle are generally higher than in brain tissue, values of PCr, PCr/P, ratio, and total ATP were baseline-adjusted by dividing the muscle concentrations by the baseline muscle-to-brain tissue ratio prior to statistical analysis. A P value < 0.05 was considered significant.

RESULTS

Circulating glucose, insulin, and lactate concentrations. After baseline $^{31}$P-MRS measurements at basal glucose levels (4.71 ± 0.12 mmol/l before hyperglycemia and 4.70 ± 0.07 mmol/l in the hypoglycemic session), blood glucose concentrations were increased by a 20% dextrose infusion to a level of 11.3 ± 0.43 mmol/l (Fig. 1A) or rapidly decreased by insulin administration to a level of 2.09 ± 0.11 mmol/l (Fig. 1B). The hyperglycemic or hypoglycemic level (mean 11.3 ± 0.42 mmol/l and 1.98 ± 0.08 mmol/l, respectively) remained for 10 min of a second series of $^{31}$P-MRS measurements. In response to the changes in blood glucose concentrations, serum insulin levels increased from 36.6 ± 4.8 pmol/l at baseline to 154.4 ± 20.3 pmol/l after 20 min of dextrose infusion and from 36.2 ± 5.1 pmol/l to 1,604.7 ± 185.0 pmol/l after bolus insulin application (Fig. 1, A and B, small insets). Plasma lactate concentrations significantly increased in both sessions (from 0.90 ± 0.08 mmol/l at baseline to 1.10 ± 0.1 mmol/l after 30 min of dextrose infusion, P = 0.036; from 0.93 ± 0.09 mmol/l at baseline vs. 1.46 ± 0.33 mmol/l after bolus insulin application, P = 0.001; Fig. 2, A and B).

$^{31}$P-MRS measurements. Absolute values of high-energy phosphate compounds are summarized in Table 1. Hyperglycemia increased PCr content in the brain compared with baseline conditions (P = 0.013; Table 1, top). A similar trend for a rise in PCr content was seen during hypoglycemia (P = 0.055; Table 1, bottom). In skeletal muscle, PCr concentration was on average reduced in both sessions, with this change per
Figure 3 shows alterations in high-energy phosphate concentrations in brain and skeletal muscle during hyperglycemia (Fig. 3A) and hypoglycemia (Fig. 3B) compared with the respective baseline values. Changes are presented as percentages for illustrative reasons.

**DISCUSSION**

We report for the first time that brief periods of hyperglycemia and hypoglycemia induce a differential energetic response in brain and skeletal muscle in healthy humans. An increase in cerebral high-energy phosphate concentrations ranging from 3.7% in total ATP to 14.8% in PCr content upon hyperglycemia was accompanied by a tendency toward a decrease in high-energy phosphate concentrations in peripheral muscle (2.9% in PCr, 3.1% in total ATP, and 3.2% in PCr/Pi ratio). Similar effects were observed during the hypoglycemic intervention displaying a rise in PCr and PCr/Pi ratio in the brain and a trend for a drop in skeletal muscle. Because our subjects were at rest throughout the study, the decrease in muscular high-energy phosphate concentrations cannot be explained by activity-related energy consumption.

Our data are in line with previous results demonstrating that cerebral energy supply rises during hyperglycemia as evaluated by measurements of intracerebral glucose concentrations in mammals (7, 16). However, glucose is only one of the energy substrates used by the brain. It has been shown that elevated blood lactate levels constitute a good fuel source for the brain and can even reduce its glucose utilization under euglycemic conditions (17). During hypoglycemia, there is evidence that

Table 1. **Values of high-energy phosphate metabolites in the hyperglycemic and the hypoglycemic sessions**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Hyperglycemia</th>
<th>P Value (change from baseline)</th>
<th>P Value (time × tissue interaction)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PCr</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>191.7±20.3</td>
<td>218.1±24.0</td>
<td>0.013*</td>
<td>0.005**</td>
</tr>
<tr>
<td>Muscle</td>
<td>1504.1±97.0</td>
<td>1459.7±99.0</td>
<td>0.149</td>
<td></td>
</tr>
<tr>
<td><strong>PCr/Pi</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>1.56±0.1</td>
<td>1.76±0.1</td>
<td>0.047*</td>
<td>0.020*</td>
</tr>
<tr>
<td>Muscle</td>
<td>5.46±0.2</td>
<td>5.29±0.2</td>
<td>0.176</td>
<td></td>
</tr>
<tr>
<td><strong>Total ATP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>653.7±50.7</td>
<td>660.6±57.9</td>
<td>0.096</td>
<td>0.018*</td>
</tr>
<tr>
<td>Muscle</td>
<td>1442.9±72.9</td>
<td>1397.3±76.9</td>
<td>0.192</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as means ± SE of phosphocreatine (PCr), PCr/Pi ratio, and total ATP in muscle and brain during baseline and hyperglycemia are shown. Because values are determined by calculating the area under the spectral peak, no units are indicated. P values refer to paired Student’s t-test comparisons and time × tissue interactions in ANOVA after baseline adjustment.

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Fig. 2. Means ± SE of plasma lactate concentrations during the hyperglycemic (A) and the hypoglycemic (B) sessions. Dextrose infusion or insulin bolus application started at 0 min. Gray areas mark the time period of 31P-magnetic resonance spectroscopy measurements. The increase in plasma lactate was significant in both sessions (P = 0.036 upon hyperglycemia and P = 0.001 upon hypoglycemia).

An ANOVA analysis of the changes from baseline in both organs revealed a highly significant difference in PCr response upon glycemic variations in brain vs. muscle (P = 0.005 and P = 0.033, respectively, for the time by tissue interaction, Table 1). The ratio of PCr/Pi confirmed the results obtained from measurements of PCr (P = 0.020 upon hyperglycemia, P = 0.034 upon hypoglycemia for the time by tissue interaction), revealing a significant increase of PCr/Pi in the brain (P < 0.05 for both conditions) and no significant change in the muscle (P > 0.1 for both conditions, Table 1). In parallel, total ATP concentrations during hyperglycemia tended to increase in the brain (P = 0.096) and to decrease in muscle (P = 0.192, Table 1, top). Although the changes in each organ per se failed to reach significance, ANOVA analysis confirmed the strong divergent response in brain and muscle upon hyperglycemia (P = 0.018 for the interaction effect, Table 1, top), which was observed in PCr and PCr/Pi content. In contrast, there was no change in total ATP concentrations upon hypoglycemia (P > 0.3 for the time effect in both organs and P = 0.393 for the time by tissue interaction; Table 1, bottom).
high-energy phosphates, as observed in our study. This reason-
would explain the concomitant rise in plasma lactate and
placement of glucose by lactate for cerebral energy supply
glucose deficit. A good candidate is bloodborne lactate. Re-
glucose supply is limited under conditions of hypoglycemia,
assumed at least upon hypoglycemia. On the other hand,
this concept, a neuroprotective rise in glucose demand can be
sure to the maintenance of brain energetics (8), while in the case
of hyperglycemia, it is known that glucose conversion into
lactate is stimulated under this condition (3). Concordantly, we
found a significant increase in plasma lactate upon both gly-
cemic variations. On this background, the technique of31P
measurements directly determining components of the
phosphate regulation in brain and skeletal muscle in the long
application, we cannot make a statement about high-energy
phosphate concentrations upon hyperglycemia in humans. However, in our study, we cannot clarify the underlying
mechanism of the increments in cerebral high-energy phos-
phate content upon varying glucose supply without blundering
into speculations. Moreover, the increase in total ATP concen-
tration was not significant during both interventions, and the
significant PCr rise rather serves as a buffer to stabilize ATP
content.

The second important finding of our study was the stable
high-energy phosphate content upon both glycemic conditions
in skeletal muscle. In consequence to the rise in insulin concen-
trations, whether glucose induced or by external insulin
application, one would expect an increased glucose uptake in
muscle and enhanced insulin-induced mitochondrial ATP pro-
duction (18). In this context, one should consider that mito-
chondrial ATP synthesis is not stimulated by insulin in type 2
diabetic subjects or their offspring (12, 18). This explanation,
however, can be ruled out as we excluded diabetic subjects and
those with diabetic parents in their medical history prior to the
study. Notwithstanding, other influencing factors may underlie
the lack in ATP rise such as plasma free fatty acids (FFAs). It
has recently been found that high FFA concentrations reduce
insulin-stimulated muscle ATP synthase flux (1), an effect that
may antagonize any insulin-stimulated increase in ATP syn-
thesis in our study. On the other hand, it is generally accepted
that resting muscle runs mainly on fatty acids and because we
included young normal weight subjects, it appears questionable
why they should display high FFA concentrations. A more
reasonable explanation for the observed stable phosphate me-
tabolite content would be, presuming an increase in muscular
glucose uptake, that some glucose would be oxidized, some
stored as glycogen, and some potentially stored as lipid. All of
these processes require ATP and would therefore potentially
decrease the opportunity to replenish the PCr store. However,
this reasoning is rather speculative at this point, as we did not
measure turnover rates to verify it.

Our study has some potential limitations. Because of our
experimental approach inducing brief periods of hyperglyce-
mia and hypoglycemia by dextrose infusion or insulin bolus
application, we cannot make a statement about high-energy
phosphate regulation in brain and skeletal muscle in the long
term. Further, investigating the course of phosphate changes
after restored blood glucose concentrations would give addi-
tional information and therefore is desirable in future studies.

Overall, our findings demonstrate a differential energetic
response of brain and skeletal muscle upon glycemic variations
in healthy humans. Also, they are compatible with our concept
of an organismic energy allocation, assuming that the brain
prioritizes adjustment of its own ATP concentration indepen-
dent of peripheral regulation (11). A failure in such a mecha-
nism would lead to compensatory activation of hypothalamic
appetite centers and thereby body weight gain, diabetes mellit-
us, and the metabolic syndrome (10). Notwithstanding, be-
cause our in vivo approach does not allow us to unravel the
underlying mechanism of the differential energetic response in
both tissues, this reasoning appears rather speculative at this
point. In any case, our data provide evidence that regulation of
energy homeostasis is more inflexible in skeletal muscle than
in the brain. Mechanistic coherences, regulation of high-energy
phosphates in patients with chronically disturbed glucose me-
tabolism, and clinical relevance of these novel data remain to
be explored in future studies.

ACKNOWLEDGMENTS

We thank Christiane Otten for laboratory assistance and Anja Otterbein for
organizational work. Further, we thank Dr. Lisa Marshall for language advice.

GRANTS

This work was supported by grants from the Deutsche Forschungsgemein-
schaft.

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