Maintenance of High-Energy Brain Phosphorous Compounds During Insulin-Induced Hypoglycemia in Men

^{31}P Nuclear Magnetic Resonance Spectroscopy Study

JANNIK HILSTED, KARL ERIK JENSEN, CARSTEN THOMSEN, STEEN LARSEN, AND OLE HENRIKSEN

^{31}P nuclear magnetic resonance (NMR) spectroscopy allows noninvasive studies of cerebral energy-rich phosphorous compounds in humans. In an attempt to characterize the relationship between peripheral blood glucose concentrations and whole-brain phosphate metabolism during insulin-induced hypoglycemia, ^{31}P NMR spectra were obtained before and after intravenous injection of insulin (0.15 IU/kg body wt) in six men. Compared with prehypoglycemic measurements, no significant changes were found in brain content of P_i, sugar phosphates, phosphocreatine, phosphodiesters, and ATP, and brain pH remained constant during the experiment. These results show that the integrated brain profile of energy-rich phosphorous compounds is unaffected by experimental insulin-induced hypoglycemia in humans. ***Diabetes*** **37:760–62, 1988**

Although peripheral metabolic and cardiovascular events during hypoglycemia are well characterized in humans (1,2), cerebral metabolism and blood-flow regulation are poorly understood. The brain is critically dependent on glucose as an energy source, and during hypoglycemia, glucose uptake in the brain decreases as blood glucose declines because the blood-brain barrier is relatively impermeable to insulin (3). The temporal relationship between blood glucose concentrations and cerebral content of glucose metabolites and energy-rich compounds has not, however, been characterized in humans. Nuclear magnetic resonance (NMR) spectroscopy allows hitherto not available noninvasive measurements of cerebral content of phosphate compounds (sugar phosphates, P_i, phosphocreatine, and ATP) and cerebral pH. The aim of this study was, therefore, to characterize these parameters during insulin-induced hypoglycemia in humans.

From the Department of Magnetic Resonance, Hvidovre Hospital, and Medical Department F, Glostrup Hospital, Copenhagen, Denmark.
Address correspondence and reprint requests to Jannik Hilsted, MD, Medical Department F, Glostrup Hospital, DK-2600 Glostrup, Denmark.
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MATERIALS AND METHODS

Subjects. Six men (mean age 28 ± 2 yr) volunteered for the study. The experiments were approved by the local ethical committee. None of the subjects had a family history of diabetes mellitus, and they took no drugs.

Protocol. The subjects met in the laboratory in the postabsorptive state, having fasted and abstained from tobacco for ≥5 h. A cannula was inserted in a cubital vein of each subject, and another cannula was inserted in the brachial artery of the subject's other arm.

Each subject was positioned supine in the magnet, a Siemens Magnetom 1.5-tesla whole-body scanner, and a Helmholtz coil (.15 cm) was placed on each side of his head. The coil was positioned with the temporal and orbicular muscles outside the sensitive volume. With this position of the coil the sensitive volume included most of the telencephalon.

The Helmholtz coil was tuned to 25.75 MHz, the resonance frequency of ^{31}P. The magnetic field was shimmed, with the proton signal (64 MHz) recorded 400 Hz off resonance. The quality of the shimming was tested by measurement of the line width at the half-maximal water peak in the resulting proton spectrum. This parameter was <0.3 ppm in all subjects. After shimming, the resonance frequency was shifted to phosphorous resonance frequency (25.75 MHz).

Measurements were performed with a partial-saturation recovery pulse sequence (90° – delay – acquisition) repeated 64 times with a repetition time of 6 s and a delay time of 0.5 ms. The scan time was 6.5 min. Two scans were obtained before induction of hypoglycemia.

Hypoglycemia was induced by a bolus injection of 0.15 IU/kg body wt of fast-acting insulin (Actrapid human, Novo, Copenhagen) in the vein catheter.

After the insulin injection, seven spectra were recorded consecutively, each followed by an arterial blood sample drawn from the brachial artery. ECG was recorded via precordial electrodes throughout the experiment.

NMR spectrum analysis. We calculated pH from the chem-

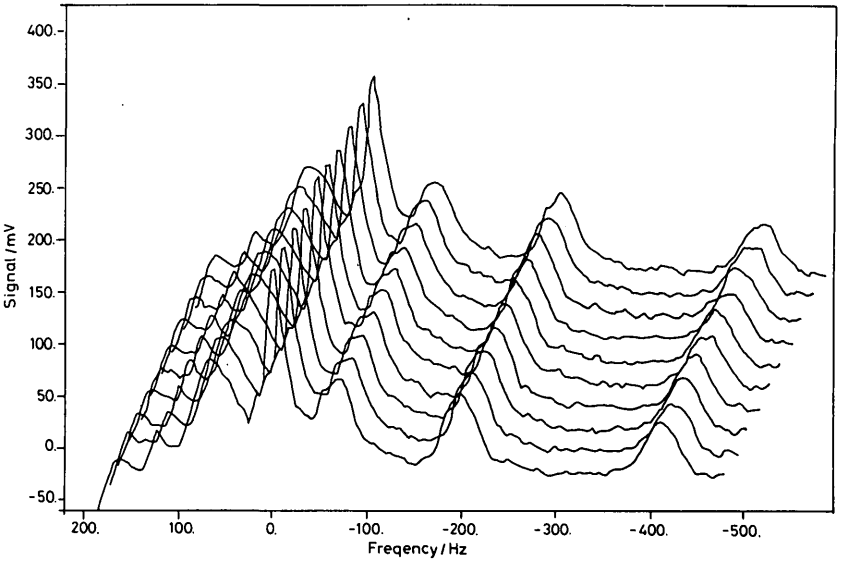

FIG. 1. Brain spectra from volunteer with 15-cm Helmholtz coil placed on each side of head. Each spectrum was obtained in 6.40 min. Lower 2 spectra were obtained before insulin injection (0.15 i.v. IU/kg body wt). Next 8 spectra were obtained 70 min later. Seven phosphorous metabolites were identified (from *left*): sugar phosphates (170 Hz), inorganic phosphates (120 Hz), phosphodiesters (80 Hz), phosphocreatine (0 Hz), and α-, β-, and γ-ATP (−70, −200, and −410 Hz, respectively). The broad underlying peak represents phosphorus in mineral bone and in cell membrane phospholipids.

ical shift between P_i and phosphocreatine (4). The detection limit for pH changes was 0.07 pH units.

Measurements of absolute phosphorous metabolite concentrations are not possible with NMR spectroscopy. The concentrations of the phosphorous metabolites, represented by the seven frequencies resolved in the spectra, were expressed as a percentage of total ^{31}P in the spectra. The broad underlying peak from the mineral phosphorus and membrane phospholipids was not included in these calculations.

During the nine measurements, the measured total ^{31}P signal decreased slightly. A linear regression analysis showed a decrease by 0.65% per measurement, giving a total fall during the nine measurements of ~5% ($r = -.742$, $P < .05$). There was no reason to expect a washout of phosphorus during the measurement, and this slight decrease was probably due to a detuning of the Helmholtz coil because of small head movements. Therefore, each spectrum was normalized with the total ^{31}P found in the spectrum. The differences not explained by the linear fall were ~3% of the total ^{31}P.

The detection limits for relative changes in phosphate compounds in brain tissue were 15% (sugar phosphates), 15% (P_i), 7% (phosphodiesters), 5% (phosphocreatine), and 5% (α-, β-, and γ-ATP).

Statistical analysis. Statistical analyses were made by analysis of variance and linear regression analysis. Level of statistical significance was $P < .05$.

RESULTS

Seven frequencies were resolved in the spectra (Fig. 1): sugar phosphates, P_i, phosphodiesters, phosphocreatine, and α-, β-, and γ-ATP. α-ADP is hidden under the α-ATP peak, whereas the β-ADP is hidden under the γ-ATP peak. $NADP^+$/NADPH is also hidden under the α-ATP peak (5). Underlying the spectra, a broad frequency can be seen (Fig. 1); this peak represents the phosphorus in the skull and in the brain phospholipids (5).

The mean areas under the seven peaks in the nine spectra obtained and mean glucose concentrations are shown in Fig. 2. Glucose concentrations fell during the first 0.5 h to ~1.4 mM and then increased toward the normal range.

All subjects had an increased heart rate during the hypoglycemic period, and all subjects experienced subjective symptoms of hypoglycemia (palpitations, hunger, and sweat).

No changes were found in the seven phosphorous me-

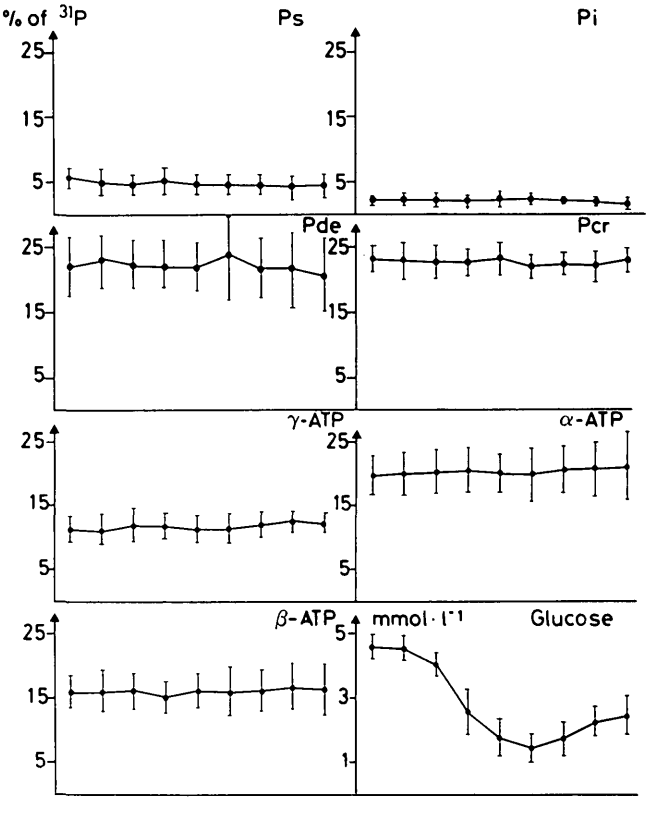

FIG. 2. Concentration of 7 phosphorous metabolites identified in spectra, given in percent of total ^{31}P. Mean concentration of cerebral phosphorous metabolites (Ps, sugar phosphates; P_i, inorganic phosphates; Pde, phosphodiesters; Pcr, phosphocreatine) and blood glucose concentrations in 6 men before and during insulin-induced hypoglycemia are given with 95% confidence limits. Time interval between measurements was ~9 min. Insulin was injected intravenously immediately after 2nd measurement of brain phosphorous metabolites and blood glucose.

tabolites. No correlation was found between glucose concentrations and P_i-to-phosphocreatine ratio or between glucose concentration and sugar phosphates. No change was found in pH during the experiment.

DISCUSSION

With ^{31}P NMR spectrum analysis, decreased cerebral content of phosphocreatine and ATP has been reported during severe, prolonged, insulin-induced hypoglycemia in vitro in the guinea pig (6) and in vivo in the rat (7). Our experiment has shown that during hypoglycemia induced by insulin in a dosage that is acceptable in experimental human research, mean cerebral concentrations of sugar phosphates, P_i, phosphocreatine, phosphodiesters, and ATP are unchanged and cerebral pH is constant compared with prehypoglycemia measurements. The difference between the former results and the outcome of our study is probably due to the fact that the changes in cerebral phosphocreatine and ATP in the animal studies were accompanied by severe irreversible neuroglucopenia.

Our finding of unchanged cerebral phosphate-compound metabolism during hypoglycemia is somewhat surprising in light of previous reports on decreased brain glucose uptake during hypoglycemia at hypoglycemic levels comparable to those induced in our experiment (8). However, cerebral glucose utilization is determined by rate of glucose metabolism rather than by rate of glucose transport, i.e., the rate of glucose transport is two- to threefold higher than the rate of glucose phosphorylation (9,10). Therefore, a transient decrement in brain glucose content as induced in this study does not necessarily induce a detectable decrease in mean cerebral content of sugar phosphates, phosphocreatine, and ATP.

In conclusion, whole-brain phosphate NMR spectroscopy did not reveal any changes in global cerebral phosphate-compound content during hypoglycemia in men. The method, although sensitive and applicable to human research is, therefore, hardly suitable for the characterization of the relationship between central and peripheral metabolic regulation in diabetic patients. Alternative methods of measuring nonphosphorylated compounds or the development of methods for a regional cerebral phosphate spectrum are needed.

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REFERENCES

1. Cryer PE, Gerich JE: Glucose counterregulation, hypoglycemia, and intensive insulin therapy in diabetes mellitus. *N Engl J Med* 313:232–41, 1985
2. Frier BM: Hypoglycemia and diabetes mellitus. *Diabetic Med* 3:215–26, 1986
3. Eisenberg S, Seltzer HS: The cerebral metabolic effects of acutely induced hypoglycemia in normal subjects. *Metabolism* 11:1162–68, 1962
4. Petroff OAC, Prichard JW, Behar KL, Alger JR, den Hollander JA, Schulman RG: Cerebral intracellular pH by ^{31}P nuclear magnetic resonance spectroscopy. *Neurology* 35:781–88, 1985
5. Glonek T, Kopp SJ, Kot E, Pettegrew JW, Harrison WH, Cohen MM: P-31 nuclear magnetic resonance analysis of brain: the perchloric acid extract spectrum. *J Neurochem* 39:1210–19, 1982
6. Cox DWG, Morris PG, Feeney J, Bachelard HS: ^{31}P-n.m.r. studies on cerebral energy metabolism under conditions of hypoglycemia and hypoxia in vitro. *Biochem J* 212:365–70, 1983
7. Prichard JW, Alger JR, Behar KL, Petroff OAC, Shulman RG: Cerebral metabolic studies in vivo by ^{31}P NMR. *Proc Natl Acad Sci USA* 80:2748–51, 1983
8. Lewis LD, Ljunggren B, Norberg K, Siesjö BK: Changes in carbohydrate substrates, amino acids, and ammonia in the brain during insulin-induced hypoglycemia. *J Neurochem* 23:659–71, 1974
9. Lund-Andersen H: Transport of glucose from blood to brain. *Physiol Rev* 59:305–52, 1979
10. Pardridge WM: Brain metabolism: a perspective from the blood-brain barrier. *Physiol Rev* 63:1481–535, 1983