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Cardiac Function, Substrate Utilization, and Myocardial Energy Metabolism Studied with 31-P NMR Spectroscopy during Acute Hypoglycemia and Hyperketonemia

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ABSTRACT. Whether severe hypoglycemia alone or in combination with hyperketonemia might cause deterioration of cardiac function has been controversial. Therefore, the influence of acute hypoglycemia (mean 33 mg/dL) with and without hyperketonemia (mean 1.3 and 3.3 mM) on cardiac function, substrate utilization, and myocardial high energy phosphate levels was studied in 10 mongrel dogs. After 45 min of hypoglycemia, mean aortic pressure, total peripheral resistance, and myocardial oxygen consumption had increased significantly, but other hemodynamic parameters and regional myocardial function had not changed. Additional infusion of 3-hydroxybutyrate did not affect hemodynamic variables significantly. During both metabolic interventions *in vivo* phosphorus-31 nuclear magnetic resonance spectroscopy showed stable levels of myocardial phosphocreatinine, ATP, as well as the phosphocreatinine/ATP (3.0–3.2) ratio. Biochemical measurements revealed that hyperketonemia led to significant alterations in arterial concentrations and arteriocoronary venous differences of selected citric acid cycle intermediates, thus confirming previous reports which suggested a blockade of the 2-oxoglutarate-dehydrogenase reaction induced by ketone body oxidation. However, despite this blockade, the energy supply to the heart was not impaired as shown by normal nuclear magnetic resonance spectroscopy and cardiac performance. It is speculated, that the blockade might be due to an enhanced NADH/NAD ratio. (*Pediatr Res* 26:536–542, 1989)

Abbreviations

a-cv, arteriocoronary venous
CBF, coronary blood flow
HG, hypoglycemia
HK, hyperketonemia
ID, internal diameter
MAP, mean aortic pressure
MPAP, mean pulmonary arterial pressure
MSE, myocardial substrate extraction
MVO_2, myocardial oxygen consumption
NMR, nuclear magnetic resonance
OD, outer diameter
OER, oxygen extraction ratio
PCr, phosphocreatinine
PVR, pulmonary vascular resistence
SV, stroke volume
TPR, total peripheral resistence
%S, systolic segmental shortening

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An association of HG and acute heart failure or cardiac enlargement has been reported in newborns, infants, children, and adults (1–8). The reason for this association is still obscure. Benzing *et al.* (3) have suggested that heart failure leads to HG because of a decreased dietary intake or malabsorption. Reid *et al.* (1) concluded that heart failure is induced by insufficient energy supply during HG (1). A decreased glucose availability should be compensated for by alternate energy substrates such as free fatty acids and ketone bodies. However, ketone bodies seem not to sustain full cardiac work-load (9, 10) because their oxidation is restricted within the citric acid cycle. Therefore, the combination of HG and a high concentration of ketone bodies which occurs in premature and dysmature infants (11, 12) may impair cardiac energy supply and performance, thereby leading to acute heart failure or cardiac enlargement (1–3). This hypothesis was tested by measuring global and regional myocardial function as well as myocardial high energy phosphates in dogs during acute HG and HG in the presence of hyperketonemia.

MATERIALS AND METHODS

Animal preparation. Experiments were performed in 10 mongrel dogs weighing 8–14 kg. After fasting for approximately 4 h, dogs were tranquilized with acepromazine maleate (1.5 mg/kg) 30 min before anesthesia with sodium pentobarbital (initial dose was 30 mg/kg; additional doses of 5 mg/kg were administered when necessary). Mechanical ventilation was adjusted to maintain normal blood gases and acid base balance, and oxygen was supplemented when necessary. Body temperature was maintained by covering and external warming at 36–38°C. ECG was monitored continously. Polyethylene catheters (1.67 mm ID; 2.41 mm OD) were inserted into the right femoral artery and vein. The arterial catheter was placed in the thoracic aorta for blood sampling and measurement of aortic pressure. The venous catheter was used for infusing drugs and fluids with a constant total infusion rate of 8–10 mL/kg/h. To prevent interference with free fatty acid and carbohydrate metabolism, the use of

heparin and solutions supplemented with lactate or glucose was strictly avoided.

A Swan-Ganz thermodilution catheter (Edwards Laboratories, Irvine, CA) was advanced into the pulmonary artery via the left femoral vein for measurement of cardiac output, pulmonary artery pressure, and right atrial pressure. Cardiac output was determined in duplicate (Cardiac Output Computer, Edwards Laboratories, Irvine, CA) and the mean of both measurements was taken. A midline sternotomy was performed and another polyethylene catheter was inserted into the coronary sinus via the right atrium for blood sampling. The correct position of the coronary sinus catheter was confirmed after completion of the experimental protocol. A silicone rubber catheter (1.27 mm ID; 2.28 mm OD) was placed directly into the left atrium for measurement of left atrial pressure. The pericardium was then sutured to the chest wall to cradle the heart. Regional myocardial function was assessed with a sonomicrometer (Triton Technologies, San Diego, CA) by measuring myocardial segmental length. A pair of ultrasonic crystals (5 mHz) was implanted into each dog according to standard techniques (13) in the anterior wall of the left ventricle near the interventricular septum.

In five dogs subject to phosphorus-31 NMR spectroscopy, the Swan-Ganz thermodilution catheter was omitted to avoid disturbance of the homogeneity of the magnetic field. Moreover, a 2.5-cm diameter double-turn circular radio frequency surface coil was sutured to the anterior wall of the left ventricle encircling the two ultrasonic crystals. These piezoelectric crystals and attached wires did not affect phosphorus-31 spectroscopy when disconnected from the sonomicrometer. Therefore, measurements of regional myocardial function and phosphorus-31 spectra were made alternately. Otherwise the preparation was the same as described above. After all preparations the chest was closed with a suture.

Biochemical studies. Arterial and coronary sinus blood samples were drawn simultaneously with heparinized syringes for measurement of oxygen saturation, blood gases, Hb, and metabolic substrates. For determination of lactate, pyruvate, ketone bodies (*i.e.* 3-hydroxybutyrate and acetoacetate) and citric acid cycle intermediates, whole blood was immediately centrifuged. The plasma was then extracted with ice-cold, 0.4 M perchloric acid, and the supernatant was stored at −70°C until it was assayed on the next day with a recently described HPLC method (14). Glucose determinations were performed by the glucose-oxidase method with an automatic analyzer (ASTRA, Beckman, Fullerton, CA). Serum samples for free fatty acid analysis were stored at −10°C and assayed within 10 d by the colorimetric method of Duncombe (15).

NMR studies. For *in vivo* phosphorus-31 NMR measurements, a 23.5-cm horizontal bore, 2.0 Tesla superconducting magnet (Oxford Instruments, Oxford, UK) and a General Electric (Milwaukee, WI) computer were used. The phosphorus-31 resonance frequency of this system was 34.5 mHz. After implantation of the surface coil the dog was wrapped into a plastic bag and positioned with its heart in the center of the magnet. The magnetic homogeneity in the region of the surface coil was optimized by shimming on the proton signal. NMR data were collected over 17 min, using a 4-s pulse delay and a 35-ms pulse width. The spectra obtained during hemodynamic and metabolic steady states were analysed by measuring the peak ht from baseline while maintaining all parameters constant throughout the experiment. Peak intensities were used, because, consistent with signal to noise ratio and spectral overlap, the intensities were found to have better reproducibility than did resonance areas (16).

Experimental protocol. After completion of surgery, the dog was allowed to stabilize for 20 min, then the first control measurement (control) was performed including all hemodynamic parameters and simultaneous blood sampling from the aorta and the coronary sinus. Then, 1 mg/kg propranolol was injected to prevent direct metabolic effects of changes in sympathetic tone, mediated via β-receptors. After 15 min, a second measurement (after propranolol) was made. Thereafter, HG was induced by an injection of 20 U/kg regular insulin; and a continous infusion of 1 U/kg/h insulin and 1.2 mmol/kg/h potassium dissolved in 0.9% saline was started. With this supplementation of potassium the serum concentration was kept constant at a normal level of 3.7 ± 0.3 mM. Blood glucose concentrations were monitored every 10–15 min after insulin injection with chemistrip-bG (Boehringer Mannheim Diagnostics, FRG) until HG was accomplished, defined as glucose levels of 30 mg/dL. Forty-five min after the first glucose determination had shown a value of 30 mg/dL, the third measurement was made. A low level of HK (low HK) was then induced by infusing sodium DL-3-hydroxybutyrate (Sigma Chemical Co., St. Louis, MO) at a rate of 6 mg/kg/min for 10 min and 3 mg/kg/min for additional 35 min. Sodium 3-hydroxybutyrate was dissolved in purified water and adjusted to a pH of 7.40 by adding appropriate amounts of 1 N hydrochloric acid. Fifteen min after starting the infusion of 3-hydroxybutyrate, a constant plasma concentration of ketone bodies was established and 30 min later the fourth measurement (HG + low HK) was performed. The infusion rate of 3-hydroxybutyrate was then increased to 6 mg/kg/min to obtain a higher level of HK (high HK). This was accomplished after another 30 min and a measurement (HG + high HK) was made 15 min later. At the last step of our protocol the dog was allowed to recover from HG and HK by ending both infusions and starting a 20% dextrose infusion with a rate of 1 to 1.5 mL/min. After 60 min the last measurement (recovery) was performed. NMR data acquisition were started after the hemodynamic and metabolic measurements had been completed leading to a 17 min prolongation of each step of the protocol.

Data acquisition and calculations. Data were recorded on a 8 channel recorder (Gould, Cleveland, OH) or on a Mingograf 91 (Elma-Schoenander, Stockholm, Sweden), and digitized manually. MAP and MPAP were either electronically derived from the pressure signal or calculated. SV, TPR, and PVR were calculated according to standard equations. %S was defined as the difference between end-systolic and end-diastolic segmental length, expressed as percentage of the end-diastolic segmental length. MVO was calculated from the formula MVO (mL O/100 g·min) = 0.00072 × systolic aortic pressure × heart rate + 1.43 (17). Blood oxygen content was calculated as O (mL/dL) = saturation × Hb × 1.36; and carbon dioxide content (mL/dL) was determined from the pCO using the Hendersson-Hasselbalch equation (18, 19) and a factor converting plasma CO content into blood CO content (20). CBF was estimated (est. CBF) from the formula est. CBF (mL/100 g·min) = MVO/a-cv oxygen difference. The myocardial respiratory quotient, *i.e.* the a-cv CO difference divided by the a-cv O difference, was also calculated. MSE was defined as the a-cv substrate difference divided by the corresponding arterial concentration. The percent contribution of different substrates to the aerobic metabolism of the myocardium was estimated by the OER, *i.e.* oxygen equivalents of a substrate divided by the a-cv O difference (21). With this ratio, the most important myocardial fuels at each experimental condition can be readily recognized. The following oxygen equivalents were used: 3 mol O/mol lactate, 2.5 mol O/mol pyruvate, 4.5 mol O/mol ketone bodies, 6 mol O/mol glucose, and 23 mol O/mol free fatty acids. Mitochondrial respiration was assessed by PCr/ATP (22–24).

Statistics. Statistical analysis was performed by using the BMDP statistical software, installed on a Siemens 7.580 mainframe. Mean values are given with the SEM. Differences between arterial and coronary venous substrate concentrations at each step of the protocol were statistically verified by paired *t* tests. A regression analysis was performed on arterial concentrations of selected citric acid cycle intermediates *versus* ketone body concentration. Variations between the six steps of the experimental protocol were statistically tested by an *a priori* analysis, the method of orthogonal contrasts (25). In advance of collecting the