Ischemia in normoglycemic and hyperglycemic rats: plasma energy substrates and hormones

JOHAN LUNDGREN, ANKE MANS, AND BO K. SIESJÖ

Laboratory for Experimental Brain Research, University of Lund, and Department of Paediatrics, University Hospital, S-221 85 Lund, Sweden

LUNDGREN, JOHAN, ANKE MANS, AND BO K. SIESJÖ. Ischemia in normoglycemic and hyperglycemic rats: plasma energy substrates and hormones. Am. J. Physiol. 258 (Endocrinol. Metab. 21): E767–E774, 1990.—Seizures are a documented complication to cerebral ischemia. After 10 min of forebrain ischemia in rats, preischemic hyperglycemia invariably leads to severe, most often fatal epileptic attacks. This outcome is related to the exaggerated lactic acidosis, which has been suggested as a possible contributor to severe membrane changes and widespread edema. To find out if circulating hormones or plasma energy substrates modulate this additive damage caused by the hyperglycemia, plasma concentrations of of corticosterone, epinephrine, norepinephrine, dopamine, glucagon, insulin, glucose, free fatty acids (FFA), 3-hydroxybutyrate, and acetoacetate were measured before and in the early recirculation period after 15 min of forebrain ischemia in the rat. Plasma corticosterone levels did not differ between the normo- and hyperglycemic groups. Although not significantly different from control, the catecholamine levels showed a tendency to be higher in the hyperglycemic groups. Therefore, because catecholamines have been reported to have a protective effect during ischemia the present result cannot explain why hyperglycemia aggravates the ischemic damage. Insulin levels seemed to increase during ischemia but not significantly. Levels quickly returned to normal after 30 min of recirculation. FFA concentrations were reduced after the induction of ischemia and appeared lower in all hyperglycemic groups. The level of one of the ketone bodies, 3-hydroxybutyrate, showed a significant decrease in hyperglycemic ischemia in all groups compared with normoglycemic ischemia. The same tendency was seen for acetoacetate. Results are compatible with a protective role of ketone bodies in ischemia. It is concluded that among the hormones and substrates studied only the ketone body concentrations qualify as a modulator of the exaggerated brain damage after ischemia in hyperglycemic subjects.

SEIZURES CONSTITUTE a well-documented complication of cerebral ischemia (for experimental verification, see Refs. 26 and 31). In experimental models the incidence of postischemic seizures dramatically increases when the preischemic nutritional state of the animal is manipulated to yield higher plasma glucose concentrations and thereby increased cerebral availability of glucose (26, 28). Hyperglycemic compared with normoglycemic animals also show aggravation of the tissue damage. There is a general increase in the number of affected neurons, widespread edema, a different distribution of the damage with a unique lesion of the substantia nigra (14), and an alteration in the evolution of the damage (33).

The worsening of the outcome after ischemia in hyperglycemic subjects is usually assumed to be related to the accumulation of lactate plus H+, using an ensuing decrease in intra- and extracellular pH (see Ref. 32). It is then postulated that somehow this exaggerated acidosis gives rise to membrane changes that cause subsequent edema and seizures. However, the possibility that changes in circulating hormones or plasma substrate levels could play a modulating role has never been excluded. For example, Sapolsky and Pulsinelli (30) found that the final damage incurred after transient ischemia was directly related to the postischemic plasma corticosterone level. Other results suggest that the final damage is inversely correlated to plasma catecholamine levels (18). The importance of tissue catecholamine levels was underscored by results showing that in starved animals inhibition of catecholamine synthesis by methyl-O-tyrosine aggravated ischemic brain damage in much the same way as hyperglycemia does (35). Finally, it appears established that plasma ketone body concentrations influence brain damage in hypoxia/ischemia (22), and the possibility cannot be excluded that changes in insulin and glucagon concentrations have an influence as well.

Because stress influences plasma levels of hormones and substrates and changes in such levels interact, ischemia must be suspected to cause a cascade of changes in the substrate-hormonal state. The aim of this study was to find out how alterations in the preischemic nutritional state, notably an increase of plasma glucose concentration to values >20 μmol/ml, affect substrate and hormonal levels in plasma in animals subjected to 15 min of reversible forebrain ischemia.

METHOIDS

Animals. All experiments were performed on 3-mo-old adult male Wistar rats (285–390 g) of a specific pathogen-free strain (Møllegaard’s Avlslaboratorium, Copenhagen, Denmark). To allow adjustment to the environment the animals were housed in groups of three in macrolon cages with wood shavings for 11–15 days. Air temperature was 20 ± 2°C, and humidity was ~35%. The animals were exposed to a diurnal light-dark cycle with lights on between 0600 h and 1800 h. Food (EWOS-ALAB, R3; Ewos Sverige, Södertälje, Sweden) and water were provided in excess. Before the experiments the animals were fasted overnight, housed alone in a grid stainless steel...
cage with water ad libitum, and then gently transported in a small macrolon cage the short distance from the animals room to the operating room.

**Groups.** Animals were divided into 10 groups (see Table 2) with four animals in each group except one group, which had five animals. Each animal was used for only one blood sampling. There were six normoglycemic and four hyperglycemic groups. In both series samples were collected before ischemia, i.e., at the end of the steady-state period (normo pre, hyper pre), at the end of the ischemic period, (i.e., before recirculation normo pre, hyper 0), after 30 min of recirculation (normo 30, hyper 30), and after 120 min of recirculation (normo 120, hyper 120). The two additional normoglycemic groups were sham operated, and plasma was sampled 30 and 120 min after the discontinuation of anesthesia (sham 30, sham 120). For ethical reasons we deemed it impossible to study a sham-operated group that would match the post-ischemic ones, primarily because this would have implied discontinuation of anesthesia. This does not seriously affect any conclusions drawn from the present results, which primarily concern differences between normo- and hyperglycemic animals.

**Operative and sampling techniques.** Anesthesia was induced with 3.5% isoflurane (Forene, Abbott) and 70% N₂O in O₂, and intubation was performed with a polyethylene tubing (ID 1.67mm, OD 2.42mm, Portex) with the help of transillumination of the neck with a fiber optic apparatus. Ventilation was continued with a respirator (7525 Rodent Ventilator; Ugo Basile, Italy) with 1.5–2.0% isoflurane. A neck incision gave access to the external jugular vein. A silicone catheter (Silastic; ID 0.076 mm, OD 0.165 mm) was inserted ~5 cm to the level of the right atrium. Common carotid arteries were isolated, vagal and sympathetic nerves following the vessels were identified and separated, and a string was placed around each artery. A tail arterial catheter (polyethylene tubing, ID 0.58 mm, OD 0.96 mm; Portex) was inserted for blood pressure measurement (Elema-Schöndner; EMT 34, blood pressure transducer), analysis of blood gases (L. Eschweiler, Kiel, FRG), pH (Radiometer; Copenhagen, Denmark), plasma glucose (Beckman Glucose Analyzer 2; Beckman Instruments, Fullerton, CA), and sampling of plasma for measurements of substrates and hormones. Needle electrodes for electroencephalogram (EEG) recording were inserted below the muscles lateral to the skull bone, a temperature probe was placed through a small skin incision on the head just outside the bone, and another temperature probe was inserted in the rectum. After the surgical procedures were completed the isoflurane in the inspired air was decreased to 1.0–1.5% to achieve a well anesthetized animal with continuous EEG activity. Animals were allowed a steady-state period of 30 min. During the first 5 min the hyperglycemic groups were given a glucose load by infusion of a 25% solution (0.92 g/kg body wt) followed by a slower infusion (0.039 g/kg body wt⁻¹.min⁻¹). Normoglycemic groups were given an identical volume of saline solution (3.7 ml/kg during the 5 min of load and 3.9 ml/kg during the following 25 min of the steady-state period). The temperature of the skull and rectum was kept between 37.0 and 37.5°C with a heating table and lamp throughout the steady-state period and during ischemia. Blood gases were kept in the normal range with partial pressure of O₂ (PO₂) 95–125 mmHg and partial pressure of CO₂ (PCO₂) 35–40 mmHg; pH was 7.35–7.40. Fifty international units of heparin (Kabi Vitrum, Sweden) was given before the first blood gas sampling. If the physiological parameters after the steady-state period not were in the desired ranges, the steady-state period was prolonged ~5–15 min. Intravenous infusion of saline or 25% glucose was then continued during this prolonged period.

After the steady-state period, when all physiological parameters were in the desired ranges, the glucose infusion was discontinued (~1 min), the patency of the jugular catheter was controlled, and isoflurane administration was discontinued (~30 s to ~45 s). At this time samples for the preischemic groups (normo pre, hyper pre) were collected. In the ischemic groups induction of ischemia was started with central venous exsanguination. Blood pressure fell rapidly, and when it was <50 mmHg, bilateral carotid clamping was performed. With additional withdrawal or reinfusion of blood blood pressure was maintained ~50 mmHg (40–60 mmHg). When EEG showed an isoelectric recording, the timer was started. After the induction of ischemia the rat was again placed in a prone position. Depth of ischemia was verified by the absence of EEG activity. After 15 min of ischemic brain circulation was restored by removal of the carotid clamps and by reinfusion of shed blood. Systemic acidosis was counteracted with iv injection of 0.5 ml of 0.6 M sodium bicarbonate. The caval catheter was quickly removed, and the neck incision was closed by sutures. After ~20 min of recirculation the rat was extubated and transferred to a Perspex cage (4). The cage was covered with aluminum foil and slowly flushed with room air. The tail artery catheter was heparinized.

In the additional normoglycemic sham-operated groups (sham 30, sham 120) isoflurane administration was discontinued after the steady-state period. The caval catheter was quickly removed, the neck incision was closed, and the animal was gently and quickly moved to a Perspex cage as described for the ischemic groups.

**Blood sampling and treatment.** Blood was sampled from the tail artery by spontaneous bleeding. The sampling procedure did not seem to induce any additional stress causing changes in hormonal levels. This was proven in a preliminary experiment by analyzing samples of catecholamines from two different rats after the minor surgical trauma of inserting an arterial tail catheter. The total sample of 3 ml, analyzed for catecholamines, was divided into three consecutive portions. Both rats were used twice. After the first sampling the rat was retransfused with fresh-frozen plasma. Results showed that norepinephrine and epinephrine levels were similar in the consecutive samples and in the first and second batches (Table 1).

In the main experiment a sample of 3 ml of blood was withdrawn for measurements from each rat. The first 0.5 ml was added to a tube on ice containing 1 µl of aprotinin (Sigma) solution (0.03 mg in distilled water) and mixed. Then 2.5 ml were withdrawn into a separate tube on ice.
Both tubes were immediately centrifuged for 10 min at 4°C, 10,000 g. Plasma was pipetted into separate plastic tubes for the different assays, and all tubes were stored at −70°C until analysis.

**Results.** Physiological variables and plasma glucose concentrations are given in Table 2. Body weight was similar in normoglycemic and hyperglycemic groups. Temperature at the skull and in the rectum, and the PO_{2} and PCO_{2} before and after ischemia were not different between the groups compared. Plasma pH differed somewhat between hyper- and normoglycemic animals at the end of ischemia, i.e., between hyper 0 and normo 0 groups. Blood glucose in the hyperglycemic groups was 20.4 μmol/ml 5 min before ischemia and 14.7 μmol/ml 10 min after ischemia. In normoglycemic groups, the corresponding values were 7.2 μmol/ml and 5.9 μmol/ml, respectively. Blood pressure fluctuations were seen in many rats in all groups during the steady-state period and pressure occasionally fell to values as low as 40 mmHg. However, repeated EEG recordings during these periods always showed a continuous normal recording. When isoflurane was discontinued, blood pressure always increased. An increase in pressure was usually seen after clamping of the carotid arteries.

**Glucose** (Fig. 1A). In the normoglycemic animals ischemia failed to raise the plasma glucose concentration (normo 0); if anything, values were reduced. After 30

**Table 1. Catecholamine levels during spontaneous bleeding from arterial catheter.**

<table>
<thead>
<tr>
<th></th>
<th>Norepinephrine, pmol/ml plasma</th>
<th>Epinephrine, pmol/ml plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st Sample</td>
<td>2nd Sample</td>
</tr>
<tr>
<td><strong>Rat 1</strong></td>
<td>4.10</td>
<td>4.04</td>
</tr>
<tr>
<td></td>
<td>4.82</td>
<td>3.67</td>
</tr>
<tr>
<td><strong>Rat 2</strong></td>
<td>3.16</td>
<td>3.54</td>
</tr>
<tr>
<td></td>
<td>3.13</td>
<td></td>
</tr>
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<td></td>
<td>3.38</td>
<td></td>
</tr>
</tbody>
</table>

Three consecutive milliliters of blood were taken during sampling events. See Methods for further details.

**Table 2. Physiological variables measured before and after normo- and hyperglycemic cerebral ischemia.**

<table>
<thead>
<tr>
<th></th>
<th>Weight (g)</th>
<th>Temp, °C</th>
<th>Glucose, μmol/ml</th>
<th>Blood Pressure, mmHg</th>
<th>PaO_{2}, mmHg</th>
<th>PaCO_{2}, mmHg</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Head</td>
<td>Rectum</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Sham 30</td>
<td>331±9</td>
<td>36.9±0.3</td>
<td>37.0±0.2</td>
<td>8.3±0.7</td>
<td>85±11</td>
<td>35.7±1.2</td>
<td>106±7</td>
</tr>
<tr>
<td>Sham 120</td>
<td>344±14</td>
<td>37.4±0.3</td>
<td>37.1±0.3</td>
<td>6.7±0.3</td>
<td>110±13</td>
<td>35.5±1.0</td>
<td>117±5</td>
</tr>
<tr>
<td>Normo pre</td>
<td>326±10</td>
<td>37.2±0.5</td>
<td>37.2±0.5</td>
<td>7.6±0.8</td>
<td>96±8</td>
<td>35.5±1.0</td>
<td>100±5</td>
</tr>
<tr>
<td>Normo 0</td>
<td>333±11</td>
<td>37.5±0.1</td>
<td>37.4±0.3</td>
<td>7.6±0.5</td>
<td>123±8</td>
<td>35.7±1.3</td>
<td>112±4</td>
</tr>
<tr>
<td>Normo 30</td>
<td>347±17</td>
<td>37.3±0.1</td>
<td>37.3±0.1</td>
<td>7.9±0.6</td>
<td>97±9</td>
<td>36.9±1.8</td>
<td>108±3</td>
</tr>
<tr>
<td>Normo 120</td>
<td>315±12</td>
<td>37.5±0.3</td>
<td>37.3±0.1</td>
<td>6.3±0.9</td>
<td>110±8</td>
<td>36.0±0.7</td>
<td>114±1</td>
</tr>
<tr>
<td>Hyper pre</td>
<td>333±6</td>
<td>37.5±0.3</td>
<td>37.2±0.3</td>
<td>20.7±1.0</td>
<td>100±20</td>
<td>37.5±1.5</td>
<td>111±5</td>
</tr>
<tr>
<td>Hyper 0</td>
<td>363±14</td>
<td>37.3±0.1</td>
<td>37.4±0.1</td>
<td>19.7±1.1</td>
<td>112±8</td>
<td>38.8±0.8</td>
<td>116±2</td>
</tr>
<tr>
<td>Hyper 30</td>
<td>333±18</td>
<td>37.3±0.1</td>
<td>37.2±0.2</td>
<td>20.3±1.6</td>
<td>132±5*</td>
<td>35.5±1.2</td>
<td>114±1</td>
</tr>
<tr>
<td>Hyper 120</td>
<td>340±9</td>
<td>37.4±0.3</td>
<td>37.9±0.3</td>
<td>20.9±1.4</td>
<td>138±7</td>
<td>35.5±0.9</td>
<td>113±2</td>
</tr>
<tr>
<td>All</td>
<td>330±4</td>
<td>37.3±0.1</td>
<td>37.3±0.1</td>
<td>13.2±1.8</td>
<td>106±7</td>
<td>36.2±0.4</td>
<td>112±1</td>
</tr>
</tbody>
</table>

Values are means ± SE, n = 4 rats except normo pre, n = 5. See Methods for definitions of groups. **PaO_{2}, arterial PO_{2}; PaCO_{2}, arterial PCO_{2}.** Temperature was registered 5 min before ischemia. Glucose and blood gases were measured 5 min before and 10 min after ischemia. Mean blood pressure was recorded when isoflurane administration was discontinued, just before ischemia, and after 5 min of recirculation. *Values significantly different, P < 0.05 (t-test), from corresponding normoglycemic group. 

**Statistics.** Statistical comparison was performed using analysis of variance and post hoc Newman-Keuls test. Within each glycemic series (normo- and hyperglycemia) the pre- and postischemic groups were compared with each other. Normoglycemic groups were also compared with sham-operated animals. Figs. 1B, 2A, 3B, 4B, postischemic groups (0, 30, and 120 min) are marked (†) if they differed from preischemic groups (P < 0.05). In addition the pre- and postischemic normoglycemic groups were compared with their corresponding hyperglycemic groups. A significant difference was marked (*). Other significant differences, if present, are mentioned in the text.
Values after 30 and 120 min postischemia were similar to those measured at comparable times in sham-operated animals. The relative changes of FFA levels appeared the same after ischemia in hyperglycemic animals. After 120 min of recovery the level was $0.69 \pm 0.05$ mmol/l and differed significantly ($P < 0.05$) from the value measured at the end of the ischemic period ($0.19 \pm 0.05$ mmol/l). In a comparison of hyperglycemic with normoglycemic ischemia, the hyperglycemic preischemic and 30-min recovery groups showed lower FFA contents than the corresponding normoglycemic groups ($P < 0.05$). The data suggest that hyperglycemia reduced the FFA contents before, during, and after ischemia.

**Insulin (Fig. 2A).** Ischemia in hyperglycemic animals significantly increased insulin levels to $4.2 \pm 0.8$ rig/ml ($P < 0.05$), but this increase in insulin levels during ischemia was transient because the 30- and 120-min recovery values increased ($P < 0.05$) from the level at the end of the ischemic period. In normoglycemic groups no significant changes were observed. Considerable interanimal variations in the insulin levels were observed. These may have concealed a corresponding increase of insulin at the end of ischemia in normoglycemic animals. A comparison of hyperglycemic with normoglycemic groups showed that the insulin level at the end of ischemia in normoglycemic animals was lower than in hyperglycemic animals.

**Free fatty acids (Fig. 1B).** Induction of ischemia in normoglycemic animals caused a reduction of plasma FFA content from $0.68 \pm 0.06$ to $0.37 \pm 0.02$ mmol/l, which was reversed after 30 and 120 min of recovery ($P < 0.05$). Values after 30 and 120 min postischemia were similar to those measured at comparable times in sham-operated animals. The relative changes of FFA levels appeared the same after ischemia in hyperglycemic animals. After 120 min of recovery the level was $0.69 \pm 0.05$ mmol/l and differed significantly ($P < 0.05$) from the value measured at the end of the ischemic period ($0.19 \pm 0.05$ mmol/l). In a comparison of hyperglycemic with normoglycemic ischemia, the hyperglycemic preischemic and 30-min recovery groups showed lower FFA contents than the corresponding normoglycemic groups ($P < 0.05$). The data suggest that hyperglycemia reduced the FFA contents before, during, and after ischemia.
Glucagon (Fig. 2B). Glucagon levels also showed large intrainidual variations. No significant changes were observed.

3-Hydroxybutyrate and acetoacetate (Fig. 3). Acetoacetate decreased during ischemia with normoglycemia to $0.36 \pm 0.07 \mu mol/ml$ compared with $0.94 \pm 0.25 \mu mol/ml$ in the preischemic group. Otherwise, ischemia did not seem to affect ketone body concentrations in normoglycemic animals. In hyperglycemic animals no changes in ketone body concentrations during or after ischemia were observed. A comparison of glycemic conditions showed that concentrations were clearly lower in the hyperglycemic subjects. After ischemia the 3-hydroxybutyrate was $0.26 \pm 0.05 \mu mol/ml$ in hyperglycemic animals and $1.71 \pm 0.18 \mu mol/ml$ in normoglycemic animals. The difference was significant for 3-hydroxybutyrate at any time compared. Although differences were not significant for acetoacetate, except the preischemic group, the combined data suggest that infusion of glucose depressed ketone body concentrations before, during, and after ischemia.

Epinephrine, norepinephrine, and dopamine (Fig. 4). As expected, ischemia was accompanied by increases in plasma epinephrine, norepinephrine, and dopamine levels. However, because of the large variability and small number of animals the changes were only significant for norepinephrine immediately after the ischemic period in the hyperglycemic group. Hyperglycemia clearly did not attenuate the catecholamine response to ischemia.

Corticosterone (Fig. 5). There were no differences among any groups. Thus hyperglycemia failed to raise plasma corticosterone levels.

DISCUSSION

As stated in the introduction, the aim of this study was to define any hormonal or nutritional factors that could conceivably contribute to the enhanced tissue damage seen after ischemia in hyperglycemic compared with normoglycemic individuals. We have provisionally assumed that any alterations in substrate and hormonal...
levels induced by hyperglycemia will not influence events during ischemia in other ways than by enhancing the lactic acidosis; an influence on the final ischemic damage should thus be found in the early or late recovery period.

The present results should be interpreted with two reservations in mind. First, as always with an animal model, problems arise with uncontrollable methodological difficulties in the experimental conditions. Even though strict criteria for including rats in the study were applied, hormonal levels were in some cases far outside normal physiological ranges, making the results sometimes difficult to interpret. This is probably unavoidable in studies of responses to stressful conditions. As a result, time differences in response may be hidden or, alternatively, they may not appear because of "exhaustion" of cells secreting the hormones. Second, although our results may help define factors responsible for the aggrava-

tion of damage in hyperglycemic animals, they can only provide information on factors operating in the first two postoperative hours.

Our results fail to indicate that the worsening of ischemic brain damage in hyperglycemic animals could be related to either a rise in corticosteroid levels or to a decrease in catecholamine levels. There were no, or only very small, differences in insulin or glucagon levels between normo- and hyperglycemic groups. In contrast, such differences were found in FFA levels and, particularly, in ketone body concentrations. We discuss these factors in turn.

Glucocorticoids. High glucocorticoid levels potentiate ischemic injury to neurons in rat brain (30) and may precipitate additional damage by causing posts ischemic seizures (17). As corticosterone exhibits a diurnal rhythm with peak levels occurring at the onset of darkness (3), we performed all experiments with rats in early morning when the lowest corticosterone levels are expected. Interestingly, steroid levels were about the same in the control and ischemic groups, and 5–10 times higher than reported in an unstressed rat at the same time of day (3). This suggests that a maximum rise may have been elicited by handling and anesthesia, as indicated in a study showing a maximum of 740 ng/ml after 30 min of anesthesia with ether and pentobarbital sodium (38). Our results showed no differences in corticosterone concentration between the normo- and hyperglycemic groups. Whether corticosterone modulates membrane damage during ischemia is not clarified by this study, and the results cannot exclude the possibility that normo- and hyperglycemic groups differ in plasma corticosterone levels after longer periods of recirculation.

Catecholamines. There is considerable evidence for a protective effect of the catecholamines during ischemia (18, 35). Even though there seemed to be increased levels of epinephrine and norepinephrine in hyperglycemic groups during the early recirculation period (hyper 0, hyper 30) compared with normoglycemic ischemia, these differences were not significant. After 120 min or recirculation no difference was found. Dopamine levels were increased by similar amounts in normo- and hyperglycemic ischemia. Thus measurements of catecholamines give no clue to why hyperglycemia aggravates ischemic damage.

Insulin. Because the brain no longer can be considered an insulin-insensitive organ (1), a possible modulation by insulin on ischemic brain damage cannot be ruled out. Insulin receptors are present in the brain (8). The origin of insulin in the central nervous system (CNS) is not quite clear. Systemic hormone levels gain access to the brain mainly through cerebrospinal fluid (CSF) (37) but may also act on the brain by activating specific receptors on the luminal side of the capillary endothelium or at the circumventricular organs, areas of the brain lacking a blood-brain barrier (6). Insulin has a possible neuromodulatory effect; for example, it inhibits norepinephrine uptake in neuronal cultures from rat brain (2). At least in some tissues, insulin exerts its anabolic action by activating cell surface transport systems for hexoses, amino acids, and ions (24, 25). In some cells it stimulates the membrane-bound Na⁺-H⁺ antiporter and changes Ca²⁺ fluxes.

In hyperglycemic animals insulin levels increased during ischemia compared with preischemic values. A similar increase may have occurred in normoglycemic animals. In both normo- and hyperglycemic animals insulin levels rose during ischemia out of proportion to plasma glucose concentration. This may be attributable to ischemia per se, to an interference with the ability of CNS to control the secretion of insulin (37), or to the net effect of the changes in the levels of different hormones on α- and β-receptors in pancreas that also regulate insulin secretion. As Fig. 2A shows, insulin levels rose in hyperglycemic animals, and at the end of ischemia the differences between normo- and hyperglycemic animals were significant. This difference, which probably reflects the hormonal response to induced hyperglycemia, is noteworthy. However, because insulin levels were similar after 30 and 120 min of recirculation in normo- and hyperglycemic animals, insulin may not be an important factor in explaining why hyperglycemic animals develop additional brain damage compared with normoglycemic animals.

Free fatty acids. There is no evidence that FFAs provide important energy fuels for brain metabolism. Although there is a considerable exchange of labeled FFAs
between the extracellular space and the brain, there is no net uptake but rather an ongoing release of FFAs into blood by the brain (27). Our results showed a significant decrease in plasma FFA concentrations during normoglycemic ischemia, that was reversed in the recovery period. A similar pattern was seen in the hyperglycemic animals. Induced hyperglycemia obviously depressed FFA concentrations. The values measured partly reflect the antilipolytic action of insulin because they mirrored animals. Induced hyperglycemia obviously depressed FFA concentrations. The values measured partly reflect the antilipolytic action of insulin because they mirrored

Ketone Bodies. The ketone bodies, β-hydroxybutyrate and acetoacetate, are substrates that can support energy metabolism in the brain (11). The capacity to use ketone bodies increases in starvation and diabetes by ~50–60% but even then ketone bodies support only a modest part of brain metabolism (10). Various structures metabolize ketone bodies to a different extent, with cortical areas having a relative preference for ketone bodies (9). In general, the telencephalon makes the greatest use of them (10). The limiting factor for access to the CNS is the blood-brain barrier (7, 9, 27), transport across the barrier occurring via a monocarboxylic acid carrier. This carrier has been suggested to be linked to the efflux of lactate. The influence of ischemia on blood-brain barrier ketone body transport is not clear.

Hyperketonemia lowers brain lactate levels in hypoxic mice (16), but acute elevation of β-hydroxybutyrate was not sufficient to provide cerebral protection in ischemia-hypoxia (21). An induction period of, for example, starvation may be required before the brain can maximally transport and metabolize ketone bodies. A ketone precursor, 1,3-butanediol, is lipophilic and diffuses passively across the blood-brain barrier. This 4-carboxylated alcohol confers cerebral protection in ischemia-hypoxia (21) and in ischemia alone (22). The latter study showed reduced morphological damage, increased energy charge, and decreased lactate accumulation in butanediol-treated ischemic rats.

Our results showing decreases in plasma concentrations of β-hydroxybutyrate and acetoacetate in hyperglycemic animals are compatible with the protective role previously assigned to ketone bodies. If the difference in outcome between normo- and hyperglycemic animals is related to these organic acids, the enhancement of blood-brain barrier transport induced by overnight starvation obviously did not counteract the negative effect of the decreased β-hydroxybutyrate (and acetoacetate) in plasma observed in hyperglycemic animals. We conclude that if any of the factors examined in the present study modulates the damage in hyperglycemic ischemia compared with normoglycemic ischemia, the best candidates are plasma ketone bodies.

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Present address of A. Mans: Dept. of Physiology and Biophysics, University of Health Sciences, The Chicago Medical School, North Chicago, IL.

Address for reprint requests: J. Lundgren, Dept. of Paediatrics, University Hospital, S-221 85, Lund, Sweden.

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