Hypoglycemic Brain Injury

I. Metabolic and Light Microscopic Findings in Rat Cerebral Cortex During Profound Insulin-induced Hypoglycemia and in the Recovery Period Following Glucose Administration

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Summary. Profound hypoglycemia causing the disappearance of spontaneous EEG activity was induced by insulin in rats. For analysis of cerebral cortical concentrations of labile phosphates, glycolytic metabolites and amino acids, the brain was frozen in situ. For microscopic analysis of the corresponding cerebral cortical areas the brain was fixed by perfusion. Hypoglycemia with an isoelectric EEG for 30 and 60 min caused severe perturbation of the cerebral energy metabolites. After both 30 and 60 min of isoelectric EEG, two microscopically different types of nerve cell injury were seen. Type I injury was characterized by angulated, darkly stained neurons with perineuronal vacuolation, mainly affecting small neurons in cortical layer 3. Type II injured neurons, mainly larger ones in layers 5–6, were slightly swollen with vacuolation or clearing (depending on the histotechnique used) of the peripheral cytoplasm, but had no nuclear changes.

Recovery was induced by glucose injection. Improvement in the cerebral energy state occurred during the 30 min recovery period even after 60 min of hypoglycemia. However, the persisting reduction in the size of adenine nucleotide and amino acid pools after 30 or 180 min recovery suggested that some cells remained damaged. In confirmation many type I injured neurons persisted during the recovery suggesting an irreversible injury. The disappearance of virtually all type II injured neurons indicated reversibility of these histopathological changes.

The microscopic changes in hypoglycemia were different from those in anoxia-ischemia suggesting a dissimilar pathogenesis in these states despite the common final pathway of energy failure.

Key words: Hypoglycemia — Nerve cell injury — Biochemistry — Light microscopy — Rat cerebral cortex

In hypoglycemia the brain is deprived of glucose, its principal substrate for energy production. When hypoglycemia is of sufficient severity it leads to gross energy failure causing functional derangement, such as abolition of electroencephalographic (EEG) potentials and clinical coma (Tews et al. 1965; Hinzen and Müller 1971; Lewis et al. 1974b; Norberg and Siesjö 1976; Agardh et al. 1978). If it is of sufficient duration it causes irreversible neuronal injury (Brierley et al. 1971a).

Energy failure also occurs in anoxia-ischemia. The light microscopic neuronal changes in hypoglycemia, as well as the pathogenesis of the nerve cell injury, have been considered identical to those in anoxia-ischemia (Brierley et al. 1971b; Brierley 1976). In some cases there are, however, certain differences in the distribution of the brain lesions in hypoglycemia as compared to ischemia both in human neuropathological cases (Kalimo and Olsson, in prep.) and in some experimental conditions (Myers and Kahn 1971). Furthermore, the biochemical point of view of the metabolic events, which in hypoglycemia precede the appearance of gross energy failure, are markedly different from those in anoxia-ischemia. In hypoglycemia the exhaustion of the carbohydrate stores, and the utilization of non-carbohydrate substrates for energy production with ample oxygen present, terminally result in decreased levels of lactate, pyruvate, citric acid cycle metabolites, NADH and certain amino acids, whereas the concentration of ammonia increases excessively as a consequence of oxidative deamination. Furthermore, as a result of the substrate depletion, the intracellular pH is not decreased (Lewis et al. 1974a; Pelligrino et al. 1979). This contrasts with anoxia-ischemia, where the lactate concentration and lactate/pyruvate ratio increase with a concomitant fall in pH and NAD⁺. In addition, the rise in ammonia level is less pronounced than in hypoglycemia. Thus, these results demonstrate that despite a common final event leading to depletion of energy-rich compounds there are important patho-
genetic differences between hypoglycemic and anoxic-ischemic brain injury.

In this communication the light microscopic alterations that occur in the rat brain during and after severe hypoglycemia are correlated to the biochemical changes to further clarify the pathogenesis of hypoglycemic brain injury.

**Material and Methods**

**Animals and Operative Procedures**

Male Wistar rats (270—405 g) of a S.P.F. Wistar strain (Møllegaard Avlaboratorium, Copenhagen) were fasted over night before the experiments but had free access to tap water. Hypoglycemia was induced by an i.p. injection of insulin (Insulin Novo Actrapid, Novo Industri AB) in a dose of 40 I.U· kg⁻¹. The insulin was dissolved in 0.75 ml of Krebs-Henseleit solution before injection. Control animals were given 0.75 ml of this solution. Anesthesia was induced with 2—3 % halothane, the animals were then tracheotomized, immobilized with i.v. injection of tubocurarine chloride (0.5 mg · kg⁻¹) and ventilated with a Starling type respirator, delivering 70 % N₂O and 30 % O₂, to yield an arterial P₉O of 30—40 mm Hg. Body temperature was maintained close to 37°C. One femoral artery was cannulated for continuous blood pressure recording with an electromanometer and for sampling of blood, and one femoral vein was cannulated for injections. The electro-corticogram (EEG) was continuously recorded in all animals from gold-plated copper bolts inserted into the skull bone in the fronto-parietal region (bipolar leads), using an Elema EEG machine.

After the operative procedures had been completed the animals were given heparin i.v. (10 I.U. · kg⁻¹) and were maintained on 70 % N₂O and 30 % O₂, to yield an arterial P₉O of 30—40 mm Hg. Body temperature was maintained close to 37°C. One femoral artery was cannulated for continuous blood pressure recording with an electromanometer and for sampling of blood, and one femoral vein was cannulated for injections. The electro-corticogram (EEG) was continuously recorded in all animals from gold-plated copper bolts inserted into the skull bone in the fronto-parietal region (bipolar leads), using an Elema EEG machine.

The pH, P₉O and P₉O, or collected directly in liquid nitrogen for later analyses of glucose.

**Sampling for Biochemistry**

A skin incision was made over the skull bone to accommodate a plastic funnel for later freezing of the brain in situ with liquid nitrogen at the termination of the experiment (Pontén et al. 1973). Cerebral cortical tissue from the area indicated in Fig. 1 a was sampled with chisel in the frozen state and the tissue was stored at −80°C until analysis.

**Sampling for Morphology**

At the termination of the experiment a quick thoracotomy was performed, and a cannula was inserted in the ascending aorta via the left ventricle. Following a quick rinse with saline, 300 ml of 3 % glutaraldehyde (GA) (Polaron Equipment Ltd., Watford, England) in 0.1 mol · l⁻¹ phosphate buffer at pH 7.4 and 37°C as well as at a pressure of 135 mm Hg was perfused through the vascular bed. To allow a comparison to a previous report (Brierley et al. 1971 a) three control rats and two animals of group C (Table 1) were similarly perfused at 20°C with FAM-fixative (40 % formaldehyde-glacial acetic acid-absolute methanol 1:1:8) (David 1955). After perfusion, the brain was allowed to stabilize in situ for 1—2 h after which it was removed and stored in the same fixative until processed for microscopy. Pairs of coronal sections were sampled at three different levels from one hemisphere (Fig. 1 a). One of the paired samples was embedded in paraffin while the other was embedded in JB-4 plastic (Sorvall, Polysciences Inc., Warrington, Pennsylvania, USA). From the opposite hemisphere two cortical samples (Fig. 1 b) were taken for electron microscopy. The tissue blocks were sectioned with a tissue sectioner (The Michle Lab. Eng., Gomshall, Surrey, England) in approximately 500 μm thick slices, postosmicated, dehydrated, and embedded in Araldite. Large semithin (1 μm) sections were cut for light microscopic analysis.

**Experimental Groups**

As summarized in Table 1, there were six different experimental groups (A—F). One was a control group (A) maintained under anesthesia for 196 ± 87 min (mean ± S.D) before tissue was frozen in situ or perfusion fixed. In two groups, the animals were kept at steady state until the EEG became isoelectric. They were then maintained with an isoelectric EEG for 30 (B) or 60 (C) min before tissue was sampled. Two other groups were allowed 30 (D) or 180 (E) min of recovery, following an isoelectric period of 30 min. In the last group (F), recovery was allowed for 30 min following an isoelectric period of 60 min. Recovery was induced by i.v. injection of 0.5 ml of a 50 % (w/v) glucose solution (in saline). The injection was followed by a slow i.v. infusion (1 ml · h⁻¹) of the glucose solution.

**Analytical Techniques for Biochemistry**

The pH, P₉O and P₉O, in arterial blood were measured immediately after sampling, using microelectrodes operated at 37°C (Radiometer, Copenhagen and Eschweiler & Co., Kiel) with appropriate tempera-