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Brain and Plasma Quinolinic Acid in Profound Insulin-Induced Hypoglycemia

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Abstract: Profound insulin-induced hypoglycemia is associated with early-onset neuronal damage that resembles excitotoxic lesions and is attenuated in severity by antagonists of *N*-methyl-D-aspartate receptors. Hypoglycemia increases L-tryptophan concentrations in brain and could increase the concentration of the L-tryptophan metabolite quinolinic acid (QUIN), an agonist of *N*-methyl-D-aspartate receptors and an excitotoxin in brain. Therefore, we investigated the effects of 40 min of profound hypoglycemia (isoelectric EEG) and 1–2 h of normoglycemic recovery on the concentrations of QUIN in brain tissue, brain extracellular fluid, and plasma in male Wistar rats. Plasma QUIN increased 6.5-fold by the time of isoelectricity (2 h after insulin administration). Regional brain QUIN concentrations increased two- to threefold during hypoglycemia and increased a further two- to threefold during recovery. However, no change in extracellular fluid QUIN concentrations in hippocampus occurred during hypoglycemia or recovery as measured using in vivo microdialysis. Therefore, the increases in brain tissue QUIN concentrations may reflect elevations of QUIN in the intracellular space or be secondary to the increases in QUIN in the vascular compartment in brain per se. L-Tryptophan concentrations increased more than twofold during recovery only. Serotonin decreased >50% throughout the brain during hypoglycemia, while 5-hydroxyindoleacetic acid concentrations increased more than twofold during hypoglycemia and recovery. In striatum, dopamine was decreased 75% during hypoglycemia but returned to control values during recovery, while striatal 3,4-dihydroxyphenylacetic acid and homovanillic acid were increased more than twofold during both hypoglycemia and recovery. The present results do not support a role for increased QUIN in extracellular fluid space during the early-phase neuronal damage of profound insulin-induced hypoglycemia. **Key Words:** Hypoglycemia—Quinolinic acid—Indoleamines—Brain—Plasma—Microdialysis. **Heyes M. P. et al.** Brain and plasma quinolinic acid in profound insulin-induced hypoglycemia. *J. Neurochem.* **54,** 1027–1033 (1990).

Profound insulin-induced hypoglycemia results in a wide spectrum of neurochemical derangements including decreased protein synthesis, increased protein and phospholipid breakdown, ammonia production, energy failure, and tissue alkalosis (Hoshino and Elliot, 1970; Norberg and Siesjö, 1976; Agardh et al., 1978; Kiessling et al., 1984, 1986; Auer, 1986; Siesjö, 1988). Of special interest are changes in excitatory amino acids because of characteristic morphologic hallmarks of excitotoxic brain damage in hypoglycemia, which begin during hypoglycemia and in the first hour after the hypoglycemic insult (Auer et al., 1985*a–c*; Kalimo et al., 1985; Auer, 1986). The demonstration that neuronal death in hypoglycemia is attenuated by local application of *N*-methyl-D-aspartate (NMDA) receptor antagonists provides further evidence for an important role played by endogenously produced excitotoxic compounds in mediating neuronal damage in hypoglycemia (Wieloch, 1985; Simon et al., 1986). One endogenous excitatory amino acid that increases during profound hypoglycemia is aspartic acid owing to a pronounced shift in the aspartate-glutamate transaminase reaction toward aspartate (Agardh et al., 1980; Butcher et al., 1987; Siesjö, 1988). In vivo microdialysis studies have revealed that the extracellular fluid concentrations of glutamic acid in brain are also increased (Butcher et al., 1987).

Agardh et al. (1979) demonstrated that following 30

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Abbreviations used: DOPAC, 3,4-dihydroxyphenylacetic acid; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine; HVA, homovanillic acid; NMDA, *N*-methyl-D-aspartate; PCA, perchloric acid; QUIN, quinolinic acid; L-TRP, L-tryptophan.

min of severe insulin-induced hypoglycemia, the concentration of L-tryptophan (L-TRP) increased in the cerebral cortex, striatum, and limbic regions of the brain. L-TRP is a precursor of quinolinic acid (QUIN), at least in systemic tissues, and it is possible, therefore, that the concentrations of QUIN increase in hypoglycemia. Studies in experimental animals have demonstrated that QUIN is an agonist of NMDA receptors (Stone and Perkins, 1981; Perkins and Stone, 1983), a convulsant (Lapin, 1978, 1982), and a neurotoxin at high concentrations (Foster et al., 1983; Schwarcz et al., 1983). If QUIN concentrations were to increase in brain sufficiently during insulin-induced hypoglycemia, QUIN might have neuropathologic effects.

To investigate whether QUIN concentrations are increased in brain during hypoglycemia, we undertook a study of QUIN and biogenic amines in brain tissue and plasma during 40 min of EEG isoelectricity and by 1–2 h of normoglycemic recovery. The neuroanatomical features of the regional brain lesions produced by this protocol have already been described (Auer et al., 1985*a–c*; Kalimo et al., 1985; Auer, 1986). Because NMDA receptors are localized on the outer membranes of neurons and are activated by compounds in the extracellular fluid, we also quantified QUIN in the extracellular fluid of the hippocampus by in vivo microdialysis (During et al., 1989*a,b*). To confirm the ability of in vivo microdialysis to detect changes in the extracellular fluid QUIN concentrations, 3-hydroxyanthranilic acid, a confirmed precursor of QUIN in brain in vivo (Heyes et al., 1988), was perfused into the brain via the microdialysis probe and increases in eluant QUIN confirmed.

MATERIALS AND METHODS

Materials

Insulin, L-TRP, 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), QUIN, Dowex 1X8 (400 mesh chloride form and converted to the formate form), heptane sulfonic acid, and suxamethonium chloride were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Hexafluoroisopropanol and trifluoroacetylimidazole were obtained from Pierce Chem. Co. (Rockford, IL, U.S.A.). The remaining compounds were obtained from Fisher (Fair Lawn, NJ, U.S.A.). [^{18}O]QUIN was prepared by heating 6 μmol of QUIN in 1 ml of 3 mol/L hydrochloric acid in [^{18}O]water at 80°C for 4 days (Heyes and Markey, 1988*a*).

Experimental procedures

Male Wistar rats (Charles River, St. Constant, Quebec, Canada) weighing 230–243 g were fasted overnight but had free access to tap water. Brains from four groups of six rats were studied: (a) control group: killed immediately on removal from their home cage; (b) sham-operated group: exposed to all procedures except injection of insulin; (c) hypoglycemic isoelectricity group: exposed to 40 min of EEG isoelectricity; (d) recovery group: exposed to 40 min of EEG isoelectricity followed by 60 min of normoglycemic recovery.

In studies of plasma, additional rats were killed 1 and 2 h after injection of insulin but prior to EEG isoelectricity and killed 1 and 2 h after beginning normoglycemic recovery. At least five rats per time point were studied.

Induction of hypoglycemic isoelectricity. For the hypoglycemia groups, rats were given an intraperitoneal injection of 10–15 IU/kg insulin 1–2 h prior to operation. The sham-operated rats received an equivalent volume of 150 mmol/L saline. Rats in the hypoglycemia and sham groups were anesthetized by 3% halothane, intubated (PE-240; Clay Adams, Parsipanny, NY, U.S.A.), and ventilated on a Starling-type ventilator (Harvard Apparatus, South Natick, MA, U.S.A.) using a 2:1 N_2/O_2 mixture containing 1% halothane. A tail vein was cannulated with PE-50 tubing and a continuous infusion of suxamethonium chloride at 2 mg/h was administered by an infusion pump to maintain continuous paralysis. The tail artery was cannulated and blood pressure continuously recorded using a Stratham transducer (Gould P50, Oxnard, CA, U.S.A.). Blood pressure readings were digitized once every 6 s and recorded with a computer. Bipolar interhemispheric EEG was monitored using a subdermal scalp electrode (Grass Instruments, Quincy, MA, U.S.A.). The EEG was monitored in real time and also stored to disk. Arterial blood glucose levels were monitored with an Ames glucometer (Elkhart, IN, U.S.A.). Arterial pH, P_{CO_2}, and P_{O_2} were monitored on a blood gas analyzer (model 1304; Instrument Laboratory, Milan, Italy). After the operation, rats were ventilated on a 2:1 N_2O/O_2 mixture throughout the study.

Cerebral isoelectricity began 60–170 min after the injection of insulin. After 40 min of cerebral isoelectricity, normoglycemic recovery was induced with an infusion of 1.5 ml of 25% glucose over a 2-min period followed by a continuous infusion of glucose at 0.056–0.078 ml/min for 1 h. Rats were decapitated under anesthesia, their brains quickly removed and cooled on a glass plate. The frontal (50–90 mg), parietal (60–100 mg), and occipital (50–95 mg) regions of the cerebral cortex, striatum (25–40 mg), thalamus (30–50 mg, including hypothalamus), hippocampus (20–30 mg), and cerebellum (180–240 mg) were isolated and collected into pre-weighed 1.5-ml polypropylene tubes. The tubes were frozen in liquid nitrogen and stored at −70°C until analysis. Brain tissue data are expressed in amount per unit wet weight.

Microdialysis. The dialysis fibers were manufactured by mounting cellulose-acetate hollow tubing (molecular weight cutoff 6,000; Spectrum Medical Industries, Los Angeles, CA, U.S.A.) onto 30-gauge stainless-steel wire. The ends of the fiber were then fixed to a hollow twin-barrel stainless-steel cannula with epoxy cement. The length of the dialysis tubing available for exchange with the interstitial fluid was 5 mm.

On the day prior to the hypoglycemia, seven rats were anesthetized by an intraperitoneal injection of 5 mg/kg pentobarbital. A superior parasagittal craniotomy (2.0 mm lateral; 3.8 mm posterior to bregma) was performed with a dental burr. The cannula was lowered 3.5 mm below the skull surface into the hippocampus and cemented into place with a self-curing plastic (Formatray; Kerr, Romulus, MI, U.S.A.). The animals were allowed to awaken and fasted overnight prior to the induction of hypoglycemia.

On the day of hypoglycemia, the two barrels of the dialysis cannula were connected to vinyl tubing and perfusion of the probes with artificial cerebrospinal fluid was begun at 1 μl/min. Dialysates were collected in 1-h aliquots during control and recovery periods and for 40 min during isoelectricity by clamping and sealing the ends of the tubing into 10-cm lengths. Hypoglycemia was induced in rats by an intraperi-

toneal injection of 8 IU/kg crystalline insulin and brain EEG activity monitored by subcutaneous bipolar electrodes.

In two additional rats, 3-hydroxyanthranilic acid (100 nmol/L) was perfused through the microdialysis probe for 1 h beginning after a 3-h control period. Samples were collected every hour until the end of the 3-hydroxyanthranilic acid perfusion. After completion of the 3-hydroxyanthranilic acid perfusion, microdialysate samples were collected for an additional 3 h in 30-min collection periods.

Neurochemical analyses

Sample preparation. All brain samples were sonicated in 1 ml of 1 mol/L perchloric acid (PCA) containing 6 pmol/ml [^{18}O]QUIN as internal standard for the quantification of QUIN by electron capture negative chemical ionization mass spectrometry (Heyes and Markey, 1988*a,b*). For the standard curve, 1 ml of the [^{18}O]QUIN in PCA solution was added to QUIN standards (0.6–60 pmol) dissolved in water. PCA extracts were centrifuged at 12,000 *g* for 20 min at 4°C. A 50-μl aliquot of the supernatant from brain was collected into 250-μl polypropylene tubes and used for the analysis of indoleamines and catecholamines. The remaining PCA extract was used in the quantification of QUIN. Plasma (10 μl) was mixed with 0.5 ml of 1 mol/L hydrochloric acid or deionized water containing 6 pmol of [^{18}O]QUIN and centrifuged at 12,000 *g* for 20 min at 4°C. Microdialysates were flushed from the tubing using deionized water and 0.6 pmol of [^{18}O]QUIN was added. All extracts and dialysates were freeze-dried overnight for the quantification of QUIN.

Gas chromatography/mass spectrometry QUIN measures. The PCA extract of brain was neutralized by 10 mol/L potassium hydroxide and washed with 2–4 ml of chloroform to remove lipid material and further precipitate protein. The aqueous extract was applied to Dowex 1X8 anion exchange resin as described previously (Heyes and Markey, 1988*b*). The Dowex was washed with 3 ml of water, 3 ml of 0.1 mol/L formic acid, and 0.3 ml of 6 mol/L formic acid; the QUIN and [^{18}O]QUIN were eluted into screw-capped tubes by 2 ml of 6 mol/L formic acid. Eluates were freeze-dried overnight. QUIN and [^{18}O]QUIN were converted to their dihexafluoroisopropanol esters by heating with 100 μl of hexafluoroisopropanol and 100 μl of trifluoroacetylimidazole for 1 h at 75°C in a heating block. After cooling, the esters were extracted into 300 μl of heptane over 250 μl of water.

Samples (1–2 μl) were injected onto a DB-5 capillary column (0.25-mm inside diameter, 15 m long; J & W Scientific, Folsom, CA, U.S.A.) housed in either a Varian 3700 (program of 95°C for 1 min, 30°C/min temperature ramp to 170°C) or a Carlo Erba (115°C, isothermal) gas chromatographic oven. The mass spectrometers used were a Finnigan 3200 quadrupole mass filter with Extrel electronics and a Teknivent data analysis system or a Kratos MS-80. Carrier gas was helium and reagent gas was methane. QUIN-hexafluoroisopropanol (mass 467 daltons) and [^{18}O]QUIN-hexafluoroisopropanol (mass 471 daltons) were analyzed by selected ion monitoring at mass/charge ratio (*m/z*) of 467 and *m/z* 471 (molecular ions) and *m/z* 316 and *m/z* 320 (major fragment ions). Endogenous QUIN was quantified by the peak area ratio of *m/z* 467:471. The minimum sensitivity at a signal/noise ratio of 10:1 in standards was at least 6 fmol (Heyes and Markey, 1988*b*).

Indoleamines and catecholamines. The high pressure liquid chromatographic system for the quantification of L-TRP, 5-HT, 5-HIAA, dopamine, HVA, and DOPAC consisted of a 4.6 × 75–mm Altech ultrasphere 3-μm ODS column, an LC-4B amperometric detector (Bioanalytical Systems, Lafayette, IN, U.S.A.), a glassy carbon electrochemical detector cell, and a Waters 510 pump. The mobile phase buffer was 100 mmol/L sodium acetate, 0.2 mmol/L disodium ethyleneamine tetraacetate, 0.15 mmol/L sodium octyl sulfate, with 50 ml acetonitrile/L deionized water at pH 4.87 and the flow rate was 1 ml/min. Oxidation voltage was set at 0.9 V relative to an Ag/AgCl reference electrode. A Wisp 710B autoinjector was used to inject 5 or 10 μl of PCA brain extract. Oxidation current was recorded on a chart recorder set at 10 or 100 nA full scale. Peak heights were used in quantification and compared with those of freshly made standards. Times of elution were as follows: DOPAC, 3.3 min; 5-HIAA, 4.6 min; dopamine, 5.7 min; L-TRP, 9.1 min; HVA, 11.0 min; 5-HT, 21.0 min.

Statistical analysis

Values presented are either means ± 1 SEM or expressed as a percentage of pooled control and sham-operated values. Results were analyzed by one-way analysis of variance and Dunnett's *t* test. Correlation coefficients were calculated by the method of least squares. Statistically significant changes in measured parameters are indicated in the figures. A p value of <0.01 was considered significant.

RESULTS

No significant differences were found between the sham, hypoglycemic, or recovery groups with respect to body temperature, blood gases, blood pH, or mean arterial pressure. These physiologic parameters were within the following ranges: body temperature, 36.7–37.8°C; arterial P_{O_2}, 88–145 mm Hg; arterial P_{CO_2}, 31–46 mm Hg; arterial pH, 7.36–7.46; mean arterial pressure, 100–120 mm Hg. The two hypoglycemia groups showed the expected transient rise in mean arterial pressure to ~110–150 mm Hg during the early hypoglycemia period.

There were no significant differences in the regional brain concentrations of QUIN, L-TRP, 5-HT, or 5-HIAA between control and sham rats. Likewise, in striatum the concentrations of dopamine, HVA, and DOPAC were not different between control and sham rats. Therefore, the data from control and sham rats were pooled for statistical comparisons to hypoglycemia and recovery groups.

Plasma, brain, and microdialysate QUIN

Plasma QUIN. Plasma QUIN concentrations were 273.2 ± 32.1 pmol/ml in control rats. Plasma QUIN had increased significantly by 2 h after injection of insulin by 3.57-fold and increased further during hypoglycemic isoelectricity to 6.47-fold higher than control. Plasma QUIN concentrations began to return to control values during the recovery period, but were still increased by 3.96-fold at 2 h (Fig. 1).

Regional brain QUIN. The values for regional brain QUIN concentrations are presented in Fig. 2. In all regions, QUIN concentrations increased two- to threefold after 40 min of hypoglycemia and increased two- to threefold further after 1 h of normoglycemic recovery.

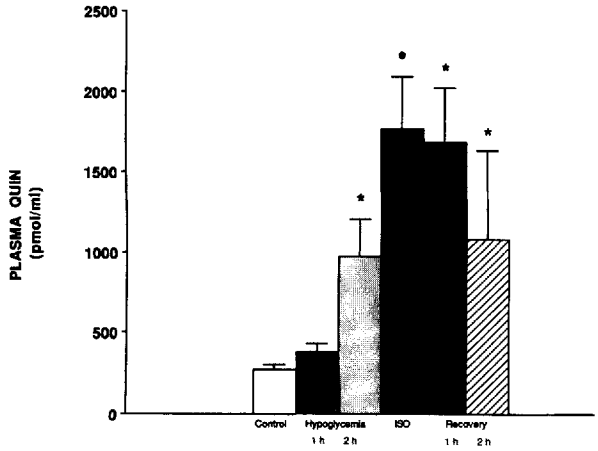

FIG. 1. Plasma QUIN concentrations in control (open bar), 1 and 2 h after injection of insulin (stippled bars), 40 min of hypoglycemic isoelectricity (filled bar), and 1 and 2 h of normoglycemic recovery (hatched bars). Values presented are means ± 1 SEM of at least five rats. *$p < 0.01$ compared with control. The data show increases in plasma QUIN during the hypoglycemic periods.

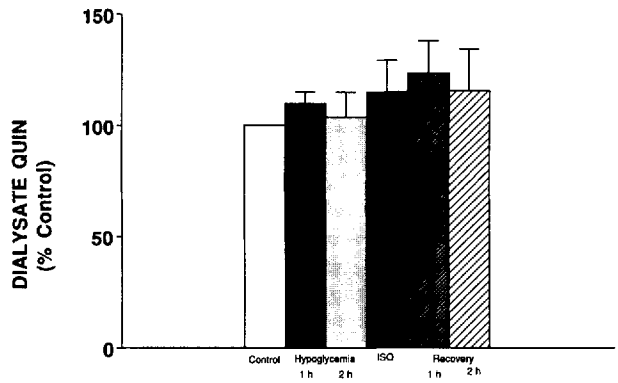

FIG. 3. QUIN recovered from in vivo microdialysis probe inserted into the hippocampus. Microdialysis probes were inserted 24 h prior to injection of insulin. Rats were exposed to 2 h of insulin-induced hypoglycemia, 40 min of isoelectricity, and 2 h of normoglycemic recovery. Results are expressed as a percentage of QUIN recovered during the control period. Bar codes as in Fig. 1. The results indicate no changes in extracellular fluid QUIN concentrations at any time point examined.

In vivo microdialysate QUIN. The concentration of QUIN in microdialysate of control samples collected from hippocampus was 20 ± 4 nmol/L. At a recovery efficiency of 30% for QUIN from the probes used, the extracellular fluid QUIN concentrations are calculated to be ~67 nmol/L. No significant change in microdialysate QUIN concentrations was observed during either cerebral isoelectricity or the recovery period (Fig. 3), which indicates that extracellular fluid QUIN concentrations were maintained at control values throughout the study. Addition of 3-hydroxyanthranilic acid to the microdialysis perfusion medium resulted in increased microdialysate QUIN concentrations of 18.1- and 29.7-fold in the two rats studied. In both rats, peak QUIN concentrations occurred 1.5–2 h after beginning the 3-hydroxyanthranilic acid infusion.

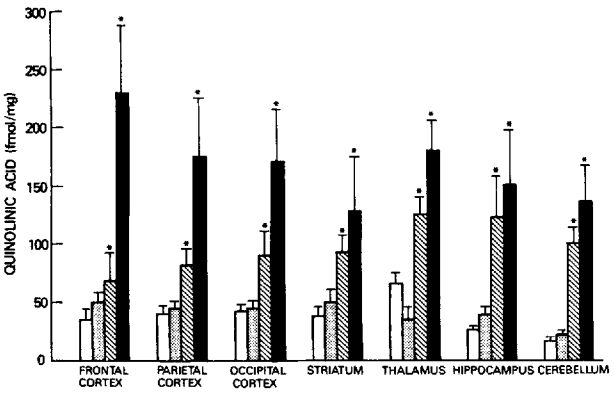

FIG. 2. Regional brain QUIN concentrations in control unoperated rats (open bars), sham-operated rats (stippled bars), rats killed immediately after 40 min of insulin-induced hypoglycemic isoelectricity (hatched bars), and rats exposed to 40 min of isoelectricity and killed after 60 min of normoglycemic recovery (filled bars). The data show apparent increases in regional brain QUIN during isoelectricity and recovery. Values presented are means ± 1 SEM of six rats per group. Statistical comparisons were made with the pooled values of control and sham-operated rats: *$p < 0.01$.

Regional brain L-TRP, 5-HT, and 5-HIAA concentrations

The regional brain concentration of L-TRP was not changed after hypoglycemia but was significantly increased 1.5- to 2-fold in all regions after 1 h of recovery (Fig. 4). Regional brain 5-HT concentrations decreased significantly after hypoglycemia by 40–60% (Fig. 5). During the recovery period, 5-HT concentrations increased as compared with hypoglycemia values, but in cortical regions were still significantly lower than control and sham. Regional brain 5-HIAA concentrations increased significantly after hypoglycemia by 76–161%

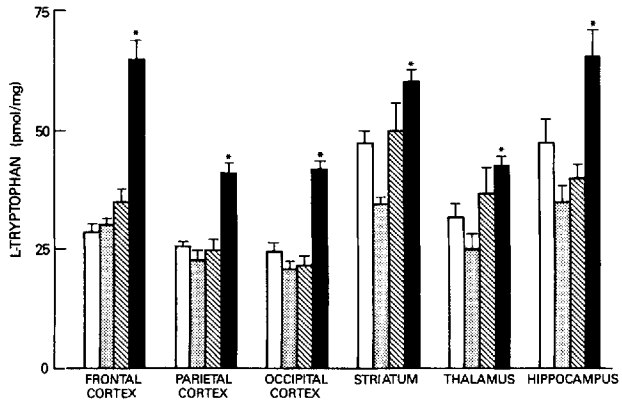

FIG. 4. Effects of profound hypoglycemia on regional brain L-TRP concentrations. Bar codes and comparisons as in Fig. 2. *$p < 0.01$. In all brain regions, L-TRP concentrations increased during the recovery period only.

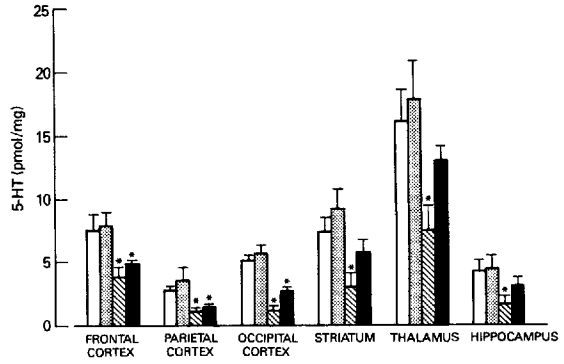

FIG. 5. Effects of profound hypoglycemia on regional brain 5-HT concentrations. Bar codes and comparisons as in Fig. 2. $*p < 0.01$. In all brain regions, 5-HT concentrations decreased during hypoglycemia and increased toward normal values during recovery.

and were still increased after 1 h of recovery in all regions examined (Fig. 6).

Striatal catecholamines

In striatum (Fig. 7) dopamine concentrations decreased by 74% after hypoglycemia, while at the same time both HVA and DOPAC concentrations increased by 252 and 281%, respectively. After recovery, dopamine concentrations had returned to control values, whereas both HVA and DOPAC concentrations remained elevated at 200 and 270%, respectively, above control and sham values.

DISCUSSION

The present data show increases in regional brain QUIN concentrations during 40 min of cerebral isoelectricity, which continued to increase during 1 h of normoglycemic recovery. Plasma QUIN concentrations also increased but reached peak values during hypoglycemia. However, there was no change in extracellular fluid QUIN concentrations as measured by in vivo microdialysis. These observations may indicate that the increases in brain tissue QUIN concentrations during hypoglycemia and recovery are restricted to the intracellular compartment of brain or are attributable to the presence of QUIN in the blood of the tissue samples.

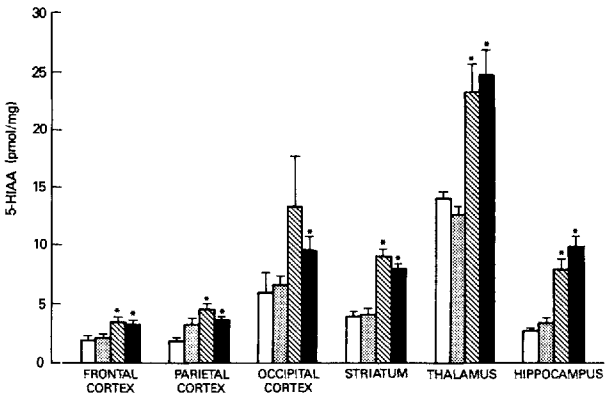

FIG. 6. Effects of profound hypoglycemia on regional brain 5-HIAA concentrations. Bar codes and comparisons as in Fig. 2. $*p < 0.01$. In all brain regions, 5-HIAA concentrations were increased during both hypoglycemia and recovery.

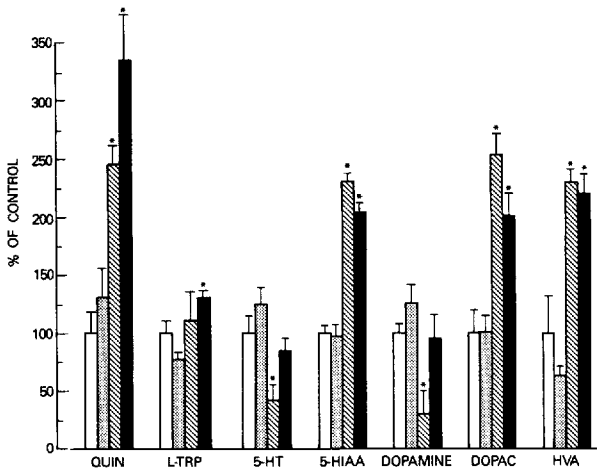

FIG. 7. Relative changes in QUIN, L-TRP, 5-HT, 5-HIAA, dopamine, DOPAC, and HVA concentrations in striatum during hypoglycemia and recovery. Values for each are expressed as a percentage of control group values. Bar codes as in Fig. 2. Pooled control and sham values were as follows: QUIN, 39.9 ± 7.1 fmol/mg; L-TRP, 40.3 ± 3.4 pmol/mg; 5-HT, 8.20 ± 0.62 pmol/mg; 5-HIAA, 3.99 ± 0.17 pmol/mg; dopamine, 79.5 ± 12.1 pmol/mg; DOPAC, 11.6 ± 6.7 pmol/mg; HVA, 8.50 ± 1.90 pmol/mg. $*p < 0.01$.

The assay we used employs purification and chromatographic procedures that isolate QUIN selectively and a mass spectrometric analysis system that monitors QUIN specifically as the molecular anion of QUIN (m/z 467) and a characteristic fragment (m/z 316). The detection system has the high sensitivity required for the low concentrations of QUIN in brain, microdialysate, and plasma (Heyes and Markey, 1988*a*). Furthermore, the internal standard, [^{18}O]QUIN, ensures accuracy in the quantification of QUIN (Heyes and Markey, 1988*b*). The increase in brain tissue QUIN concentrations (Fig. 2) could be interpreted as being consistent with the notion that QUIN, via agonist effects on NMDA receptors, may be involved in the neuropathology of hypoglycemia. The type of neuronal damage produced by profound hypoglycemia is characteristic of excitotoxic damage and includes dendritic swelling, mitochondrial disruption, subsequent acidophilic staining of neurons, and necrosis (Auer et al., 1985*a–c*; Auer, 1986). Neuronal damage is observed within minutes of isoelectricity (Auer, 1986), which emphasizes the importance of early changes in the concentrations of potential neurotoxic candidates. Excitotoxic lesions produced by QUIN have similar features to hypoglycemic damage and result when, for example, 12 nmol of QUIN is injected into the striatum (Schwarcz et al., 1983). Assuming a wet weight of 50

mg for a rat striatum, 12 nmol may be expected to produce peak QUIN concentrations of 240 μmol/L. This resultant concentration of QUIN far exceeds the levels of QUIN observed in brain tissue in hypoglycemia (Fig. 2), which dilutes the potential for QUIN to mediate hypoglycemic brain damage.

It is likely that access to NMDA receptors, which are localized on neuronal membranes, by agonists is from the extracellular fluid space. Therefore, in view of the increased brain QUIN concentrations (Fig. 2), we used in vivo microdialysis to quantify changes in QUIN in the extracellular fluid during hypoglycemia, a technique presumed to sample the extracellular fluid space (During et al., 1989*a*,*b*). The hippocampus was chosen because of its susceptibility to hypoglycemic damage (Auer et al., 1985*b*,*c*). The microdialysate data indicate that extracellular fluid QUIN concentrations were not increased during either hypoglycemia or the recovery period (Fig. 3). This observation does not support a direct role for increased extracellular fluid QUIN concentrations in early hypoglycemic brain damage. The failure of extracellular QUIN concentrations to change with hypoglycemia contrasts with the reported increases in extracellular fluid aspartate and glutamate concentrations during hypoglycemia (Butcher et al., 1987), two excitatory amino acids previously implicated in hypoglycemic brain damage. The fact that 3-hydroxyanthranilic acid added to the microdialysate perfusion medium increased QUIN synthesis indicates that the procedure employed would have been able to detect changes in extracellular fluid QUIN concentrations. Therefore, increases in brain tissue QUIN during hypoglycemia may be localized to the intracellular fluid compartment, including astroglia, where 3-hydroxyanthranilate-3,4-dioxygenase is localized (Okuno et al., 1987). However, our results do not exclude the possibility that delayed increases in extracellular fluid QUIN concentrations do occur following hypoglycemia and contribute to neuronal cell loss at a later time.

The concentration of QUIN in plasma increased following administration of insulin and concomitant hypoglycemia. Assuming a brain plasma volume of 0.9% (quantified by the ^{125}I-albumin technique) (Heyes and Markey, 1988*a*), the contribution of plasma QUIN to the measured regional brain QUIN content in control and sham rats was ~4%, whereas in hypoglycemic rats the contribution was ~12%. The ^{125}I-albumin measure of brain plasma volume may not include the small perivascular fluid volume, which presumably contains QUIN, and the calculations of the contribution of intravascular QUIN do not include QUIN that may be present within the red blood cells. Therefore, it is likely that the contribution of QUIN in the vasculature compartment in the hypoglycemic rats is >12%, and the changes in brain QUIN concentrations (Fig. 2) may be due to the presence of increased amounts of QUIN in the blood compartment within the brain tissue sample. Experimentally, it would be difficult to differentiate QUIN in the different compartments of a brain tissue sample.

QUIN is formed predominantly in the liver from L-TRP via metabolism through the kynurenine pathway. The metabolic origin of QUIN in the brain is unclear. However, because the blood–brain barrier is relatively impermeable to QUIN (Foster et al., 1984), it is likely that this compound is actually synthesized within brain, although the physiologic transfer of QUIN from blood to brain remains to be quantified. The fact that extracellular fluid QUIN concentrations did not change during either hypoglycemia or recovery (Fig. 3), despite increases in plasma QUIN concentrations (Fig. 1), is consistent with a low permeability of the blood–brain barrier to QUIN. Although regional brain L-TRP concentrations were increased during the first hour of the recovery period (Fig. 3), there were no changes in extracellular fluid QUIN concentrations as measured by the microdialysis probe. This observation may indicate that intracerebral L-TRP is a poor substrate for the synthesis of QUIN in brain or that increases in brain QUIN concentrations were restricted to the intracellular compartment.

There are several studies on the effects of systemic L-TRP administration and kynurenine pathway metabolites on hepatic glucose metabolism per se (see Bender, 1982). Systemic L-TRP administration reduces the rate of hepatic gluconeogenesis, most likely because of increased hepatic QUIN concentrations, chelation of iron by QUIN, and concomitant inhibition of hepatic phosphopyruvate carboxylase (Snoke et al., 1971). Gluconeogenesis is also reduced during hyperglycemia resulting from glucose administration. During hyperglycemia, plasma insulin concentrations are increased. If insulin per se increases the synthesis of QUIN in liver, as indicated by the plasma data (Fig. 1), QUIN may have a physiologic role in the inhibition of hepatic gluconeogenesis at the site of phosphopyruvate carboxylase during the hyperglycemia-induced secretion of insulin. Further studies are required to separate the effects of hypoglycemia and insulin in the present results (Fig. 1).

During hypoglycemia, regional brain 5-HT concentrations decreased at the same time as 5-HIAA concentrations increased. This increase in 5-HIAA may reflect the release of 5-HT from storage granules due to reduced energy stores to maintain the integrity of vesicular transport of the transmitter (Agardh et al., 1979). The same mechanism may account for the release of dopamine and concomitant increased synthesis of DOPAC and HVA in the striatum. The restoration of 5-HT concentrations during the recovery period may be related to increases in tryptophan hydroxylase activity in some brain regions (Agardh et al., 1979) as well as increases in the concentrations of L-TRP (Fig. 3) and conversion to 5-HT by a mass-action ratio effect (Fernstrom, 1983).

We conclude that although insulin-induced hypoglycemia is associated with increased systemic synthesis

of QUIN and perhaps an increase in QUIN concentrations within the intracellular compartment of brain, the concentrations of QUIN in the extracellular fluid space within brain are most likely unchanged. Our data do not support a direct role for QUIN in the early excitotoxic lesions associated with profound insulin-induced hypoglycemia and cerebral isoelectricity.

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