The Effect of Pronounced Hypoglycemia on Monoamine Metabolism in Rat Brain

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SUMMARY

The cerebral metabolism of catechol and indole amines was studied in paralyzed and lightly anesthetized (70% N_2O) rats in which severe hypoglycemia was induced by insulin. When blood glucose concentrations fell towards 1 μmol·g^{-1} and the electrocorticogram (EEG) showed high-amplitude slow waves with interspersed polyspikes, the tissue contents of dopamine (DA), norepinephrine (NE), and 5-hydroxytryptamine (5-HT) were reduced. The rate of hydroxylation of tyrosine, measured as accumulation of dihydroxyphenylalanine (dopa) following inhibition of aromatic L-amino acid decarboxylase with NSD 1015, rose in cortex and striatum, while tryptophan hydroxylation increased moderately in cortical tissue.

Aggravation of the hypoglycemia with cessation of spontaneous EEG activity was accompanied by progressive reduction in tissue contents of DA, NE, and 5-HT and by accumulation of 5-hydroxyindole acetic acid (5-HIAA). In this period, tyrosine hydroxylation rate was reduced in limbic areas and in striatum, but not in the cortex, while tryptophan hydroxylation was reduced in limbic areas only.

When recovery was induced for 45 min by i.v. glucose following 30 min recording of isoelectric EEG, there was extensive recovery of tissue concentrations of DA and 5-HT but incomplete recovery of NE concentrations. Tyrosine hydroxylation rate was increased in all areas, but tryptophan hydroxylation either remained at control levels (striatum and cortex) or fell (limbic area).

It is concluded that severe hypoglycemia leads to a gross derangement of brain monoamine metabolism, which seems to include an increase in NE turnover during both the precomatose phase and the recovery phase. **DIABETES 28:804–809, September 1979.**

Progressive hypoglycemia leads to a series of changes in brain function that culminates in coma and death. It is now well known that many of these functional changes and their electrocorticogram (EEG) correlates occur without a deterioration of cerebral energy stores, as judged from the tissue concentrations of adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP).[1–4] It is therefore tempting to assume that hypoglycemia interferes with the synthesis, storage, or release of cerebral transmitters. In support of this assumption it has been reported that hypoglycemia causes a pronounced perturbation in brain tissue concentrations of excitatory and inhibitory amino acids[3,5,6] and a reduction in acetylcholine synthesis.[7]

There is relatively meagre information on the effect of hypoglycemia on cerebral monoamine metabolism. The only systematic studies that have been published deal with the effect of insulin on tissue and blood concentrations of tryptophan and tyrosine.[8–13] These studies have shown that insulin, whether administered by injection or released endogenously, increases concentrations of tryptophan and tyrosine in the brain. The elevated tryptophan concentrations in tissue are accompanied by an increased 5-hydroxytryptamine (5-HT) turnover.[8,12,14] In one of these studies,[12] the authors found that moderate insulin-induced hypoglycemia had no effect on brain tissue concentrations of dopamine (DA) and norepinephrine (NE).

In the present experiments we studied the effect of severe insulin-induced hypoglycemia on the metabolism of catechol and indole amines in the brain. Two stages of hypoglycemia were studied. In one, the EEG showed high-amplitude slow waves with interspersed polyspikes. Previous results demonstrated that, in this state, tissue concentrations of labile organic phosphates are maintained close to normal levels.[3,4,6] In the other stage of hypoglycemia, all spontaneous EEG activity had ceased for 15 or 30 min. At this stage there is extensive deterioration of cerebral energy state. In separate groups of animals the hypoglycemia was reversed by injecting glucose at the end of a 30 min period of EEG silence. In all three main groups—slow wave–polyspike, isoelectric, and recovery—monoamine metabolism was

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Received for publication 13 December 1978 and in revised form 9 May 1979.

studied by analysis of both steady state levels and rate of synthesis.

METHODS

ANIMALS, OPERATIVE AND SAMPLING TECHNIQUES

Male rats (260 to 380 g) of an S.P.F.* Wistar strain (Møllegaard Avelslaboratorium, Copenhagen) were fasted for 24 h before the experiments but had free access to tap water. One hour before operation they were given 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 3% halothane; the animals were then tracheotomized, immobilized with i.v. injection of tubocurarine chloride (0.5 $mg \cdot kg^{-1}$), and ventilated with a Starling-type respirator, delivering 75% N_2O and 25% O_2, to yield an arterial P_{CO_2} of 30 to 40 mm Hg.

Body temperature was maintained close to 37 °C. One femoral artery was cannulated for blood pressure recording with an electromanometer and for sampling of blood, and one femoral vein was cannulated for injections. After the operative procedures had been completed the animals were maintained on the N_2O-O_2 gas mixture. Repeated arterial samples were anaerobically taken from the catheter in glass capillaries for determinations of pH, P_{CO_2}, and P_{O_2} or collected directly in liquid nitrogen for later analyses of glucose.

The EEG was continuously recorded in all animals from gold-plated copper bolts inserted into the skull bone in the frontoparietal region (bipolar leads) using an Elema EEG machine. Previous results from the laboratory have established the correlations between blood glucose concentration, EEG pattern, and cerebral metabolic state.[3,4,6,15] In the present experiments, monoamine metabolism was studied in two stages of hypoglycemia. In one, the EEG shows a pattern of pronounced slowing with some polyspike activity, which occurs at blood glucose concentrations of about 1 to 2 $\mu mol \cdot g^{-1}$. There is a pronounced perturbation of tissue concentrations of carbohydrate substrates and amino acids but cerebral energy state, as judged from the tissue concentrations of ATP, ADP, and AMP, is upheld. In the other stage, spontaneous EEG activity ceases (isoelectric EEG). This occurs at blood glucose concentrations of below 1 $\mu mol \cdot g^{-1}$. At this stage, cerebral energy state is extensively deranged.

EXPERIMENTAL GROUPS

There were two series of animals. In series A, consisting of four groups with six animals in each, the rate of hydroxylation of tyrosine and tryptophan in different brain regions was measured after inhibiting the aromatic L-amino acid decarboxylase with NSD 1015 (3-hydroxybenzylhydrazine HCl, 100 $mg \cdot kg^{-1}$, i.p.). This method for estimating rate of synthesis of biogenic amines was used because of its simplicity and because it does not require measurements of specific activities in precursor pools. Thirty minutes later, the animals were killed by decapitation and brain tissue was taken for analysis of dihydroxyphenylalanine (dopa) and 5-HT (see below). Before the injection of NSD 1015 and 30 min later, arterial blood was drawn for measurements of P_{O_2}, P_{CO_2}, pH, and glucose content.

Group A1. Control animals injected with Krebs-Henseleit solution. After 90 min of anesthesia, NSD 1015 was given i.p.

Group A2. Hypoglycemia, first stage. NSD 1015 was injected when the EEG showed a slow wave–polyspike pattern, which appeared 136 ± 8 min (SD) after administration of insulin. This pattern persisted during the 30 min experimental period and no animal showed periods of isoelectricity.

Group A3. Hypoglycemia, second stage. NSD 1015 was injected when spontaneous EEG activity ceased (167 ± 12 min after insulin injection). In all animals included, the EEG record remained isoelectric during the 30 min before decapitation.

Group A4. Recovery. After a 30 min period of isoelectric EEG recovery was induced by an i.v. injection of 0.5 ml 50% (wt/vol) glucose followed by a slow i.v. infusion of the glucose solution (1 $ml \cdot h^{-1}$) (201 ± 30 min after insulin injection). NSD 1015 was administered 15 min after the initial injection of glucose.

In series B, endogenous concentrations of catecholamines were measured. There were five groups with six animals in each. In four groups, the experimental procedures were identical to those of series A with the exception that no NSD 1015 was injected. In addition, an extra group was included. In this, the animals were killed by decapitation when spontaneous EEG activity had ceased for 15 min. The time sequences were the same as in series A.

ANALYTIC TECHNIQUES

After death, the brains were quickly removed and dissected on an ice-cold Petri dish according to Carlsson and Lindqvist.[16] The cerebral hemispheres were divided in three portions: limbic forebrain, striatum, and the remaining part of the hemispheres (in the following, called cortex). After dissection, the brain samples were frozen on dry ice, weighed, and stored at −80 °C until analysis. The extraction of amines, precursors, and metabolites was done after homogenization in 25 ml plastic tubes, containing 10 ml of ice-cold 0.4 N perchloric acid, 0.2 ml of 10% EDTA, and 0.1 ml of 5% $Na_2S_2O_5$. The extract was purified on a strongly acidic cation exchange column (Dowex 50, X-4) according to Atack and Magnusson[17] and Kehr et al.[18] Concentrations of NE, DA, dopa, 5-hydroxyindole acetic acid (5-HIAA), 5-HT, 5-hydroxytryptophan (5-HTP), tyrosine, and tryptophan were measured with fluorometric techniques. For references, see Carlsson et al.[19]

STATISTICS

Statistics were done by Student's *t* test or by one-way analysis of variance followed by Student's *t* test. The following symbols were used: *$P < 0.05$, †$P < 0.01$, and ‡$P < 0.001$.

RESULTS

Since the objective of the study was to evaluate the influence of pronounced hypoglycemia on brain monoamine metabolism, it was essential to exclude an influence of systematic variables. Table 1 shows that neither blood pressure nor arterial P_{O_2} fell during the period of isoelectric EEG or in the recovery period following glucose injection. Arterial P_{CO_2} was between 33 and 43 mm Hg in all groups, and body temperature was within 0.5 °C of control. Arterial pH fell moderately in the recovery groups.

* S.P.F. = specific pathogen-free.

TABLE 1
Physiologic parameters during insulin-induced hypoglycemia and in the recovery period that followed glucose injection. Series A with NSD 1015.

Group	No. of animals	MABP* (mm Hg)	Pa_{O_2} (mm Hg)	Pa_{CO_2} (mm Hg)	pH	Temp. (°C)
Series A						
Controls	6	144 ± 7	91 ± 4	40.3 ± 1.4	7.304 ± 0.008	37.4 ± 0.2
Slow wave–polyspike EEG	6	146 ± 8	109 ± 6	43.0 ± 0.9	7.279 ± 0.019	37.5 ± 0.2
Isoelectric EEG (30 min)	6	148 ± 9	97 ± 5	33.4 ± 2.1	7.243 ± 0.025	36.9 ± 0.2
Recovery	6	145 ± 8	105 ± 4	36.0 ± 2.8	7.198 ± 0.042	37.4 ± 0.1
Series B						
Controls	6	159 ± 6	103 ± 5	37.0 ± 0.5	7.362 ± 0.009	37.1 ± 0.2
Slow wave–polyspike EEG	6	133 ± 4	111 ± 4	36.4 ± 1.4	7.350 ± 0.028	36.8 ± 0.2
Isoelectric EEG (15 min)	6	171 ± 13	109 ± 4	34.3 ± 1.5	7.379 ± 0.028	37.2 ± 0.1
Isoelectric EEG (30 min)	6	168 ± 12	109 ± 5	36.4 ± 2.7	7.343 ± 0.036	37.4 ± 0.2
Recovery	6	150 ± 3	116 ± 9	33.7 ± 1.4	7.283 ± 0.041	37.2 ± 0.2

* MABP: mean arterial blood pressure.
The values are means ± SEM.

In each experiment the EEG pattern was correlated to blood glucose concentrations (Figure 1). During the 30 min period of slow waves and polyspikes, blood glucose fell from 1.6 ± 0.1 to 1.01 ± 0.1 $\mu mol \cdot g^{-1}$ (M ± SEM). Spontaneous EEG activity disappeared when blood glucose concentrations decreased to values around or below 1 $\mu mol \cdot g^{-1}$ (cf. 4,15). During recovery, most animals had blood glucose concentrations in the range of 8.5 to 10 $\mu mol \cdot g^{-1}$.

ENDOGENOUS LEVELS OF MONOAMINES, PRECURSORS, AND 5-HIAA

Table 2 shows the endogenous concentrations of tyrosine, DA, and NE. Except for a small decrease in limbic forebrain concentration in the slow wave–polyspike group, the tyrosine concentrations remained unchanged in all hypoglycemic groups. Recovery following a 30 min period of isoelectric EEG recording was accompanied by a significant rise in tissue concentrations of tyrosine.

Hypoglycemia was accompanied by pronounced decreases in DA and NE concentrations. In all groups but one these decreases were statistically significant already in the slow wave–polyspike group and progressive decreases occurred during the period of abolished EEG activity. After 30 min of isoelectric EEG recording, striatal DA content was reduced to 30% of control and cortical NE content to 45% of control. After injection of glucose, there was extensive repletion of DA. Partial repletion of NE occurred in striatum and cortex but not in the limbic area.

The corresponding values for tryptophan, 5-HT, and 5-HIAA are shown in Table 3. Tissue concentrations of tryptophan remained unchanged in the hypoglycemic animals but rose during recovery (cf. tyrosine values in Table 2). Tissue concentrations of 5-HT fell progressively with the severity of the hypoglycemia and two regions showed decreases during the slow wave–polyspike phase. Injection of glucose gave rise to complete (limbic area, striatum) or

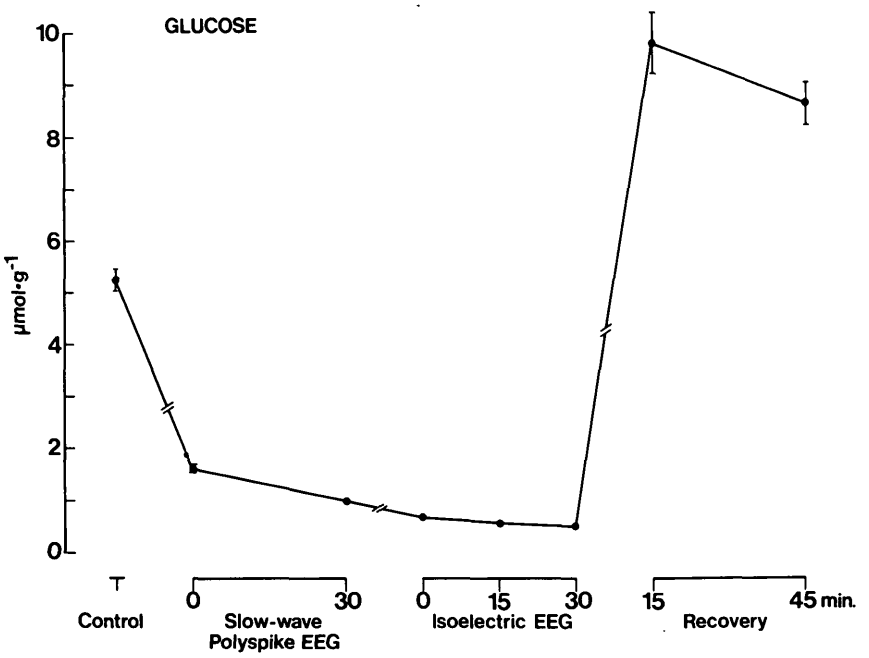

FIGURE 1. Blood glucose concentrations in the different stages of hypoglycemia and in the recovery period following an isoelectric period of 30 min. The results are means. SEM is given as vertical bars, if larger than symbol.

TABLE 2
Tyrosine and catecholamine concentrations during hypoglycemia and in the recovery period following 30 min of isoelectric EEG

	Control (saline)	Slow wave polyspike	ISO-EEG (15 min)	ISO-EEG (30 min)	Recovery
Tyrosine (μg/g)					
Limbic	14.2 ± 0.6	11.8 ± 0.6*	14.0 ± 0.6	15.6 ± 0.4	20.4 ± 0.6†
Striatum	13.9 ± 1.1	11.7 ± 0.9	12.5 ± 0.2	15.1 ± 1.5	23.2 ± 1.3†
Cortex	12.2 ± 0.8	12.1 ± 1.1	12.5 ± 0.6	13.2 ± 0.9	22.7 ± 1.2†
Dopamine (ng/g)					
Limbic	1176 ± 16	973 ± 29*	549 ± 23‡	501 ± 19‡	849 ± 111†
Striatum	3122 ± 156	2588 ± 152†	1316 ± 66‡	843 ± 30‡	3205 ± 36
Cortex	58 ± 13	21 ± 6†	19 ± 6†	6 ± 4‡	41 ± 5
Norepinephrine (ng/g)					
Limbic	554 ± 16	445 ± 9‡	311 ± 17‡	297 ± 7‡	286 ± 9‡
Striatum	187 ± 9	161 ± 13	129 ± 16†	115 ± 10†	152 ± 11
Cortex	298 ± 7	219 ± 18†	157 ± 13‡	125 ± 5‡	183 ± 13‡

* $P < 0.05$. † $P < 0.01$. ‡ $P < 0.001$. Shown are the means ± SEM. N = 3. Each experiment comprises pooled brain parts of two rats.

partial (cortex) recovery. The 5-HIAA concentrations increased during hypoglycemia although statistical significance was not reached in the striatum. The 5-HIAA values were still increased in the recovery group.

MONOAMINE SYNTHESIS

Table 4 shows the activity of the tyrosine and tryptophan hydroxylases in vivo, measured as the accumulation of dopa and 5-HTP following administration of NSD 1015 30 min before sampling of tissue. During the period with slow waves and polyspikes in the EEG, there was a significant increase in tyrosine hydroxylation in striatum ($P < 0.05$) and cortex ($P < 0.001$) but no change in the limbic area. In the isoelectric period, tyrosine hydroxylation decreased significantly in the dopamine-rich regions, i.e., the striatum and the limbic area (by 43% and 59%, respectively), but no change from control occurred in the cortex. During recovery the rate of tyrosine hydroxylation increased considerably in all three regions. In contrast, tryptophan hydroxylation did not change much. In the limbic area, the rate of hydroxylation fell in the isoelectric and the recovery groups. The results suggest that cortical 5-HT synthesis was enhanced in the slow wave–polyspike period.

In view of the possibility that large doses of insulin could by themselves influence turnover or steady state levels of amines, these were studied in a few preliminary experiments in which hypoglycemia was prevented by glucose infusion. Our results indicate that the effects of insulin per se are small and that the results presented here were mainly an effect of hypoglycemia.

DISCUSSION

The present results have shown that pronounced insulin-induced hypoglycemia is accompanied by marked changes in brain monoamine metabolism that affect both the steady state levels of amines and their rates of synthesis. Since the two levels of hypoglycemia studied differ markedly with respect to the accompanying effects on cerebral energy state, it seems profitable to discuss them separately.

TABLE 3
Tryptophan, 5-HT, and 5-HIAA concentrations during hypoglycemia and in the recovery period following 30 min of isoelectric EEG

	Control (saline)	Slow-wave polyspike	ISO-EEG (15 min)	ISO-EEG (30 min)	Recovery
Tryptophan (μg/g)					
Limbic	7.0 ± 0.2	6.2 ± 0.5	6.0 ± 0.1	6.8 ± 0.7	9.7 ± 0.1†
Striatum	5.9 ± 0.5	5.0 ± 0.3	4.5 ± 0.4	5.4 ± 0.6	7.4 ± 0.6*
Cortex	4.6 ± 0.1	4.4 ± 0.5	3.9 ± 0.1	4.6 ± 0.5	8.0 ± 0.2‡
5-HT (ng/g)					
Limbic	368 ± .3	309 ± 9*	196 ± 6‡	158 ± 5‡	323 ± 24
Striatum	232 ± 37	252 ± 22	116 ± 10†	110 ± 0*	201 ± 29
Cortex	255 ± 7	188 ± 3‡	101 ± 6‡	88 ± 7‡	176 ± 14‡
5-HIAA (ng/g)					
Limbic	399 ± 8	487 ± 8	728 ± 16‡	765 ± 35‡	625 ± 59‡
Striatum	361 ± 55	449 ± 43	500 ± 53	471 ± 87	562 ± 72
Cortex	282 ± 11	322 ± 21	450 ± 13‡	424 ± 26‡	385 ± 24†

* $P < 0.05$. † $P < 0.01$. ‡ $P < 0.001$. Shown are the means ± SEM. N = 3. Each experiment comprises pooled brain parts of two rats.

TABLE 4
Dopa and 5-HTP accumulation during hypoglycemia and in the recovery period following 30 min of isoelectric EEG

	Dopa (ng/g)				5-HTP (ng/g)			
	Control (NSD 1015)	Slow-wave–polyspike EEG	Isoelectric EEG (30 min)	Recovery	Control (NSD 1015)	Slow-wave–polyspike EEG	Isoelectric EEG (30 min)	Recovery
Limbic	283 ± 14	300 ± 19	167 ± 5*	426 ± 44†	232 ± 17	247 ± 20	174 ± 6*	166 ± 6†
Striatum	369 ± 8	480 ± 17*	160 ± 35†	717 ± 50‡	163 ± 15	189 ± 12	126 ± 7	153 ± 11
Cortex	51 ± 2	78 ± 5‡	59 ± 4	140 ± 2‡	119 ± 4	152 ± 8*	106 ± 11	98 ± 8
No. of experiments	3	3	3	3	3	3	3	3

* $P < 0.05$. † $P < 0.01$. ‡ $P < 0.001$. All animals received an i.p. injection of NSD 1015, 100 mg·kg^{-1}, 30 min before death. Shown are the means ± SEM. Each experiment comprises pooled brain parts of two rats.

Period of slow waves and polyspikes. In this period, the tissue concentrations of NE, DA, and 5-HT fell. Since hypoglycemia is considered a stressful situation,[12] changes in NE metabolism are particularly interesting. In the cortex there was a highly significant reduction in NE content and a highly significant increase in rate of tyrosine hydroxylation, demonstrating that NE turnover was enhanced. Since changes in striatal DA content and tyrosine hydroxylation rate were similar, it can be concluded that DA turnover was increased as well. It is less clear if there was a corresponding change in 5-HT turnover. Thus, only cortical tissue showed a significant fall in 5-HT content and an increase in tryptophan hydroxylation rate, but only limbic areas showed a significant rise in 5-HIAA content. Thus, it seems probable that there was an imbalance between rates of synthesis and degradation of 5-HT in the pre-experimental period. Alternatively, the storage of 5-HT could have been inadequate.

Period of EEG flattening. In this period the tissue contents of NE, DA, and 5-HT fell to low values. Changes in NE content in cortical tissue occurred in spite of an unchanged rate of synthesis, suggesting enhanced release and metabolism of the amine. Since striatal (and limbic) tyrosine hydroxylation rate fell, it seems probable that reduced synthesis contributed to the marked reduction in DA content. The fall in 5-HT content occurred in spite of an unchanged or only slightly reduced rate of tryptophan hydroxylation, and, since tissue concentrations of 5-HIAA rose substantially, the results suggest that the release and subsequent metabolism of 5-HT were enhanced.

Period of recovery. The recovery was characterized by increased tissue levels of tyrosine and tryptophan and by varying recovery of the tissue concentrations of NE, DA, and 5-HT. However, the data indicate that recovery of metabolism of these three amines differed. The markedly slow normalization of NA content in spite of a pronounced enhancement of tyrosine hydroxylation rate also in the cortex suggests that an accelerated release of NE may have persisted in this period. In the striatum, DA content was restored with an increased rate of synthesis of about 100%. In the limbic areas the dopaminergic system seemed to recover more slowly, with transmitter levels still significantly reduced during the early recovery phase. The dopa values after NSD 1015 were still elevated at this stage, indicating a rapid turnover. The 5-HT content, finally, was normalized in the limbic and striatal samples though not in the cortex. The levels of 5-HIAA tended to remain high in the recovery group in spite of a normal or reduced tryptophan hydroxylation rate. The indoleamine data during recovery, thus, represent a complex pattern that does not lend itself readily to interpretation in terms of turnover.

Relationships to cerebral energy metabolism. Using similar techniques, the present laboratories have previously studied brain monoamine metabolism in a postischemic situation and in bicuculline-induced status epilepticus.[20,21] In both conditions, the results were compatible with a marked acceleration of NE turnover. Similarly, results obtained in hypercapnia showed an increase in tyrosine hydroxylation rate that was not caused by the increased tissue oxygen tensions.[22] These results make it tempting to conclude that norepinephrine turnover is increased in conditions of stress, e.g., with tissue acidosis. The present results show, however, that cellular acidosis cannot be the only triggering factor. Hypoglycemia leads to energy failure in the absence of acidosis.[3] It is possible that the large decreases in tissue contents of NE, DA, and 5-HT during the period of EEG flattening were partly caused by energy failure and lack of ATP for sequestration of amines in storage granules. Previous results have shown that, in the anesthetized rat, the period of EEG flattening is accompanied by a rise in cerebral blood flow.[6] Since tissue oxygenation is upheld, conditions would thus favor degradation of amines by both COMT and MAO. It is more difficult to explain why the reduced amine contents did not trigger an increased rate of hydroxylation of tyrosine and tryptophan, since substrate and oxygen must have been present in adequate amounts. Possibly, the inability to synthesize amines may be a result of the particular metabolic defect occurring in hypoglycemic coma, i.e., the combination of energy failure and reduction of cellular oxidation/reduction systems.[3] Whether such a condition can lead to reduced availability of the cofactor tetrahydrobiopterin, perhaps caused by the inhibition of pteridine reductase, remains to be investigated.

Relationships to previous work. Since most previous data pertain to degrees of hypoglycemia that are less severe than those studied presently, the results are not directly comparable. Gordon and Meldrum,[14] who injected 10 I.U. insulin into unanesthetized rats, 150 to 180 g, found that blood glucose concentrations fell progressively to reach levels of 15 to 20 mg/dl blood after 4 h. At 1 to 3 h, brain 5-HT content rose by about 10% to reach control levels after 4 h. Since 5-HIAA content increased at 2 to 4 h, it was

concluded that 5-HT turnover was enhanced. This conclusion is corroborated by the present results obtained on cortical tissue.

Previous results in rats, showing that insulin-induced hypoglycemia does not affect tissue concentration of DA and NE, were obtained with a less severe degree of hypoglycemia, one that did not induce convulsions.[12] These results, and the present ones, suggest that pronounced alterations in catecholamine metabolism only occurs when blood glucose concentrations fall below 2 $\mu mol \cdot g^{-1}$ and when gross symptoms such as convulsions and stupor are present.

ACKNOWLEDGMENTS

The skilful technical assistance of Kerstin Beirup, Barbro Jörblad, and Gerd Leonsson is gratefully acknowledged. The study was supported by grants from the Swedish Medical Research Council (155 and 14x-263), from the USPHS (5 RO1 NS 07838-8 from NIH), from the Swedish Diabetes Foundation, and from the Knut and Alice Wallenberg Foundation.

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