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Brain 3-Methoxytyramine Varies Inversely With Blood Glucose in Decapitated Rats[1]

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CHANCE, W. T., L. CAO AND J. E. FISCHER. *Brain 3-methoxytyramine varies inversely with blood glucose in decapitated rats.* PHARMACOL BIOCHEM BEHAV 32(2) 553–556, 1989.—Concentrations of the dopamine metabolite, 3-methoxytyramine, were decreased significantly in the corpus striatum and nucleus accumbens of rats 30 min after the IP injection of D-glucose (2 g/kg). Conversely, 90 min after the administration of regular insulin (6 U/kg), significant increases in the concentrations of 3-methoxytyramine were observed in these two brain regions. Brain levels of the major metabolites of dopamine, 3,4-dihydroxyphenylacetic acid and homovanillic acid, did not correlate well with blood glucose concentration. The significant negative correlation of blood glucose with striatal and accumbens 3-methoxytyramine suggests an inverse relationship between dopamine metabolism and blood glucose concentration, that may be secondary to decapitation-induced anoxia.

Glucose Insulin Dopamine 3-Methoxytyramine

CONSIDERING that glucose is the major fuel of brain tissues, one would expect that neurotransmitter metabolism might be sensitive to abrupt changes in concentration of this substrate in circulating blood. Investigations of the effect of blood glucose upon brain neurotransmitter metabolism have focused upon dopamine (DA) and have thus far produced rather inconsistent results. Thus, Saller and Chiodo (13) reported that the administration of glucose suppressed the firing of DA neurons within the substantia nigra. In addition, Montefusco *et al.* (11) observed decreased concentrations of the DA metabolite, 3, 4-dihydroxyphenylacetic acid (DOPAC) in the corpus striatum and olfactory tubercle 15 min after the IV injection of glucose. These results have been challenged, however, by reports of the lack of effect of injected glucose upon DA neurons in the awake cat (15,17). In addition, Westerink and Spaan (21) observed no alteration in DA metabolism following IV plus IP administration of glucose.

Since these experiments have provided inconsistent results concerning the effect of hyperglycemia upon DA metabolism in the brain, in the present experiment we have extended these observations by examining the effects of both hypoglycemia and hyperglycemia on DA metabolism in specific brain regions.

METHOD

Forty-two male Sprague-Dawley rats (340 to 450 g) were obtained from Zivic-Miller Laboratories (Zelienople, PA) and employed as subjects in this experiment. These animals were adapated to our laboratory environment for several weeks and were group-housed under ad lib conditions.

Experimental or control treatments were assigned randomly across seven groups of six rats each. D-glucose (dextrose) was administered to three groups of rats at a dose of 2 g/kg, IP, while three additional groups were treated with 6 U/kg regular insulin (Squibb, Princeton, NJ), SC. The six remaining rats were treated (IP) with normal saline as a control procedure. Six rats from each of these treatment groups were sacrificed 30 min after the injections, while additional groups of glucose-treated and insulin-treated rats were decapitated 60 and 90 min after the treatments. Blood was collected from the cervical wound for the analysis of plasma glucose concentrations using a Yellow Springs Instruments glucose analyzer (Yellow Springs, OH). The brains were removed rapidly from the calvaria and dissected over ice into the hypothalamus (60 to 90 sec), corpus striatum (90 to 120 sec), and nucleus accumbens (90 to 120 sec) regions according to our previous report (3) prior to freezing in liquid nitrogen.

Concentrations of DA, norepinephrine (NE), tyrosine (Tyr) and the DA metabolites, DOPAC, 3-methoxytyramine (3-MT) and homovanillic acid (HVA) were determined in each of the brain regions by high performance liquid chromatography (HPLC) according to our previous report (3). Each sample was homogenized in 3 ml of 1 N formic acid/acetone (15:85, vol:vol) containing N-methyl-dopamine as an internal standard. After centrifugation (10,000×g, 10 min, 4°C), 7.5 ml of heptane/chloroform (8:1, vol:vol) was added to 2.5 ml of each supernatant. Following shaking and centrifugation

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(2,500×g, 10 min, 4°C), the organic phase of each sample was discarded. The remaining aqueous phase of the samples was reconstituted in 0.8 ml of the mobile phase HPLC buffer (8% acetonitrile and 92% of 0.1 M KH_2PO_4, 0.185 mM sodium octylsulfate and 0.195 mM EDTA, pH=2.90). Fifty microliters of each sample was injected onto the HPLC column (Altex, reverse phase, C-18, ultrasphere), with the amines being quantified by amperometric detection (Bioanalytical Systems Model LC-4, West Lafayette, IN) at a potential of 0.90 V and a flow rate of 1.0 ml/min.

All data were evaluated by analysis of variance (ANOVA) techniques, with individual means being compared post hoc by preplanned *t*-tests.

RESULTS

Plasma glucose concentrations were increased significantly ($p<0.01$) for only 30 min following the IP injection of dextrose (30 min=243±34 mg%, 60 min=145±2 mg%, 90 min=150±7 mg%, control=137±mg%). Levels of plasma glucose were significantly ($p<0.01$) reduced in all insulin-treated groups, with the animals sacrificed 90 min after the insulin injection exhibiting the lowest plasma glucose concentrations (30 min=67±3 mg%, 60 min=59±4 mg%, 90 min=54±4 mg%).

Figure 1 illustrates the alterations in regional brain catecholamine metabolism as percent of control values. The regional brain concentrations of these compounds in control rats are presented in Table 1.

As may be observed from Fig. 1 (panels A and B), only the DA metabolite, 3-MT, exhibited consistent alterations that paralleled hyperglycemia and hypoglycemia. Thus, 30 min after the injection of glucose, when the rats were the most hyperglycemic, 3-MT concentrations were decreased significantly in the corpus striatum (panel A) and nucleus accumbens (panel B). Conversely, when the rats were the most hypoglycemic 60 and 90 min after the insulin injections, 3-MT levels were increased significantly in these two brain regions.

Additional changes in neurochemistry were observed, with Tyr concentrations being elevated in each brain region 30 min after the insulin injections, DOPAC being elevated in the hypothalamus (panel C) 90 min after the insulin treatment, and DA being decreased in the corpus striatum and nucleus accumbens 30 min after the injection of glucose. However, DA was also decreased in the corpus striatum 30 min after the insulin treatments. Therefore, the only alteration in brain chemistry that was well correlated with circulating glucose levels was 3-MT. Thus, plasma glucose concentration correlated significantly ($p<0.01$) with brain 3-MT in the corpus striatum ($r=-.77$) and nucleus accumbens ($r=-.76$) across the 42 samples.

DISCUSSION

The present results demonstrate that concentrations of 3-MT in the corpus striatum and nucleus accumbens vary inversely with circulating blood glucose levels. However, the transient nature of this metabolite of DA complicates the interpretation of these observations. 3-MT is formed from the O-methylation of DA, being catalyzed by the enzyme catechol-O-methyltransferase (COMT) (14). Since COMT is localized primarily extraneuronally (7,18), concentrations of 3-MT were suggested to reflect the metabolism of DA within the area of the synapse (2,8). Monoamine oxidase (MAO) mediates the oxidation of 3-MT to HVA (10) which is then

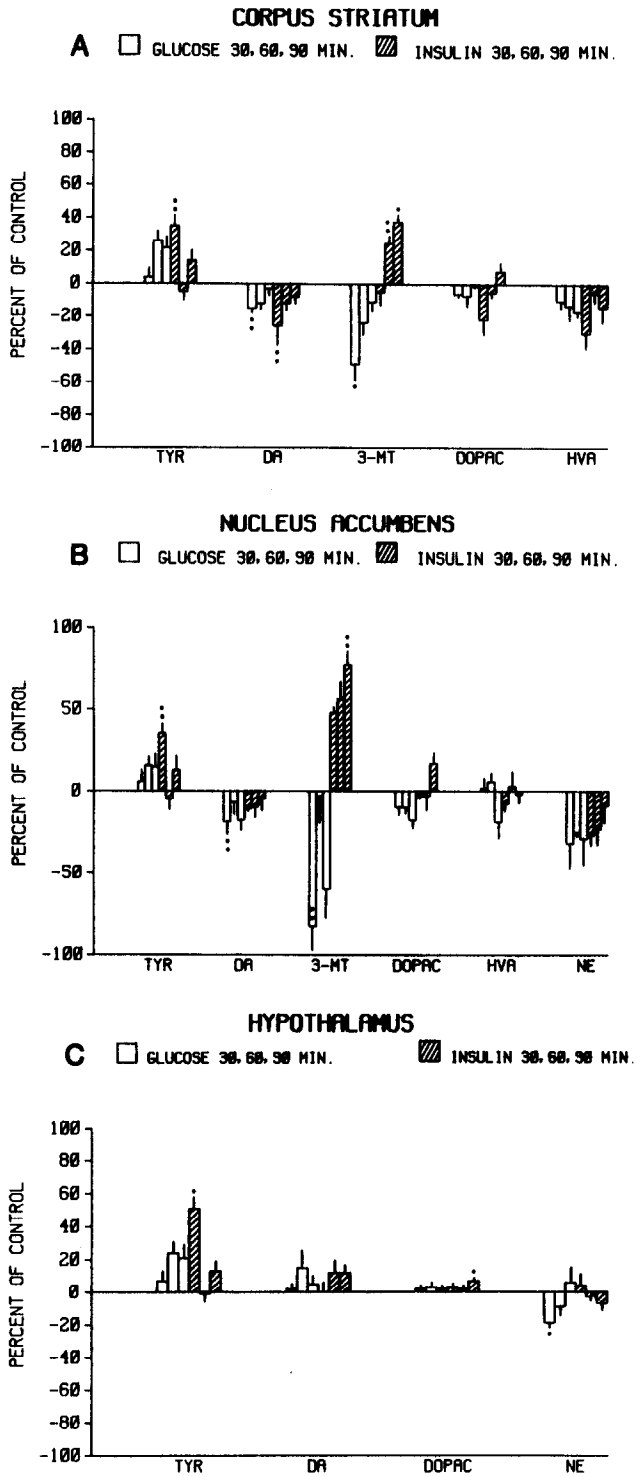

FIG. 1. Mean (±SEM) concentrations of catecholamine neurotransmitters (DA and NE), precursor (Tyr) and DA metabolites (3-MT, DOPAC and HVA) in three brain regions following glucose or insulin treatments. Data are presented sequentially and represent mean values for six rats presented as percentage of control means. *$p<0.01$; **$p<0.05$ vs. control group.

TABLE 1

MEAN (±SEM) CONCENTRATIONS OF THE CATECHOLAMINES (DA, NE), PRECURSOR (Tyr) AND DA METABOLITES (3-MT, DOPAC, HVA) IN THREE BRAIN REGIONS OF CONTROL RATS

	Tyr (μg/g)	DA (ng/g)	3-MT (ng/g)	DOPAC (ng/g)	HVA (ng/g)	NE (ng/g)
Corpus striatum	14.6 ± 1.3	7,475 ± 488	106 ± 9	807 ± 64	621 ± 67	N.D.
Nucleus accumbens	10.7 ± 0.8	4,568 ± 247	62 ± 15	784 ± 67	398 ± 23	571 ± 86
Hypothalamus	9.0 ± 0.8	409 ± 26	N.D.	548 ± 7	N.D.	1,749 ± 91

N.D.=not detectable.

transported out of the brain. Since MAO requires oxygen as a reactant, activity of this enzyme is much reduced during hypoxia. Therefore, concentrations of 3-MT accumulate rapidly after death unless the enzymes are denatured by microwave irradiation (20).

The present study examined the accumulation of 3-MT that occurs when rats are sacrificed by decapitation. Although the striata were typically frozen within 2 min of sacrifice, considerable accumulation of 3-MT had occurred, as evidenced by the concentration being roughly 3 to 5 times that observed in microwave-sacrificed rats (20,22). In spite of these post-mortem alterations, however, major differences between the various treatment groups were observed. In addition, these changes were both below and above control levels, suggesting differential effects of the treatments upon the formation or catabolism of 3-MT.

The differences in 3-MT concentration may be caused by alterations in the synthesis and release of DA or by changes in the activity of COMT. Since it is unlikely that moderate hyperglycemia or hypoglycemia would alter COMT, the alterations in 3-MT level may reflect increases and decreases in the release or reuptake of DA. The concentration of 3-MT has been suggested to be a good indicator of newly-released DA (20,22); however, this idea has been challenged by recent results suggesting that measurable 3-MT is merely an artefact of sacrificing the animal (19). Regardless of whether the increase in 3-MT exists prior to or only after death, it does suggest differential metabolism of DA in response to varying glucose concentrations.

These alterations in 3-MT are in the opposite direction of what would be predicted by a simple energy availability hypothesis. Thus, if DA continued to be released and 3-MT formed at a faster rate in the presence of excess glucose, elevated 3-MT should be associated with hyperglycemia.

Another problem in interpreting these results is the absence of a relationship between brain DOPAC concentration and blood glucose level. An explanation for this differential change in DA metabolites may be related to post-mortem anoxia. Since COMT does not require oxygen for continued activity, the metabolic pathway of DA metabolism is shifted toward the formation of 3-MT in decapitated rats. In addition, the reuptake of DA into the nerve terminals is an energy-requiring event (6). Therefore, the hypoglycemia-induced elevation in 3-MT may be related to decreased DA reuptake during the mild energy deficiency prior to sacrifice. Conversely, an energy surfeit induced by hyperglycemia may favor more efficient DA reuptake just prior to sacrifice and result in reduced 3-MT concentrations.

Support for an effect of glucose or insulin on DA metabolism comes from research with diabetic rats. Thus, DA synthesis and metabolism are reduced in diabetic rats and normalized upon insulin therapy (12,16). However, brain levels of the DA precursor, Tyr, are also reduced in diabetic rats and are increased to normal concentrations following the injection of insulin (4). Therefore, in these investigations in diabetic rats, decreased brain availability of Tyr may be a major factor in the altered DA metabolism (9). In the present study, concentrations of Tyr were altered significantly only 30 min after the injection of insulin. Since the increase in brain Tyr concentration did not coincide with the increase in DA metabolites, the impact of precursor availability on the observed alterations in 3-MT appear minimal. Therefore, these changes in DA metabolism appear to be related specifically to alterations in blood glucose concentration.

Although several reports indicate an increase in DA metabolism associated with feeding (1, 3, 5), the only study demonstrating an alteration of DA metabolism associated with glucose treatment reported decreased DOPAC concentrations shortly after glucose administration (11). Since the experiments reporting elevated DA metabolism associated with feeding assayed DA and metabolites one hour after presenting the food to the rats, these studies may be observing elevated DA metabolism secondary to elevated insulin release or increased precursor availability. However, these studies may not be comparable to the present observations, since the only alteration in DOPAC in the present experiment was an increase in hypothalamic concentration 90 min after the injection of insulin. An additional problem in interpreting these results is the reported absence of effect of glucose on striatal DOPAC, HVA and 3-MT in microwave-irradiated rats (21). Therefore, the significance of these alterations in 3-MT to glucose metabolism remains to be determined and will require further study.

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