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Effects of Short Term Experimental Diabetes on Brain Serotonin Metabolism

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Serotonin metabolism was studied in several brain regions of control and Streptozotocin-treated male Wistar rats. After induction of diabetes, the animals were killed at 24 hours. Concentrations of brain tryptophan show a generalized increase in all brain regions, being only significant in medulla-pons. Serotonin levels do not change, while 5-HIAA concentrations, as well as the ratio 5-HIAA/5-HT, show significant increases in medulla-pons and midbrain.

Key words: Experimental diabetes, Serotonin metabolism.

It is well documented that diabetic patients always suffer from behavioral and psychological disorders (15, 21). The cause of these abnormalities is somewhat uncertain. Studies in experimental animals indicate that these disorders might result in changes of metabolism of neurotransmitters in brain. Several authors, for example reported 30-50 % decreases in brain tryptophan levels but no accompanying changes in the concentration of brain serotonin in diabetic rats (17, 18, 25). However, a significant decrease in the synthesis rate of brain serotonin was reported in long-term experimental diabetes (8, 25). As the previous works were the result of long-term studies from the beginning of the diabetes development, they did not study variations in short times. The present work, therefore aims to study the monoamine content of different brain regions in untreated streptozotocin-diabetic rats after 24 hours from its induction.

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Materials and Methods

Male Wistar rats weighing 250-300 g were housed in groups of 5 per cage under a controlled temperature (22±1 °C), with a schedule of 12 h light and 12 h darkness (light on at 08.00 h). Feed (standard lab-

oratory, Purina chow) and water were provided *ad libitum*. The animals were fasted overnight and diabetes was induced by a single injection of Streptozotocin (STZ) (Sigma) (75 mg/kg b.w., i.p.) dissolved in citrate buffer pH 4.5 (23). Control animals only received a buffer injection.

Rats were killed by decapitation 24 h after STZ injection. The brains were quickly removed and dissected on an iced-surface into cortex, striatum, hippocampus, hypothalamus, midbrain and medulla-pons, according to the method of MACEWEN and PFAFF (16). Mean tissue weights ± SD obtained by this procedure expressed in mg were: cortex: 808 ± 36; striatum: 95 ± 6; hippocampus: 106 ± 8; hypothalamus: 44 ± 3; midbrain: 137 ± 6, and medulla-pons: 194 ± 9. After weighing, each region was homogenized into acidified butanol and employed for the tryptophan, serotonin and 5-hydroxiindolacetic acid (5-HIAA) determination, according to the methods of BLOXAM and WARREN (4) and CURZON and GREEN (10) respectively, with minor modifications.

Blood was collected at the time of death for determination of glucose levels, using the *o*-toluidine method of COOPER and MACDANIEL (6).

Statistical analysis of results were performed using the Student's *t* Test.

Results

Rats with serum glucose levels in excess of 250 mg % were considered diabetic. Serum glucose concentrations in the diabetic rats were 551 ± 2 mg % (mean ± SD) and the control animals had 150 ± 3 mg %. Both serotonin and 5-HIAA levels in the studied brain regions, as well as the effect of STZ-induced diabetes on these biogenic amines, are presented in table I, which also shows the tryptophan levels in control and treated rats. The diabetic state induced significant increases, just at 24 h from its induction, in medulla-pons tryptophan content, as well as in medulla-pons and midbrain 5-HIAA levels. The ratios of 5-HIAA to serotonin in rat brain regions are also shown in table I; significant

Table I. *Changes in tryptophan, serotonin and 5-HIAA concentrations (μg/g) in various brain regions of control and diabetic male Wistar rats.*

Values are means ± S.D. N = number of rats. 5-HIAA/5-HT are the ratio of 5-HIAA levels to 5-HT levels of control and diabetic groups. The statistical significance of the differences between means was calculated by Student's t-test. The significance of the differences is indicated as follows: * $p < 0.050$; ** $p < 0.025$; *** $p < 0.001$.

Group	N	Medulla-pons	Midbrain	Hypothalamus	Striatum	Hippocampus	Cortex	Whole brain
Tryptophan								
Control	(9)	2.35 ± 0.19	3.16 ± 0.29	5.77 ± 0.34	3.56 ± 0.23	3.12 ± 0.16	0.93 ± 0.11	2.05 ± 0.14
Diabetic	(12)	3.00 ± 0.11**	3.69 ± 0.19	6.52 ± 0.48	3.90 ± 0.15	3.51 ± 0.20	0.97 ± 0.05	2.20 ± 0.05
Serotonin								
Control	(9)	0.73 ± 0.08	0.87 ± 0.04	1.06 ± 0.13	0.71 ± 0.06	0.52 ± 0.03	0.28 ± 0.02	0.61 ± 0.04
Diabetic	(12)	0.71 ± 0.04	0.89 ± 0.03	1.12 ± 0.13	0.71 ± 0.05	0.57 ± 0.05	0.26 ± 0.01	0.64 ± 0.02
5-HIAA								
Control	(9)	0.68 ± 0.05	1.25 ± 0.09	1.11 ± 0.19	1.24 ± 0.22	1.05 ± 0.11	0.29 ± 0.03	0.78 ± 0.05
Diabetic	(12)	1.14 ± 0.06***	1.62 ± 0.10**	1.39 ± 0.19	1.59 ± 0.11	1.15 ± 0.14	0.33 ± 0.02	0.90 ± 0.04
5-HIAA/5-HT								
Control	(9)	1.00 ± 0.12	1.44 ± 0.10	1.18 ± 0.27	1.78 ± 0.35	2.09 ± 0.27	1.04 ± 0.10	1.32 ± 0.12
Diabetic	(12)	1.67 ± 0.15**	1.81 ± 0.10*	1.43 ± 0.27	2.17 ± 0.20	2.37 ± 0.41	1.27 ± 0.08	1.43 ± 0.06

changes are found in medulla-pons and midbrain.

Discussion

Previous studies (1) have shown that in short-term STZ-diabetes a decreased level of total serum tryptophan, with a large decrease in the amount of tryptophan bound to albumin appears. However, free serum tryptophan and the concentrations of serum and brain branched-chain amino acids (valine, leucine and isoleucine), all showed large significant increases. In the present study, brain tryptophan concentrations show a generalized increase (about 14 % above control group) in all brain regions, although it is only significant in medulla-pons. Several investigators (3, 11, 13, 19, 20) have suggested that brain tryptophan levels vary with changes in free serum tryptophan, and our results confirm this hypothesis.

Serotonin levels do not change in all the studied brain regions. With respect to 5-HIAA, the major serotonin metabolite, significant increases are obtained in medulla-pons and midbrain, which were almost parallel to the increases in the tryptophan levels. The changes in brain serotonin and 5-HIAA levels, however, do not necessarily follow changes in brain tryptophan concentrations. In long-term studies the diabetic rats exhibit a fall in brain tryptophan but unchanged serotonin and 5-HIAA levels (9, 17, 18). Other authors, however have shown low concentrations in brain 5-HIAA (2, 7), or serotonin (5), under similar experimental conditions.

Neurotransmitter turnover rate alterations are more sensitive indicators of altered brain functions than changes in steady-state levels of neurotransmitters (22). It is becoming clear that the use of the concentration ratio of the major metabolite of a brain monoamine to the brain monoamine concentration itself provides a good index of the synaptic neurotransmitter turnover rate (24), the ratio 5-HIAA/serotonin being presumed to reflect the rate of the neuronal release of serotonin and its degradation to 5-HIAA (14). In medulla-pons and midbrain a significant increase in this ratio appears.

From these results, it could be deduced that the serotoninergic activity increased in these regions, in short-term STZ-diabetes. Due to the evidence that brain serotonin metabolism is regulated largely by the availability of brain tryptophan (11, 12), diabetes-related changes in the serotoninergic system may be secondary to the increased serum free tryptophan, which in turn results in an increased availability of tryptophan for brain uptake.

In conclusion, we propose that previously described alterations in peripheral biochemistry increase the tryptophan and 5-HIAA levels in brainstem, probably the most sensitive region in a diabetic state, and presumably, the rate of serotonin synthesis in short-term STZ-diabetes.

Resumen

Se estudia el metabolismo serotoninérgico en diferentes regiones cerebrales de ratas machos Wistar, controles y tratadas con estreptozotocina. Los animales son sacrificados 24 horas después de la inducción de la diabetes. Las concentraciones cerebrales de triptófano muestran un incremento generalizado en todas las regiones, aunque sólo es significativo en la médula puente. Los niveles de serotonina no muestran cambios, mientras que los de 5-HIAA se incrementan significativamente en médula puente y cerebro medio, así como el cociente 5-HIAA/5-HT.

Palabras clave: Diabetes experimental, Metabolismo serotoninérgico.

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