Brain serotonin depletion attenuates diabetogenic effects of streptozotocin

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Yang, Y. F., and M. T. Lin. Brain serotonin depletion attenuates diabetogenic effects of streptozotocin. *Am. J. Physiol.* 268 (*Endocrinol. Metab.* 31): E839–E844, 1995.—The diabetogenic effects of streptozotocin (STZ) were studied on blood glucose, plasma insulin, feeding and drinking, body weight, islet morphology, and hypothalamic serotonin (5-HT) release in vehicle-pretreated rats and in rats pretreated with either intracerebroventricular injection of 5,7-dihydroxytryptamine (5,7-DHT; a 5-HT nerve fiber depletor), intraperitoneal injection of *p*-chlorophenylalanine (PCPA; a tryptophan hydroxylase inhibitor), or intraperitoneal injection of *p*-chloroamphetamine (PCA; a neurotoxin for 5-HT nerve fiber). At four days after STZ administration, vehicle-treated rats displayed hyperglycemia, polydipsia, polyphagia, decreased plasma insulin level, derangement of islet morphology (few insulin cells, accumulation of glucagon cells), and elevated 5-HT release in the hypothalamus. The above diabetogenic effects of STZ were attenuated by brain serotonin depletion induced by 5,7-DHT, PCPA, or PCA. Furthermore, the STZ-induced hyperglycemia or derangement of islet morphology was attenuated by peripheral sympathectomy or adrenalectomy. It is concluded that brain serotonin depletion attenuates diabetogenic effects of STZ by reducing sympathetic efferent activity in rats.

hypothalamus; diabetes mellitus; voltammetry

IN RECENT YEARS, the effect of experimental diabetes mellitus on brain neurochemistry has been under an extensive investigation. In most of these studies, diabetes was produced by a peripheral administration of streptozotocin (STZ) or alloxan (6). The results were, at least in the case of serotonin (5-HT), decreased precursor levels in the brain coupled with a subsequent decreased synthesis of 5-HT (7). However, studies have indicated that the levels of brain 5-HT were decreasing (4), increasing (15, 16), or unaltering (7, 8) during the diabetic state.

Most studies that have evaluated serotoninergic dynamics have depended on neurochemical tools, such as enzyme activity or neurotransmitter levels. In recent years, in vivo voltammetry has made possible the measurement of extracellular synaptic release of 5-HT; this enables a more direct assessment of neuronal activity than was previously available (2, 5, 11, 17). In the present studies, we have evaluated the effects of STZ-induced diabetes on 5-HT release in the hypothalamus with the use of in vivo voltammetry. The hypothalamus is an area in which 5-HT levels were found to be increased during the hyperglycemia induced by stimulating the ascending 5-HT system (19). Moreover, to determine the importance of the brain 5-HT system in experimental diabetes, we have also studied the effects of brain 5-HT depletion on the STZ-induced diabetogenic effects in rats.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 305 ± 10 g were used. They were kept on a standard pellet diet and tap water ad libitum. The rats were not fasted before the experiments. Light was turned on at 0600 and turned off at 1800. The following eight groups of experimental animals were studied. *1*) The first group was 5,7-dihydroxytryptamine (5,7-DHT) centrally pretreated rats. The rats, under pentobarbital sodium anesthesia, received a single injection of 5,7-DHT in the lateral cerebral ventricle (Sigma Chemicals, St. Louis, MO; 200 μg in 5 μl) that was dissolved in 0.9% saline plus 0.1% ascorbic acid. *2*) The second group included *p*-chlorophenylalanine (PCPA)-pretreated rats. The rats received a single injection of PCPA (Sigma; 250 $mg \cdot ml^{-1} \cdot kg^{-1}$) that was dissolved in 0.9% saline in the peritoneal cavity. *3*) The third group included *p*-chloroamphetamine (PCA)-pretreated rats. The rats received a single injection of PCA (10 $mg \cdot ml^{-1} \cdot kg^{-1}$) that was dissolved in 0.9% saline in the peritoneal cavity. *4*) The fourth group included 5,7-DHT peripherally pretreated rats. The rats received an intraperitoneal dose of 5,7-DHT (300 mg/kg). *5*) The fifth group included 6-hydroxydopamine (6-OHDA)-pretreated rats. The rats received an intraperitoneal dose of 6-OHDA (100 mg/kg). *6*) The sixth group included α-methyl-*p*-tyrosine (AMPT)-pretreated rats. The rats received an intraperitoneal dose of AMPT (250 mg/kg). *7*) The seventh group included adrenalectomized rats. The rats received bilateral adrenalectomy under pentobarbital sodium anesthesia. *8*) The last group included vehicle-pretreated rats. The control rats were of the same sex, were weight matched, and were treated with an intracerebroventricular dose of 5 μl saline plus 0.1% ascorbic acid. Seven days after 5,7-DHT, 6-OHDA, or adrenalectomy, 3 days after PCPA or PCA, or immediately after AMPT pretreatment, STZ (Sigma) dissolved in a citrate buffer (0.1 M with pH 4.5) was injected intravenously in these eight groups of animals at the dose level of 65 $mg \cdot ml^{-1} \cdot kg^{-1}$.

All rats were examined throughout the 11-day study period by regular measurements of the morning blood glucose, body weight, food intake, and water intake. After STZ administration (4 days), both the hypothalamic 5-HT release and plasma insulin level were measured. In addition, the pancreas was examined by immunocytochemistry at 4 days after STZ administration. In another separate experiment, 7 days after 5,7-DHT, 3 days after PCPA, or 3 days after PCA treatment, the animals were killed, and their brains were removed for monoamine assay.

At 4 days after STZ administration, rats were killed by ether overdose, and the abdomen was opened. The pancreas was resected, fixed in 4% formaldehyde, dehydrated in alcohol, paraffin embedded, and then exposed to either guinea pig anti-human insulin (1:500) for 1 h at room temperature or rabbit anti-human glucagon (1:1,600) antibodies overnight at 4°C. The antibodies were purchased from Milab (Malmo, Sweden). After the incubation with the primary antibody, the sections were incubated for 30 min with peroxidase-antiperoxi-

Table 1. *Effects of 5,7-DHT, PCPA, and PCA on brain monoamine concentrations in rats*

Treatment	5-HT Concentration			DA Concentration		
	Frontal cortex	Striatum	Hypothalamus	Frontal cortex	Striatum	Hypothalamus
Rats treated with vehicle control icv						
Vehicle	0.85 ± 0.07	0.44 ± 0.02	0.64 ± 0.08	9.43 ± 0.47	11.2 ± 0.35	0.31 ± 0.03
STZ	0.88 ± 0.09	0.63 ± 0.04*	1.07 ± 0.09*	9.12 ± 0.32	7.4 ± 0.26*	0.16 ± 0.02*
Rats treated with 5,7-DHT (200 μg icv)						
Vehicle	0.34 ± 0.06†	0.22 ± 0.08†	0.18 ± 0.02†	9.42 ± 0.29	10.8 ± 0.33	0.29 ± 0.04
STZ	0.37 ± 0.05†	0.31 ± 0.07†	0.20 ± 0.03†	9.25 ± 0.28	7.7 ± 0.31*	0.14 ± 0.03*
Rats treated with PCPA (250 mg/kg ip)						
Vehicle	0.22 ± 0.04†	0.06 ± 0.01†	0.21 ± 0.05†	9.26 ± 0.24	9.5 ± 0.18†	0.15 ± 0.02†
STZ	0.31 ± 0.06†	0.08 ± 0.01†	0.25 ± 0.03†	9.34 ± 0.35	6.3 ± 0.15†	0.16 ± 0.04
Rats treated with PCA (10 mg/kg ip)						
Vehicle	0.41 ± 0.06†	0.11 ± 0.01†	0.35 ± 0.07†	9.35 ± 0.31	10.9 ± 0.37	0.17 ± 0.02†
STZ	0.39 ± 0.05†	0.20 ± 0.03†	0.42 ± 0.08†	9.39 ± 0.35	7.1 ± 0.28*	0.13 ± 0.03
Rats treated with 5,7-DHT (300 mg/kg ip)						
Vehicle	0.81 ± 0.05	0.39 ± 0.02	0.58 ± 0.07	9.44 ± 0.45	10.7 ± 0.33	0.30 ± 0.02
STZ	0.79 ± 0.07	0.61 ± 0.03*	0.99 ± 0.08*	9.41 ± 0.33	7.9 ± 0.36*	0.19 ± 0.03*

Values are expressed as means ± SE (μg/g tissue) of 8 rats for each group. 5,7-DHT, 5,7-dihydroxytryptamine; PCPA, *p*-chlorophenylalamine; PCA, *p*-chloroamphetamine; DA, dopamine; STZ, streptozotocin; icv, intracerebroventricular injection; ip, intraperitoneal injection. *$P < 0.05$, significantly different from corresponding control values (vehicle controls), analysis of variance (ANOVA). †$P < 0.05$, significantly different from corresponding control values (rats treated with vehicle control), ANOVA.

dase complex (Dakopatts A/S, Copenhagen, Denmark; see Ref. 3). This was followed by incubation with 0.05% diaminobenzidine hydrochloride and 0.01% hydrogen peroxide in tris(hydroxymethyl)aminomethane buffer (pH 7.6) for 1 h.

Plasma levels of insulin were determined with radioimmunoassay, using commercially available guinea pig anti-porcine insulin (Milab) and ^{125}I-labeled porcine insulin (Novo, Bagsvaerd, Denmark). Porcine insulin was used as a standard. Separation of free and bound tracer was performed with the dextran-charcoal separation technique (12).

Seven days after 5,7-DHT treatment, 3 days after PCPA treatment, or 3 days after PCA treatment, the rats were killed by decapitation, and the brains were rapidly removed and placed on an ice-chilled petri dish. Frontal cortex, hypothalamus, and striatum were dissected out, wrapped in foil, and frozen at −80°C until neurochemical determinations were made. Concentrations of 5-HT and dopamine were determined with high-performance liquid chromatography (HPLC). The HPLC system consisted of an isocratic solvent delivery system (BAS, West Lafayette, IN) with a glassy carbon electrode and a C-18 stationary phase (flow rate = 0.8 ml/min; applied potential = +0.70 V). Sampling was carried out with a TosoHaas refrigerated autosampler, and a single pen chart recorded peak heights. The mobile phase consisted of 0.1 M monochloroacetic acid (pH 3) containing 120 mg/l sodium octylsulfate, 2 mM Na_2EDTA, 3% acetonitrile, and 0.1% tetrahydrofuran. Tissues were homogenized in ice-cold perchloric acid (0.1 M) containing either isoproterenol or dihydroxybenzylamine as the internal standard, centrifuged at 4°C for 10 min at 10,000 revolutions/min, filtered under centrifugation, and the filtrate was injected onto the HPLC system. Concentrations of the monoamine were calculated on the basis of comparison with internal and external standards.

In another separate experiment, animals were anesthetized with urethane, held in a stereotaxic frame, and implanted with nafion-coated carbon fiber electrodes in the hypothalamus using the coordinates of Paxinos and Watson (21). Auxiliary (silver wire) and reference (Ag/AgCl) electrodes were placed on the dura surface of the parietal bone. Differential pulse voltamograms were recorded automatically every 25 s as detailed previously (5, 18). At the end of each experiment, an electrolytic lesion was performed by applying a continuous potential (+5 V) for 3 s to the carbon fiber electrode. The current passing through the electrode was ~0.4 nA. The brain was dissected, then frozen and kept at −20°C. The brain was cut into 20-μm coronal slices. Every third section was collected for Nissl's staining.

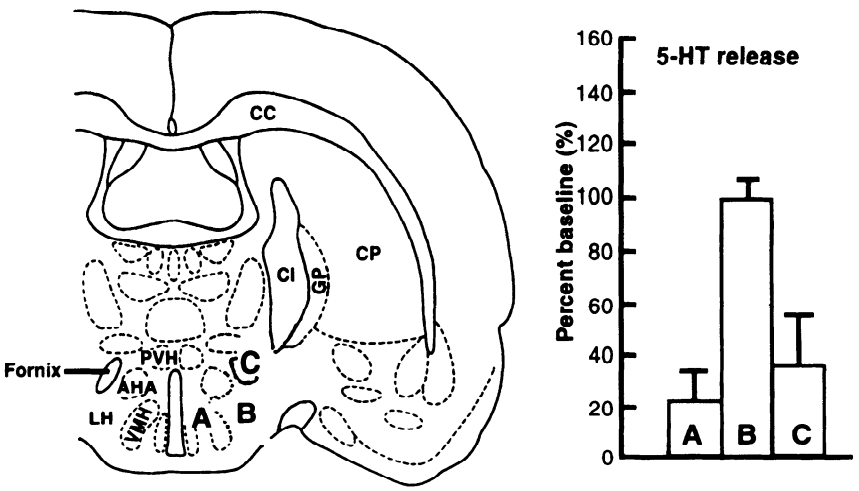

Fig. 1. Coronal section through rat hypothalamus at the level of the ventromedial hypothalamus to illustrate the location of carbon fiber electrodes. Hypothalamic serotonin (5-HT) release was recorded at 3 depth points (*A*, *B*, and *C*). CC, corpus callosum; CP, caudate putamen; CI, internal capsule; GP, globus pallidus; AHA, anterior hypothalamus; PVH, paraventricular hypothalamus; LH, lateral hypothalamus; VMH, ventromedial hypothalamus.

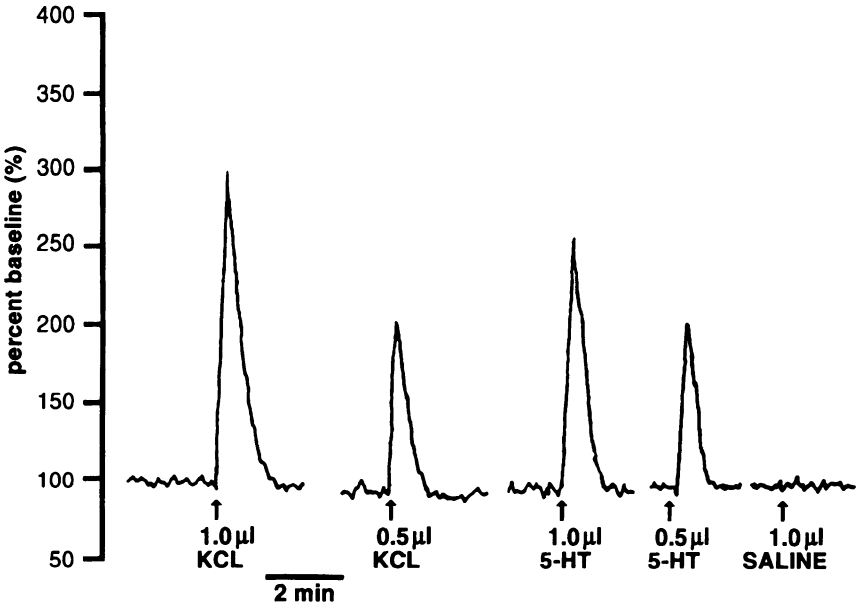

Fig. 2. Representative 5-HT release obtained in hypothalamus before and after local injection of 5-HT, KCl, or saline close to nafion-coated working electrode.

Results are reported as means ± SE. Two-way analysis of variance and Student's *t*-test were used for statistical analysis of the differences in the STZ-induced diabetogenic effects.

RESULTS

Table 1 shows both the 5-HT and dopamine concentrations of the frontal cortex, striatum, and hypothalamus for the vehicle-pretreated rats, the 5,7-DHT centrally pretreated rats, PCPA-pretreated rats, PCA-pretreated rats, and 5,7-DHT preipherally pretreated rats. Compared with those of the vehicle-pretreated rats, the 5,7-DHT centrally pretreated rats, PCPA-pretreated rats, or PCA-pretreated rats had a lower level of 5-HT concentration in the frontal cortex, striatum, and hypothalamus. On the other hand, the dopamine levels of different cerebral structures in the 5,7-DHT centrally pretreated rats were not different from those of the vehicle-pretreated rats. However, the dopamine levels of the striatum and hypothalamus in the PCPA-pretreated rats were significantly lower than those of the vehicle-pretreated rats. The hypothalamic dopamine levels of the PCA-pretreated rats were also lower than those of the vehicle-pretreated rats. Nevertheless, the monoamine concentrations of different brain regions were not affected by peripheral injection of 5,7-DHT. Table 1 also shows that the hypothalamic or striatal 5-HT concentrations increased, whereas the hypothalamic or striatal dopamine concentrations decreased in the vehicle-pretreated rats or the 5,7-DHT peripherally pretreated rats during the STZ diabetic state.

To determine the highest concentration of hypothalamic 5-HT, 5-HT release was recorded in different regions of the hypothalamus. As indicated in Fig. 1, 5-HT release increased gradually from *point A* (anterior hypothalamus) to *point B* (lateral hypothalamus). Subsequently, the amplitude of the 5-HT release decreased from *point B* to *point C* (ventromedial hypothalamus). Therefore, in the present experiments, all 5-HT releases were recorded at *point B* of Fig. 1. Figure 2 shows the representative voltammograms obtained in the hypothalamus of rat brain before and after local injection of either 5-HT (0.5–0.1 µl of 1 µM solution for 20 s) or KCl (0.5–1.0 µl of 90 mM solution for 20 s) very close to the Nafion-coated working electrode. Note that the amplitude of voltammograms increased in proportion to the imposed doses of 5-HT or KCl solutions, suggesting the electrode was sensitive to rapid changes in 5-HT concentration.

Table 2 shows body weight, food intake, water intake, blood glucose, plasma insulin, and hypothalamic 5-HT

Table 2. *Effect of 5,7-DHT, PCPA, and PCA on physiological responses produced by intravenous injection of 65 mg/kg STZ in rats*

Treatment	Body Weight at the Day Before STZ, g	At 4 Days After STZ Treatment					
		Body weight, g	Food intake, g	Water/food, ml/g	Blood glucose, mg/dl	Plasma insulin, ng/ml	Hypothalamic 5-HT release, % baseline
Vehicle + saline	300 ± 10	335 ± 11	22 ± 1	1.5 ± 0.2	125 ± 7	0.91 ± 0.13	100 ± 5
Vehicle + STZ	303 ± 17	261 ± 15	41 ± 2*	4.6 ± 0.2*	396 ± 15*	0.01 ± 0.01*	184 ± 11*
5,7-DHT (icv) + STZ	305 ± 9	381 ± 12	21 ± 2	1.5 ± 0.1	60 ± 5*	0.88 ± 0.17	80 ± 4*
PCPA (ip) + STZ	302 ± 11	340 ± 14	23 ± 1	1.6 ± 0.1	68 ± 6*	1.35 ± 0.25	78 ± 5*
PCA (ip) + STZ	304 ± 7	341 ± 15	24 ± 2	1.6 ± 0.2	66 ± 3*	1.48 ± 0.24	70 ± 5*
5,7-DHT (ip) + STZ	301 ± 8	237 ± 19	39 ± 4*	4.4 ± 0.3*	368 ± 27*	0.02 ± 0.01*	181 ± 14*

Values are expressed as means ± SE of 8 rats for each group. *$P < 0.05$, significantly different from corresponding control values (vehicle + saline), ANOVA.

release values for the vehicle-pretreated rats, the 5,7-DHT centrally pretreated rats, the PCPA-pretreated rats, the PCA-pretreated rats, and the 5,7-DHT peripherally pretreated rats. The body weight of the vehicle-pretreated rats decreased by ~12% at 4 days after STZ administration. In contrast, the body weight of either the 5,7-DHT centrally pretreated, the PCPA-pretreated, or the PCA-pretreated rats increased by ~12% at 4 days after STZ administration. In addition to a severe hyperglycemic state, the vehicle-pretreated rats or the 5,7-DHT peripherally pretreated rats displayed polydipsia, polyphagia, decreased plasma insulin level, and increased hypothalamic 5-HT release at 4 days after STZ administration. However, at 4 days after STZ administration, either the 5,7-DHT centrally pretreated, the PCPA-pretreated, or the PCA-pretreated rats did not display polydipsia, polyphagia, hyperglycemia, hypoinsulinemia, and the increased hypothalamic 5-HT release.

Figure 3, *A* and *B*, shows that, at 4 days after saline administration, islet insulin and glucagon immunoreactive cells are normal in control rats receiving normal saline. In contrast, there was a reduction in the number

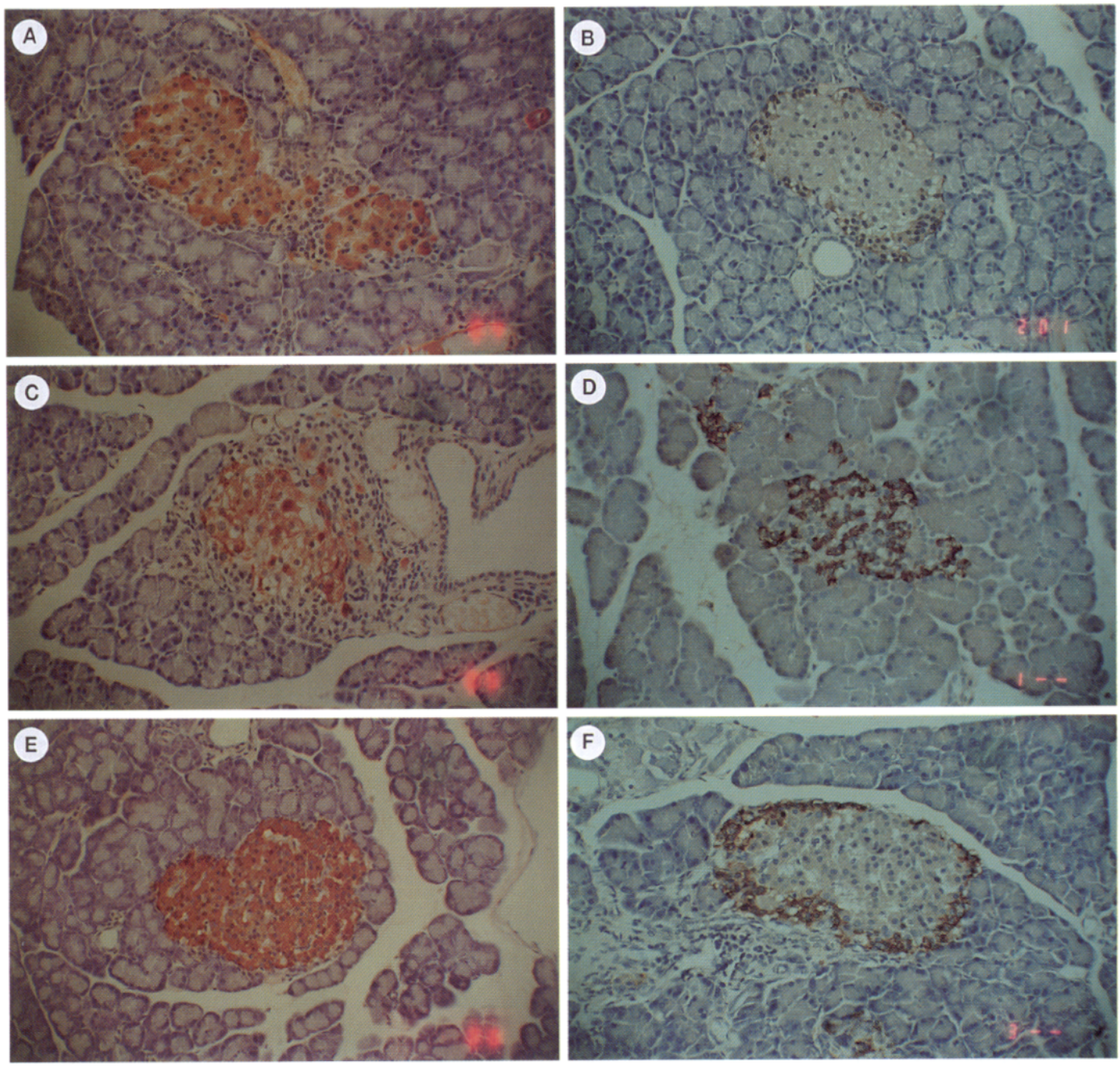

Fig. 3. Photomicrograph of section of pancreas of healthy control rat (*A* and *B*), rat receiving streptozotocin (STZ) at 65 mg/kg (*C* and *D*), and rat receiving STZ at 65 mg/kg and 5,7-dihydroxytryptamine at 200 μg (*E* and *F*) 7 days before STZ injection. Results were taken at 4 days after STZ administration and were stained for insulin immunoreactivity (*A*, *C* and *E*) and glucagon immunoreactivity (*B*, *D* and *F*). Magnification ×80.

of insulin-immunoreactive cells and a relative increase in the number of glucagon-immunoreactive cells in vehicle-pretreated rats receiving STZ at a dose level of 65 mg/kg (Fig. 3, *C* and *D*). However, at 4 days after STZ administration, islet insulin and glucagon immunoreactive cells were still normal in 5,7-DHT-pretreated rats receiving STZ administration at the same dose level (Fig. 3, *E* and *F*). Furthermore, the derangement of islet morphology (few insulin cells, accumulation of glucagon cells) of STZ was attenuated by pretreatment with peripheral sympathectomy or adrenalectomy.

Table 3 shows blood glucose values for the normal control rats and the peripherally sympathectomized rats. The blood glucose of normal control rats increased by about four times the control value at 4 days after STZ administration. However, the hyperglycemic effects of STZ were attenuated by pretreatment with peripheral sympathectomy or adrenalectomy.

DISCUSSION

Our previous results showed that either electrical or chemical stimulation of the ascending serotoninergic system in brain produced hyperglycemia in rats under general anesthesia (19). The stimulation-induced hyperglycemia in rats could be attenuated by depleting the hypothalamic 5-HT produced by local injection of 5,7-DHT (a serotoninergic nerve fiber depletor) or by prior transection of the spinal cord at C_7. In the present studies, the data show that the recent onset diabetic animal not only released more 5-HT in the hypothalamus than the nondiabetic animal under basal conditions, but the STZ-induced diabetic animal also displayed a lower level of body weight gain or plasma insulin, as well as a higher level of blood glucose, food intake, and water intake than its nondiabetic counterpart. In fact, it was also found that the STZ diabetic animal released more 5-HT in the corpus striatum or the hypothalamus than the nondiabetic animal under basal conditions (2, 15, 16). Similar changes in the hypothalamic 5-HT have already been observed in diabetic patients (16). These changes may be related to the reduction of monoamine oxidase activity observed in experimental diabetes (20). In diabetic animals, there was also a presumptive hyperaminoacidemia (6, 15). These results imply that either hypothalamic or striatal 5-HT neurons, in the (recent onset) acutely diabetic animal, show an enhanced ability for neuronal uptake of 5-HT or its precursor and subsequent conversion and release of 5-HT in brain. The current hypothesis is not supported by the findings that the brain 5-HT level decreased or was unaltered during the diabetic state (4, 7, 8). It is not clear what caused the discrepancy between these two groups of results.

Table 3. *Effects of chemical sympathectomy on STZ-induced hyperglycemia in rats*

Treatment	Blood Glucose 4 Days After STZ, mg/dl
Vehicle controls (iv)	119 ± 9
Vehicle + STZ (65 mg/kg iv)	408 ± 21*
6-OHDA (100 mg/kg ip) + STZ (65 mg/kg iv)	138 ± 8†
AMPT (250 mg/kg ip) + STZ (65 mg/kg iv)	194 ± 9†
Adrenalectomy + STZ (65 mg/kg iv)	215 ± 13†

Values are expressed as means ± SE of 8 rats for each group. 6-OHDA, 6-hydroxydopamine; AMPT, α-methyl-*p*-tyrosine. *$P < 0.05$, significantly different from control values (vehicle controls), ANOVA. †$P < 0.05$, significantly different from control values (vehicle + STZ), ANOVA.

In the present studies, the effects of STZ were studied on blood glucose, plasma insulin, body weight gain, islet morphology, and hypothalamic 5-HT release in rats with normal brain 5-HT levels and in rats with brain 5-HT depletion. The brain or the hypothalamic 5-HT was severely depleted by pretreatment of animals with intracerebroventricular injection of 5,7-DHT (9) or intraperitoneal injection of either PCPA (10) or PCA (14). STZ administration at a dose level of 65 mg/kg resulted in hyperglycemia, decreased plasma insulin, decreased body weight gain, increased hypothalamic 5-HT release, and marked derangement of islet morphology (few insulin cells, accumulation of glucagon cells) in normal control rats. However, the previously mentioned diabetogenic effects of STZ were not observed in rats with brain or hypothalamic 5-HT depletion produced by either 5,7-DHT, PCPA, or PCA. This is the first report showing a correlation between the activity of brain 5-HT systems and the induction of experimental diabetes mellitus. The results are by no means unexpected. The data are consistent with our previous findings (19) in which we showed that the hyperglycemia induced by stimulating the brain ascending 5-HT system in rats with normal brain 5-HT levels could not be duplicated in rats with brain 5-HT depletion. It is unlikely that the increased brain or hypothalamic 5-HT levels seen in the STZ-induced diabetic state are due to hyperglycemia stress. Our recent data show that, in normal control rats, intravenous injection of high doses of glucose failed to increase 5-HT release in the hypothalamus (unpublished observations). Our previous results showed that the hyperglycemia induced by stimulating the brain 5-HT pathways was attenuated by reducing sympathetic efferent activity (19). The present results also showed that the STZ-induced hyperglycemia or derangement of islet morphology was attenuated by reducing sympathetic efferent activity in rats. Thus it appears that brain 5-HT depletion attenuates the diabetogenic effects of STZ by reducing sympathetic efferent activity. In the present results, STZ administration may have produced an enhanced 5-HT release in the hypothalamus, may have resulted in enhancement of efferent sympathetic activity (9), may have led to derangement of islet morphology (17), and may have induced diabetogenic effects in rats. Brain or hypothalamic 5-HT depletion may have destroyed the previously mentioned pathways or links and resulted in attenuation of the diabetogenic effects of STZ.

Alternatively, another likely explanation for our findings is that the 5-HT-depressed animals have a decreased blood flow to the islets. Because STZ has a very short half-life, probably only sufficient for a single pass of the active drug, any reduction of islet blood flow

could diminish the effect of the drug. Indeed, our previous results showed that the 5-HT-depressed animals had an increased blood flow to the skin (18). However, it is not known whether an increased blood flow to the peripheral skin area would lead to a reduction of blood flow to the islets or other internal organs.

STZ is a specific cytotoxic agent for pancreatic insulin cells (13). Thus, as early as 1 h after its administration, β-cell degranulation occurs, and after 7 h, necrosis of β-cells is seen. In contrast, both present and previous (1) results showed that STZ administration resulted in accumulation of glucagon cells in pancreatic islets. It has been suggested that DNA breakage and depletion of islet cell NAD levels are central to the immediate diabetogenicity of the drug (22). Therefore, further studies should be carried out to ascertain any possible effects of brain 5-HT depletion on the DNA fragmentation by STZ in the future.

Finally, it should be mentioned that intracerebroventricular administration of a nondiabetogenic dose of STZ produces an increased content of brain 5-HT that is almost indistinguishable from those described in the experimental diabetes produced by peripheral administration of STZ in rats (15). This observation apparently suggests that the central nervous system effect of STZ is not necessarily related to a diabetogenic β-cytotoxic action of STZ. This comes as a puzzle. A clarification to this puzzle awaits further experiments.

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REFERENCES

1. **Ar'Rajab, A., and B. O. Ahren.** Long-term diabetogenic effect of streptozotocin in rats. *Pancreas* 8: 50–57, 1993.
2. **Broderick, P. A., and J. H. Jacoby.** Central monoamine in diabetes: psychotherapeutic implications: electroanalysis by voltammetry. *Acta Physiol. Scand.* 39: 211–225, 1989.
3. **Bruss, M. L., and A. L. Black.** Enzymatic microdetermination of glycogen. *Anal. Biochem.* 84: 309–312, 1978.
4. **Chu, P. C., M. T. Lin, L. R. Shian, and S. Y. Leu.** Alterations in physiologic functions and brain monoamine content in streptozotocin-diabetic rats. *Diabetes* 35: 481–485, 1986.
5. **Chuang, J. I, S. S. Chen, and M. T. Lin.** Melatonin decreases brain serotonin release, arterial pressure and heart rate in rats. *Pharmacology* 47: 91–97, 1993.
6. **Crandall, R. A., and J. D Fernstrom.** Acute changes in brain tryptophan and serotonin after carbohydrate or protein ingestion by diabetic rats. *Diabetes* 29: 461–464, 1980.
7. **Crandall, R. A., M. A. Gillis, and J. D. Fernstrom.** Reduction in brain serotonin synthesis rate in streptozotocin-diabetic rats. *Endocrinology* 109: 310–312, 1981.
8. **Fernstrom, J. D., D. V. Faller, and H. Shashelowitz.** Acute reduction of brain serotonin and 5-HIAA following to the sum of competing neutral amino acids. *J. Neural Transm.* 36: 113–117, 1975.
9. **Fuller, R. W.** Neurochemical effects of serotonin neurotoxins: an introduction. *Ann. NY Acad. Sci.* 305: 178–181, 1978.
10. **Fuller, R. W.** Effects of *p*-chloroamphetamine on brain serotonin neurons. *Neurochem. Res.* 17: 449–456, 1992.
11. **Gerhardt, G. A. A., F. Oke, G. Nagy, and R. N. Adams.** Nafion-coated electrodes with high selectivity for CNS electrochemistry. *Brain Res.* 290: 390–395, 1984.
12. **Herbert, V., K. S. Lau, C. W. Gottlioeb, and S. J. Bleicher.** Coated charcoal immunoassay of insulin. *J. Clin. Endocrinol.* 25: 1875–1884, 1965.
13. **Junod, A., A. E. Lambert, L. Orci, R. Pictet, and A. E. Gonet.** Studies of the diabetogenic action of streptozotocin. *Proc. Soc. Exp. Biol. Med.* 126: 201–205, 1967.
14. **Koe, B. K., and A. Weissman.** Chlorophenylanine, a specific depletor of brain serotonin. *J. Pharmacol. Exp. Ther.* 154: 499–516, 1966.
15. **Lackovic, Z., and M. Salkovic.** Streptozotocin and alloxan produce alterations in rat brain monoamines independently of pancreatic beta cells destruction. *Life. Sci.* 46: 49–54, 1990.
16. **Lackovic, Z., M. Salkovic, Z. Kuci, and M. Belja.** Effect of long-lasting diabetes mellitus on rat and human brain monoamines. *J. Neurochem.* 54: 143–147, 1990.
17. **Liao, B. Y., H. P. Lio, C. Y. Wang, M. S. Young, M. T. Ho, and M. T. Lin.** A simplified method for selecting a carbon-fiber electrode in pulse voltammetry. *J. Neurosi. Methods* 50: 291–299, 1993.
18. **Lin, M. T.** Effect of hypothalamic injections of 5,6-dihydoxtryptamine on thermoregulation in rats. *Experientia Basel* 35: 359–361, 1979.
19. **Lin, M. T., and L. R. Shian.** Stimulation of 5-hydroxytryptamine nerve cells in dorsal and median raphe nuclei elevates blood glucose in rats. *Pfluegers Arch.* 417: 441–445, 1991.
20. **Mayanil, C. Z., S. M. Kazmi, and N. Z. Baquer.** Changes in monoamine oxidase activity in rat brain during alloxan diabetes. *J. Neurochem.* 38: 179–183, 1982.
21. **Paxinos, G., and C. Watson.** *The Rat Brain in Stereotaxic Coordinates.* New York: Academic, 1982.
22. **Yamamoto, H., Y. Uchigata, and H. Okamoto.** Streptozotocin and alloxan induced DNA strand breaks and ploy (ADP-ribose) synthetase in pancreatic islets. *Nature Lond.* 294: 284–285, 1981.