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The Effect of Streptozotocin-Induced Diabetes on Dopamine$_2$, Serotonin$_{1A}$ and Serotonin$_{2A}$ Receptors in the Rat Brain

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The effect of streptozotocin (STZ)-induced diabetes and a combination of chronic treatment with haloperidol (HPD) on dopamine (DA)D_2, serotonin (5-HT) 5-HT_{1A} and 5- HT_{2A} receptors was investigated in rat brain. Rats were randomly assigned to one of four groups: vehicle-vehicle, STZ-vehicle, vehicle-HPD, and STZ-HPD groups. Four weeks after single administration of STZ (65 mg/kg IV) or vehicle (citrate buffer), rats received depot HPD (4 mg/kg IM) or vehicle (sesame oil) once a week for 4 weeks. Sixteen days after the last injection of HPD or vehicle, rats were sacrificed, and the density of binding sites was determined using [^{3}H]spiperone as ligand in the striatum (D_2), [^{3}H]8-hydroxy-2-(di-n-propyl)-aminotetraline in the hippocampus (5-HT_{1A}), and [^{3}H]ketanserin in the frontal cortex (5-HT_{2A}). The density of D_2 receptors was significantly increased in the vehicle-HPD compared to vehicle-vehicle controls. However, striatal D_2 receptor density of the STZ-HPD and the STZ-vehicle were not significantly different from the vehicle-vehicle group. A significant increase in cortical 5- HT_{2A} receptor density was observed only in the group of STZ-vehicle. Treatment with STZ, HPD, or the combination thereof, did not affect the density of 5-HT_{1A} receptors. The affinity constants for D_2, 5-HT_{1A}, and 5-HT_{2A} receptors were not affected by any treatment. These results suggest that diabetic state may affect brain serotonergic activity via an increase in the density of 5-HT_{2A} receptors. This may indicate an increased vulnerability to major depression in patients with diabetes. The lack of an effect of the combined chronic treatment with STZ and HPD on the D_2 receptor density may correspond to the increased risk to develop tardive dyskinesia in patients with diabetes. © *1997 American College of Neuropsychopharmacology* ***[Neuropsychopharmacology 16:183-190, 1997]***

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Streptozotocin- (STZ) induced hyperglycemic state has been used as an animal model for diabetes mellitus (DM; Tarui et al. 1987; Bradberry et al. 1989; McCall 1992). STZ selectively destroys pancreatic islet beta-cells and causes hypoinsulinemia, leading to hyperglycemia (Arison et al. 1967; Hohenegger and Rudas 1971). STZ itself does not enter the brain (Bhuyan et al. 1974). Studies of the chronic effect of STZ-induced DM on the concentrations of dopamine (DA), its metabolites, and on DA receptors in rodent brain have shown: (1) reduced accumulation of 3,4-dihydroxyphenylalanine (DOPA, a DA precursor) (Bradberry et al. 1989); (2) decreased concentrations of DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) (Bellush and Reid 1991; Lackovic et al. 1990); (3) increased DA-D_2 receptors (Lozovsky et al. 1981; Serri et al. 1985; Truson and Himmel 1983); and (4) decreased D_1 receptors (Salkovic and Lackovic 1992) in rats with STZ- or alloxan-induced DM.

Changes in serotonin (5-hydroxytryptamine, 5-HT)

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neurotransmission have also been demonstrated: (1) reduced tryptophan, 5HT, and 5-hydroxyindoleacetic acid (5-HIAA) concentrations (Kwok and Juorio 1987); (2) no change in the number of [^{3}H]5-HT binding sites in the forebrain and brainstem of STZ-induced DM rats (Truson and MacKenzie 1981). However, no studies investigating DM-related changes in brain 5-HT receptor subtypes, such as the 5-HT_{1A} and 5-HT_{2A} receptors, have been reported. Clarification of changes in these 5-HT receptor subtypes in experimental DM is of interest, as alterations of these receptors may play a role in the pathogenesis of major depression (Meltzer and Lowry 1987) and DM has been reported to be a risk factor for this disease (Lustman et al. 1992; Popkin et al. 1988). For example, the 5-HT_{2A} receptor number is reported to be higher in postmortem frontal cortex from depressive subjects (Stanley 1983; Mann et al. 1986). The upregulation of 5-HT_{2A} receptors could be expected in subjects with DM. Plasma glucose levels increase following stimulation of 5-HT_{1A} (Chaouloff and Jeanrenaud 1987) or 5-HT_{2A} (Chaouloff et al. 1990) receptors, both of which have been suggested to be abnormal in major depression or to be affected by some antidepressant drugs (Meltzer and Lowry 1987).

Diabetes mellitus also has been reported to be one of the risk factors for tardive dyskinesia (Mukherjee et al. 1985; Ganzini et al. 1991; Woerner et al. 1993), which is a basal ganglia disorder caused by chronic treatment with neuroleptics such as haloperidol (HPD), and is possibly related to abnormalities in dopaminergic function (Gerlach and Casey 1988). As it has been suggested that typical antipsychotic drugs such as HPD exert their clinical effect via D_2 receptors (Seeman et al. 1976), it is speculated that responses (changes in the affinity and/or the number) of these receptors to chronic treatment with antipsychotic drugs are altered in subjects with DM, leading to the higher risk to develop tardive dyskinesia. To our knowledge, there has been no report investigating whether DM modulates the effect of chronic treatment with antipsychotic drugs, such as HPD, on D_2 receptors.

Taken together, all the considerations lead to the hypothesis that aspects of DA and 5-HT neurotransmissions are altered via changes in D_2, 5-HT_{1A} or 5-HT_{2A} receptors in the brains of subjects with DM. To further elucidate possible dysfunction in DA and 5-HT activity in relation to altered sensitivity to chronic antipsychotic treatment in subjects with DM, the current study investigated adaptive changes in brain D_2, 5-HT_{1A}, and 5-HT_{2A} receptors in STZ- induced DM rats, with or without combined HPD treatment.

MATERIAL AND METHODS

Animals

Male Sprague-Dawley rats (Zivic Miller Laboratories, Pittsburgh, PA) weighing 200 to 250 g on arrival in the laboratory were used. The rats were housed in groups of five or six in standard floor net cages in a light- and temperature-controlled room and had free access to food and water.

Administration of STZ

Animals were rendered diabetic according to an established protocol (Bradberry et al. 1989). STZ (65 mg/kg, in 0.01 M citrate buffer, pH 4.5, 1.3 ml/kg) was injected into the lateral tail vein. An IV dose of 65 mg/kg STZ was chosen based on previous studies investigating neurochemical changes in brain DA, 5-HT, and other neurotransmitters (Chu et al. 1986; Bradberry et al. 1989; Lackovic et al. 1990). Diabetes was verified by glucosuria (Chemstrip^R, Boehringer Mannheim Co., Indianapolis, IN) and hyperphagia. Testing for hyperglycemia was conducted 1 week following the injection of STZ with a Glucometer Encore (Model #5885A, Miles Inc., Elkhart, IN) and tail stick blood samples.

Depot HPD Treatment

Four weeks after STZ treatment, both DM and non-DM rats were allocated into HPD- treated groups (STZ-HPD or vehicle-HPD group) or vehicle-treated groups (STZ-vehicle or vehicle-vehicle group). HPD-treated groups received an IM injection of the long-acting depot HPD (Halodol decanoate^R) 4 mg/kg (0.08 ml/kg), once a week for 4 weeks, according to a previous study (Debonnel et al. 1990). With this protocol, an increase in the striatal D_2 receptor density was expected (Debonnel et al. 1990). Vehicle groups received sesame oil. The subjects were decapitated 16 days after the last HPD injection.

Receptor-Binding Studies

The brains were removed immediately from the skull following decapitation, and the striatum, frontal cortex and hippocampus were dissected and stored at −80°C until use.

The tissue preparation and binding assays for D_2, 5-HT_{1A}, and 5-HT_{2A} receptors were performed according to Creese et al. (1977), Hall et al. (1985) and Leysen et al. (1982), respectively. Assay conditions used for these receptor sites are summarized in Table 1. Briefly, membranes from the striatum (the whole caudate-putamen, not including the pallidum), frontal cortex (anterior to the optic chiasmus, including the cingulate cortex), and hippocampus (both the dorsal and the ventral portions, not including the subiculum) were incubated with different concentrations of radioligand ([^{3}H]spiperone for D_2, [^{3}H]8-hydroxy-2-(di-n-propyl)-aminotetraline ([^{3}H]8-OH-DPAT) for 5-HT_{1A}, and [^{3}H]ketanserin for 5-HT_{2A} receptors). The incubations were stopped by rapid filtration through Whatman GF/B glass filters and washed three

Table 1. Assay Conditions for Dopamine-D_2, 5-HT_{1A}, and 5-HT_2 Receptor Binding

	3H-Ligand	Membranes	Nonspecific Binding	Assay Conditions
Receptor	Name (final concentration)	Area (mg of tissue per assay)	Compound Concentration	Buffer (volume, temperature, time)
D_2	[^{3}H]Spiperone, 0.05–2.0 nM	Striatum, 6	(+)-Butaclamol, 10 μM	A, pH 7.7, 1.0 ml, 37°C, 20min[a]
5-HT_{1A}	[^{3}H]8-OH-DPAT, 0.1–2.0 nM	Hippocampus, 7.5	5-HT, 10 μM	B, pH 7.4, 0.5ml 37°C, 12 min[b]
5-HT_{2A}	[^{3}H]Ketanserin, 0.1–3.0 nM	Frontal cortex, 9	Methysergide, 2 μM	C, pH 7.7, 1.0 ml 37°C, 20 min[c]

[a] Buffer A: 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1mM $MgCl_2$, 10 μM pargyline, 0.1% ascorbic acid, 1 μM mianserin (to occlude 5-HT_{2A} sites).
[b] Buffer B: 50 mM Tris-HCl, 1 mM $MnCl_2$.
[c] Buffer C: 50 mM Tris-HCl.

times with 4 ml ice-cold 50 mM Tris-HCl buffer (pH 7.4 or 7.7 at 25°C) with a Brandel cell harvester. All determinations were done in duplicate. Under these conditions, 4 to 6 (for D_2 receptors), 6 (for 5-HT_{1A} receptors), or 9 to 11 (for 5-HT_{2A} receptors) Scatchard plots were obtained from each group (STZ-HPD, STZ-vehicle, vehicle-HPD, or vehicle-vehicle) consisting of 9 to 12 rats.

Chemicals

The following drugs were either purchased or supplied by the manufacturers: (+)- butaclamol-HCl, methysergide-maleate, and mianserin-HCl (Research Biochemical Inc., Natick, MA); haloperidol decanoate (McNeil, Spring House, PA); 5-HT-creatinine sulfate and streptozotocin (Sigma, St. Louis, MO); [^{3}H]spiperone (24 Ci/mmol), [^{3}H]8-OH-DPAT (137 Ci/mmol), and [^{3}H]ketanserin (77.1 Ci/mmol) (New England Nuclear, Boston, MA).

Data Analysis

The number of receptor binding sites (B_{max}), and the dissociation constants (K_d) were obtained by Scatchard analysis (Scatchard 1949). Statistical comparisons were made by analysis of variance (ANOVA) followed by Fisher's least-significant difference (PLSD) test. A p value of less than 0.05 was considered significant.

RESULTS

Establishment of DM

Figure 1 shows the time course of mean body weight of rats for each group. While non-DM rats (vehicle-HPD and vehicle-vehicle groups) continued to gain weight throughout the study period, weight gain was suppressed in DM rats (STZ-HPD and STZ-vehicle groups). Blood glucose levels in DM rats (STZ-HPD and STZ-vehicle groups) 1 week after STZ treatment were about four times higher than in non-DM rats (vehicle-HPD and vehicle-vehicle groups) (Figure 2). All rats treated with STZ (DM groups) were found to be hyperglycemic (blood glucose >332 mg/dl).

Effect of DM and Combined HPD Treatment on D_2, 5-HT_{1A}, and 5-HT_{2A} Receptors

Representative Scatchard plots for D_2, 5-HT_{1A}, and 5-HT_{2A} receptors are shown in Figure 3. There was no significant difference in dissociation constants (K_d) among the four groups (STZ-HPD, STZ-vehicle, vehicle-HPD, and vehicle-vehicle) for all of the receptor sites (Table 2).

In non-DM rats, HPD treatment caused increase in D_2 receptor density (B_{max}) of about 31% (vehicle-HPD vs. vehicle-vehicle group, Figure 3, top panel, and Table 2). There was no significant effect of DM on the density of D_2 receptors (vehicle-vehicle vs. STZ-vehicle group). Chronic

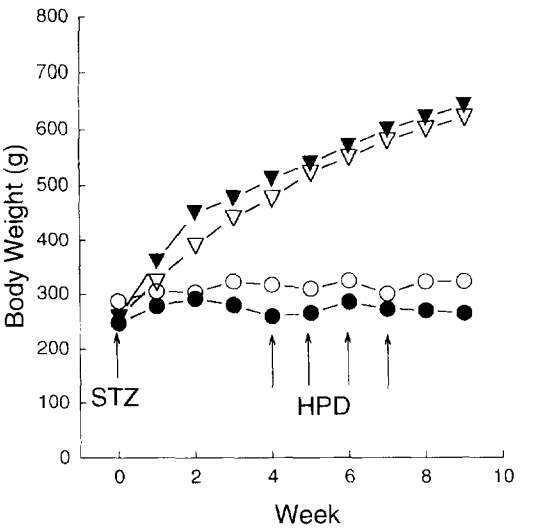

Figure 1. Body weight change of rats. Rats were treated with either STZ (65 mg/kg IV) or corresponding vehicle (0.01 M citrate buffer). Four weeks after STZ administration, the animals were treated with depot HPD (Halodol decanoate, 4 mg/kg/wk, IM × 4 weeks) or vehicle (sesame oil). *Open circles*, STZ-HPD group (n = 12); *solid circles*, STZ-vehicle group (n = 12); *open triangles*, vehicle-HPD group (n = 9); *solid triangles*, vehicle-vehicle group (n = 10).

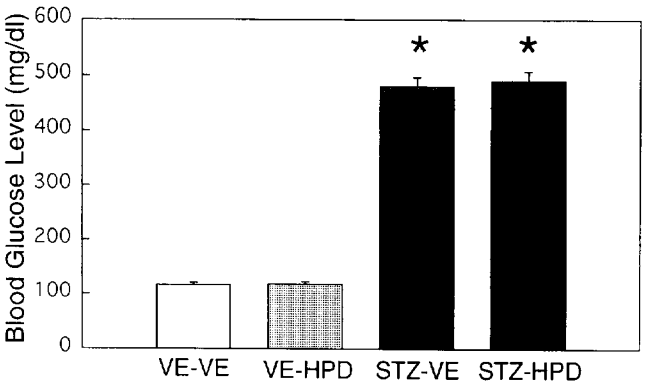

Figure 2. Effect of STZ (65 mg/kg IV) on blood glucose levels. Blood was drawn from the tail vein of rats one week after STZ or vehicle (0.01 M citrate buffer) administration. Values are expressed as mean ± SEM. *VE-VE*, vehicle-vehicle group (n = 10); *VE-HPD*, vehicle-haloperidol group (n = 9); *STZ-VE*, STZ-vehicle group (n = 12); *STZ-HPD*, STZ-haloperidol group (n = 12). *Significantly different from VE-VE or VE-HPD group ($p < .0001$, one-way ANOVA followed by Fisher's PLSD test).

HPD treatment did not affect the density of D_2 receptors in DM rats (STZ-vehicle vs. STZ-HPD group).

No significant difference in the density of 5-HT_{1A} receptors was observed among the four treatment groups (Figure 3 middle, Table 2).

The density of 5-HT_{2A} receptors in STZ-vehicle group was significantly higher (26%) than in vehicle-vehicle and vehicle-HPD groups (Figure 3 lower, Table 2). The 5-HT_{2A} receptor density in vehicle-vehicle and vehicle-HPD groups was not significantly different. No significant difference in the density of 5-HT_{2A} receptors was observed between STZ-HPD and vehicle-HPD and vehicle-vehicle groups.

DISCUSSION

The major findings of the present study are that: (1) STZ-induced DM caused an increase in the density of 5-HT_{2A} receptors (STZ-vehicle vs. vehicle-vehicle group); and (2) the increase in the density of D_2 receptors after chronic HPD treatment (vehicle-HPD vs. vehicle-vehicle group) was not observed in DM rats (STZ-HPD vs. STZ-vehicle group).

Methodological Considerations

Bradberry et al. (1989) found hyperglycemia (433 mg/dl) in rats treated 21 days before with STZ, which was markedly higher than control rats (152 mg/dl). The present results confirmed the results of Bradberry et al. (1989) in that we found significantly higher blood glucose levels in rats that received STZ (65 mg/kg IV) 1 week prior as compared to non-DM rats (Figure 2). The hyperglycemic state in STZ-induced diabetic rats was accompanied by polydypsia, polyphagia, glycosuria, and a

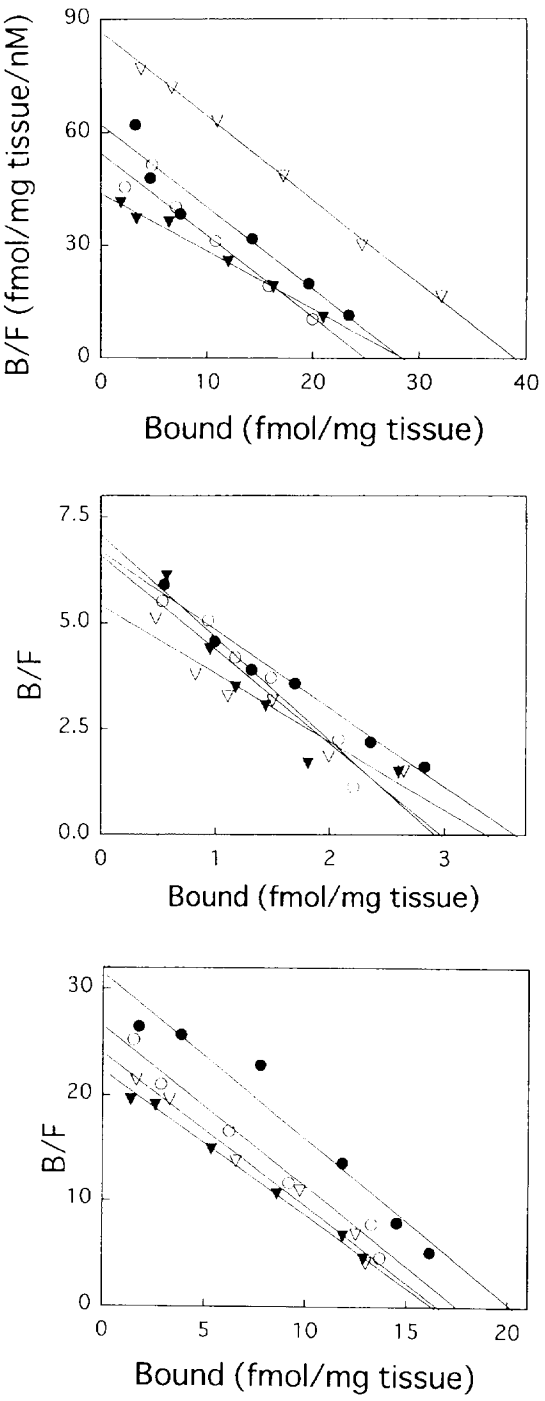

Figure 3. Effect of STZ (65mg/kg IV)-induced diabetes on rat brain receptors; [^{3}H]spiperone binding sites in the striatum (D_2 receptors, top panel), [^{3}H]8-OH-DPAT binding sites in the hippocampus (5-HT_{1A} receptors, middle panel), and [^{3}H]ketanserin binding sites in the frontal cortex (5-HT_{2A} receptors, bottom panel). Depot HPD (Halodol decanoate, 4mg/kg/wk x 4 wk) or vehicle (sesame oil) was treated 4 weeks after STZ administration followed by a 16–day withdrawal period. Binding assays were carried out as described in the Methods section, with six concentrations of [^{3}H]spiperone ranging from 0.05 to 2.0 nM, [^{3}H]8-OH-DPAT from 0.1 to 2.0 nM, or [^{3}H]ketanserin from 0.1 to 3.0 nM. *open circles*, STZ-HPD rat; *solid circles*, STZ-vehicle rat; *open triangles*, vehicle-HPD rat, *solid triangles*, vehicle-vehicle rat. Data are from one representative experiment performed in duplicate (see Materials and Methods).

Table 2. Effect of STZ-induced Diabetes on Brain Dopamine D_2, 5-HT_{1A}, and 5-HT_{2A} Receptors in Rats with and without Combined HPD Treatment

	D_2 Receptor			5-HT_{1A} Receptor			5-HT_{2A} Receptor		
Treatment	B_{max}	K_d	n	B_{max}	K_d	n	B_{max}	K_d	n
Vehicle-vehicle	28.2 ± 1.1	0.54 ± 0.10	4	2.63 ± 0.44	0.45 ± 0.08	6	16.7 ± 0.9	0.67 ± 0.05	10
Vehicle-HPD	37.0 ± 2.3[a]	0.51 ± 0.03	5	3.57 ± 0.45	0.45 ± 0.06	6	16.8 ± 1.0	0.67 ± 0.04	9
STZ-vehicle	26.3 ± 1.3	0.40 ± 0.03	5	3.34 ± 0.50	0.48 ± 0.05	6	21.0 ± 1.2[b]	0.82 ± 0.08	11
STZ-HPD	28.4 ± 1.6	0.40 ± 0.02	6	2.12 ± 0.41	0.50 ± 0.04	6	18.0 ± 1.2	0.82 ± 0.06	10

[a]Significantly different from the other three groups ($p < .01$, analysis of variance followed by Fisher's PLSD test).
[b]Significantly different from vehicle-vehicle or vehicle-HPD group ($p < .03$, analysis of variance followed by Fisher's PLSD test).

resultant loss of weight gain that continued throughout the study period (Figure 1). These results indicate the validity of STZ-treated rats in the current study as a conventional animal model for DM. Several previous studies reported that insulin normalizes blood glucose levels in STZ-induced DM rats (Bellush and Reid 1991; Chu et al. 1986; Kwok and Juorio 1987). Insulin treatment also normalizes such DM-related changes as: (1) a decrease in amphetamine-induced stereotypy (Bellush and Reid 1991); (2) an increase in the concentration of DA in the striatum (Chu et al. 1986); and (3) the reduced concentration of 5-HT and 5-HIAA in the striatum (Kwok and Juorio 1987). These results suggest that alterations in DA and 5-HT neurotransmission in STZ-induced DM rats are secondary to the hyperglycemic state that results from hypoinsulinemia. Therefore, DM rats were used to determine the possible effects of STZ treatment on brain D_2, 5-HT_{1A}, and 5-HT_{2A} receptors.

Although there have been little data on the effect of the emaciation that accompanies STZ treatment on D_2, 5-HT_{1A}, and 5-HT_{2A} receptors in the brain, the lack of difference in the density of D_2 and 5-HT_{1A} receptors between DM and non-DM rats in the present study suggests that the effect of emaciation itself (Figure 1) on D_2 as well as 5-HT_{1A} and 5-HT_{2A} receptors is minimal.

It should be noted that [^{3}H]8-OH-DPAT and [^{3}H]ketanserin may reveal more than one binding site (Nénonéné et al. 1994; Roth et al. 1987). Further studies employing wider concentration range of these ligands and other methods to analyze binding data could resolve this issue.

Effects of STZ-Induced Diabetes on 5-HT Receptors

STZ-induced DM caused a 26% increase in the density of 5-HT_{2A} receptors in the frontal cortex (Figure 3, Table 2). Christ et al. (1994) reported that 5-HT-induced contraction of rat aortic rings, which is mediated by 5-HT_{2A} receptors, is enhanced at 8 weeks following STZ-induced diabetes. Although it is difficult to determine the relationship between the increased sensitivity in the peripheral measure of 5-HT_{2A} receptor-mediated response and the observed increase in 5-HT_{2A} receptors in the frontal cortex in our study, these findings as a whole indicate that STZ-induced DM modulates 5-HT_{2A} receptor-mediated transmission in both central and peripheral levels.

So far, only a limited number of treatments have been reported to increase 5-HT_{2A} receptors. Repeated electroconvulsive shock (ECS) has been reported to produce a 21% to 57% increase in 5-HT_{2A} receptors in the cortex (Kellar et al. 1981; Vetulani et al. 1981; Stockmeier and Kellar 1986; Butler et al. 1993). Repeated treatment with reserpine (0.5 mg/kg × 12 day IP), which reduces 5-HT content in the brain by disrupting its storage in vesicles (Brodie et al. 1965) increases 5-HT_{2A} receptors (Stockmeier and Kellar 1986), although Scott and Crews (1985) failed to observe this effect before a shorter period of reserpine treatment (5 mg/kg × 4 days IP). Selective lesioning of 5-HT neurons by intracerebroventricular administration of a 5-HT neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT, 50 μg), increased 5-HT_{2A} receptors labeled by [^{3}H]ketanserin in the mouse cortex (Heal et al. 1985). Application of a smaller dose of 5,7-DHT (7 μg) into the raphe nuclei did not affect the density of 5-HT_{2A} receptors in the rat frontal cortex (Stockmeier and Kellar 1986). Chronic exposure to certain kinds of stress has also been reported to increase cortical 5-HT_{2A} receptors (Papp et al. 1994; Takao et al. 1995).

Stockmeier and Kellar (1986) have suggested that an interaction between 5-HT and other neurotransmitters may be responsible for the homeostatic control of 5-HT_{2A} receptors and that intact 5-HT axons are necessary to the upregulation of 5-HT_{2A} receptors. They reported that the reduction of 5-HT content and subsequent impaired 5-HT neurotransmission in brain by reserpine are associated with 5-HT_{2A} receptor upregulation (Stockmeier and Kellar 1986). Reduced tryptophan (Kwok and Juorio 1987), 5-HT (Chu et al. 1986) and 5-HIAA (Bellush and Reid 1991; Lackovic et al. 1990) levels, as well as decreased 5-HT turnover (5-HIAA/5-HT) (Bellush and Reid 1991) have been reported in DM rats, which are consistent with decreased 5-HT neuronal function. Therefore, it is plausible that the observed upregulation of 5-HT_{2A} receptors in STZ-induced DM rats is due to a mechanism(s) comparable to that of reserpine, which decreases brain 5-HT content, rather than ECS treatment which has no apparent effect on 5-HT content or turnover in brain (Modigh 1976). Further studies investigating the function of 5-HT_{2A} receptors (e.g., DOI-induced head-twitch response, 5-HT_{2A} receptor-linked phosphoinositide turnover, and 5-HT_{2A} receptor mRNA

levels,) will clarify the nature of the 5-HT_{2A} receptor upregulation found in the brain of STZ-induced DM rats.

In the context of the present results, it is interesting to note that both the reserpine treatment and patients with DM (Lustman et al. 1992) have the increased risk for major depression and that 5-HT_{2A} receptor upregulation is observed in postmortem brain from depressive subjects (Stanley 1983; Mann et al. 1986). Whether modulation of 5-HT_{2A} receptor density occurs in other brain regions and could be relevant to the increased risk to develop major depression in patients with DM requires further study.

STZ-induced DM did not affect the density of 5-HT_{1A} receptors labeled by [^{3}H]8-OH-DPAT in the hippocampus in the present study (Figure 3, Table 2). Truson and MacKenzie (1981) previously reported no changes in the density of [^{3}H]5-HT binding sites that include 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2C} receptors (Peroutka 1986) in the STZ-induced diabetic rat. The present results provide additional evidence for the lack of change in the density of 5-HT_{1A} receptors in DM rats.

Effects of STZ-Induced zDiabetes on D_2 Receptors

The increase in D_2 receptor sensitivity has been taken as a compensatory response to diminished firing of DA neurons and the DA synthesis in DM (Saller and Chiodo 1980; Lozovsky et al. 1981; Truson and Himmel 1983). The present results did not confirm former reports of an increase in the density of striatal D_2 receptors in STZ- or alloxan-induced DM rats (Lozovsky et al. 1981; Truson and Himmel 1983; Serri et al. 1985). The difference in experimental designs between the former reports and ours should be noted here. The previous studies (Saller and Chiodo 1980; Lozovsky et al. 1981; Truson and Himmel 1983) measured D_2 receptor densities after a shorter hyperglycemia (i.e., 4 to 6 weeks). In contrast, the rats studied here had been diabetic for more than 9 weeks. It has been reported that 4 to 5 weeks of diabetes has no effect on rat brain DA content (Bellush and Reid 1991), whereas increased DA content has been observed following longer periods (12 weeks) after STZ treatment (Lackovic et al. 1990). Therefore, the effect of diabetic state on D_2 receptors may depend on the duration of hyperglycemic state. It could be argued that the initial D_2 receptor upregulation disappears in the long-lasting diabetic state. Another factor to explain the discrepancy noted may be the difference in the amount of STZ administered. Previous investigations (Lozovsky et al. 1981; Truson and Himmel 1983) used an IP dose of 75 mg/kg STZ, whereas an IV dose of 65 mg/kg was applied in this study. However, the difference in the dose of STZ is unlikely to explain the discrepancy in D_2 receptor upregulation, as blood glucose levels reach a plateau at an IP dose of 60 mg/kg (Ohkuwa et al. 1995), which suggests that the IV dose of 65 mg/kg STZ used in the present study was sufficient to produce maximal increase in blood glucose levels.

Effect of HPD Treatment

In accord with a previous report that found a 44% increase in the striatal D_2 receptors following 5 weeks of depot HPD (4 mg/kg/week IM) (Debonnel et al. 1990), chronic treatment with depot HPD (4 mg/kg/week IM for 4 weeks) caused a 31% increase in the density of D_2 receptors labeled by [^{3}H]spiperone (vehicle-HPD vs. vehicle-vehicle group). This effect of HPD was not associated with changes in the D_2 affinity constants (K_d). The lack of change in the affinity constants in HPD-treated rats (STZ-HPD and vehicle-HPD groups) indicates that the effect of residual HPD following the 16 days of withdrawal period is negligible. The lack of change in the density of hippocampal 5-HT_{1A} and frontal 5-HT_{2A} receptors after chronic HPD treatment (vehicle-HPD vs. vehicle-vehicle group) is in agreement with previous reports (Andree et al. 1986; Matsubara and Meltzer 1989; Hashimoto et al. 1993).

Effect of Combined Treatment with STZ and HPD

HPD no longer produced an increase in the density of D_2 receptors in the striatum of STZ-induced DM rats. The precise mechanism underlying the absence of effects of chronic HPD treatment on D_2 receptors in the DM rat is currently unclear. The chronic hyperglycemic state with subsequent loss of weight may have affected the pharmacokinetics and bioavailability of HPD that reaches the brain, as cerebral blood flow is reported to be lower in animals with DM (see McCall 1992 for review). Therefore, the availability of HPD in the brain of STZ-induced DM rats could be reduced. However, the STZ-induced 5-HT_{2A} receptor upregulation was reversed by chronic HPD, which indicates that some pharmacological effects of HPD are still preserved in the brain of DM rats. An alternative interpretation could be that HPD treatment prevents the STZ-induced 5-HT_{2A} receptor upregulation. Studies on the time course of the 5-HT_{2A} receptor upregulation would clarify the interaction between STZ and HPD.

The failure of D_2 receptors to upregulate following chronic HPD treatment in STZ-induced DM rats may be relevant to the increased risk of tardive dyskinesia in DM (Mukherjee et al. 1985; Ganzini et al. 1991; Woerner et al. 1993). Upregulation of D_2 receptors by typical neuroleptics such as HPD may be an adaptive response to maintain dopaminergic function that is impaired in patients with DM. The inability of D_2 receptors to respond to chronic HPD treatment and increased S(-)-apomorphine-induced vacuous chewing movements in STZ-induced DM rats (Sumiyoshi et al., in press) may repre-

sent part of the underlying mechanisms for the increased risk of tardive dyskinesia in patients with DM.

An increase in the density of 5-HT_{2A} receptors in the cortex was not observed in DM rats that received subsequent treatment with HPD (STZ-HPD vs. vehicle-HPD and vehicle-vehicle groups). Because HPD has only a weak affinity for 5-HT_{2A} receptors (pK_i = 7.7 compared to 9.0 for D_2 receptors; Meltzer et al. 1989), some other effects of this drug, such as the inactivation of mesocortical DA neurons and increased cholinergic and GABAergic neurotransmission associated with the cortex, may be responsible for the reversal of 5-HT_{2A} receptor upregulation in DM rats.

In conclusion, the present study demonstrated that STZ-induced DM increased the density of 5-HT_{2A} receptors, but did not affect D_2 or 5-HT_{1A} receptor densities in the rat brain. The increase in the density of D_2 receptors by chronic HPD treatment was abolished in STZ-induced DM rat. The 5-HT_{2A} receptor upregulation and the inability of D_2 receptors to respond to chronic neuroleptic treatment may be further evidence of altered DA and 5-HT neurotransmission in the diabetic state and suggest a possible basis for the vulnerability of patients with DM to develop tardive dyskinesia or major depression.

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