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Changes in the Central Dopaminergic Systems in the Streptozotocin-induced Diabetic Rats

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The behavioral response, dopamine metabolism, and characteristics of dopamine subtypes after developing the hyperglycemia were studied in the striata of rats. In animals developed hyperglycemia, the on-set and duration of cataleptic behavior responded to SCH 23390 injection was delayed and shortened, respectively. However, the cataleptic responses to spiperone occurred significantly earlier in on-set and prolonged in duration. Dopamine metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were significantly reduced in the striata of hyperglycemic rats. However, level of DA was significantly increased. It is noted that the ratios of DOPAC and HVA to DA were decreased, suggesting decreased turnover of DA. The affinity of striatal D-1 receptors was significantly increased without changes in the number of binding sites, while the maximum binding number of D-2 receptors was significantly increased without affecting its affinity in the diabetic rats. These results indicate that the dopaminergic activity in the striata was altered in hyperglycemic rats. Furthermore, it suggests that the upregulation of dopamine receptors might be due to the decreased dopamine metabolism.

Key words: Hyperglycemia, Catalepsy, Dopamine metabolism, D-1 and D-2 receptors

INTRODUCTION

It is well documented that retinopathy, neuropathy and nephropathy are serious complications of diabetes mellitus. The occurrence of the central neuropathy in diabetes mellitus is rare, probably because of the undetectable changes in the brain functions.

It has been reported that drug metabolism (Watkins and Sherman, 1992) as well as various neuronal activities (Bitar and DeSouza, 1990; Carrier and Aronstam, 1990; Shimomura *et al.*, 1988, 1990) are altered in the diabetes mellitus. It has also been reported that behavioral responses related to dopaminergic nervous activity are decreased in diabetes mellitus (Marshall *et al.*, 1976; Shimomura *et al.*, 1990). Saller (1984) has reported that striatal dopamine metabolite levels are reduced after alloxan administration. Although hypothalamic norepinephrine metabolites were markedly reduced in diabetes mellitus (Bitar and DeSouza, 1990), the maximum velocity of tyrosine hydroxylase was markedly reduced and norepinephrine content was significantly increased (Bitar *et al.*, 1986). Furthermore, striatal [^{3}H]spiperone binding (Lozovsky *et al.*, 1981) and hypothalamic beta-1 adrenoreceptors (Bitar and DeSouza, 1990) were reported to be increased in this disease state. These studies indicate that central neuronal activities might be changed in diabetes mellitus.

It has been reported that there are more than two central dopamine receptors, D-1 and D-2 receptors (Stoof and Kebabian, 1984). Furthermore, their locations as well as the physiological and biochemical role are different and their interactions are controversial (Barone *et al.*, 1988; Robertson and Robertson, 1987; Saller and Salama, 1986; Stoof and Kebabian, 1984). Although the decrease in dopamine turnover rates might be induced by diabetes-induced alterations in the characteristics of dopamine receptor subtypes, little is known about the receptor alterations or the behavioral responses of diabetic animals to specific dopamine receptor antagonists.

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Therefore we undertook a systematic investigation of the behavioral responsiveness to D-1 and D-2 specific antagonists, dopamine turnover rates, and the characteristics of dopamine receptors in STZ-induced diabetic rats.

METHODS AND MATERIALS

Animals and Materials

Male Sprague-Dawley rats (SNU animal house, Seoul, Korea and Charles River Lab, Wilmington, MA) weighing 200-250 g were used throughout the study. The animals were housed four to a cage with free access to food and water in a temperature-regulated room having a 12/12 hour light-dark cycle. SCH-23390, sulpiride and spiperone were purchased from Research Biochemical Inc. (Wayland, MA). The tritium labeled ligand, [^{3}H]sulpiride (specific activity, 78.6 Ci/mmol), was obtained from New England Nuclear (Boston, MA). The ligand, [^{3}H]SCH-23390, was purchased from Amersham Corp. (Arlington Height, IL). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Treatment Protocol

Rats which were made diabetic received intraperitoneal injections of 40 mg/kg streptozotocin (STZ) freshly dissolved in citrate buffer (pH 4.5) for three successive days according to our preliminary study and the modification of Hoskins and Scott (1984). In this treatment, no mortality was occurred within two weeks. Blood samples were spun at 5,000×g for 10 min at 4℃. The plasma glucose levels were measured by the glucose hexokinase method using a commercial kit from Sigma (St Louis, MO). Twenty four hr or two weeks after the last administration of STZ, blood glucose levels of STZ-treated rats were more than 350 mg/dl.

In behavioral studies, the specific D-1 antagonist, SCH-23390 (0.05 and 0.1 mg/kg) (Hyttel, 1983), and the relatively specific D-2 antagonist, spiperone (0.5 mg/kg) (Ryall, 1989), were injected subcutaneously one day and two weeks, respectively, after the last administration of STZ. The on-set and duration of the drug-induced catalepsy were recorded for each animal. In our preliminary study, the more specific D-2 antagonist, sulpiride, did not induce catalepsy even at a dose of 40 mg/kg.

For biochemical studies, levels of dopamine and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and tyrosine hydroxylase activity were determined 24 hr and two weeks after the last injection of STZ. Since dopamine metabolisms were not different between one day and two weeks after the last injection of STZ, the characteristics of the dopamine receptors were determined one day after the STZ treatment only.

Measurement of Catalepsy

Catalepsy was assessed by the bar method as described by Meller *et al.* (1985) with minor modification. After administration of antagonist, the front paws of each treated rat were gently placed on a horizontal metal bar 1.0 cm in diameter which was suspended 10 cm above the work surface. Placement on the bar was repeated until the animal remained in position. The interval between antagonist administration and this time point was recorded as the latency to onset of catalepsy. The latency to the on-set and the duration of catalepsy were recorded for placement of both paws on the floor up to a 60 seconds.

Determination of DA and Its Metabolites

Levels of DA, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were assayed using the method described by Mayer and Shoup (1983) with minor modification. The striata were dissected out according to the procedure of Glowinski and Iversen (1966). Immediately following decapitation and dissection, tissue samples were homogenized in 2 ml of ice-cold 0.05 M perchloric acid (PCA). Following centrifugation (20 min, 15,000 g at 4℃), the supernatants were diluted 1 : 10 with ice-cold PCA. An aliquot of 100 μl was injected into a high performance liquid chromatography-electrochemical detection (HPLC-EC) system to determine DA, DOPAC and HVA. The column employed was a Spheri-5 RP-18, 5 μm, 100×4.6 mm, from Bioanalytical Systems. An electrochemical detector (M460, Waters Systems) with a glassy carbon electrode, at a sensitivity of 10 nA, was used to monitor the column eluate. The applied potential was 700 mV vs Ag/AgCl-3M NaCl. Peaks were integrated using a Hewlett Packard M7458 integrator. The mobile phase was 10% acetonitrile/monochloroacetate buffer, pH 3.0 with 0.7 mM EDTA and 0.86 mM sodium octyl sulfate. The flow rate was maintained at 0.8 ml/min. The determinations of DA, DOPAC and HVA content were performed by direct comparison of peak heights between samples and an external standard. The external standards were checked by HPLC once every day prior to the start of sample assays.

Determination of Tyrosine Hydroxylase (TH) Activity

TH activity in the striata was assayed using the procedure of Horwitz and Perlman (1984) with a minor modification. The accumulation of DOPA was measured in the presence of brocresine, an inhibitor of DOPA decarboxylase (Emy *et al.*, 1981). The dissected

striata were homogenized in 2 ml of an ice-cold solution containing 10 mM sodium phosphate, 5 mM sodium pyrophosphate, 5 mM EDTA, and 0.2% Triton X-100, pH 6.7. Following centrifugation (5 min, 18,000 g at 4℃), 100 μl of supernatant were added to a 100 a10ml reaction solution consisting of 160 μM L-tyrosine, 300 μM brocresine, 0.4 mM 6-methyl-5,6,7,8,-tetrahydropterin, 10,000 units of catalase, 200 μM $FeSO_4$, 200 mM 2-mercaptoethanol and 200 mM 2-[N-morpholino]-ethanesulfonic acid (pH 6.8). Reactions were carried out for 10 min at 37℃ and were terminated by addition of 300 μl of 0.28 M trichloroacetic acid containing 90 pmol of epinephrine. After centrifugation, the supernatant was added to 25 mg of acid-washed alumina and the pH was adjusted to 8.6-8.7 with 3 M Tris containing 10 mM EDTA and 0.1 mM Na_2SO_3. The alumina was washed four times with water, and DOPA was eluted with 200 μl of 100 mM PCA. The eluent was assayed using HPLC-ECD as described above except that acetonitrile was deleted from the mobile solution and the flow rate was 1.2 ml/min. Epinephrine was used as an internal standard to correct for the recovery of DOPA, which was 85-90%. TH activity was estimated after subtraction of the DOPA produced in enzyme-free incubations and was expressed as pmol DOPA produced/protein/min.

Membrane Preparation for Binding Assays

Rats were decapitated and the brains were rapidly removed. The striata were dissected out according to the method of Glowinski and Iversen (1966). Membranes for D-1 receptor binding assays were prepared according to the method of Zukin *et al.* (1974) with minor modifications as follows: the pooled samples were homogenized in 15 volumes of ice-cold 0.32 M sucrose using a Brinkmann Polytron PT-10 at low speed. The homogenate was centrifuged at 1,000 g for 10 min; the pellet was discarded and the supernatant was centrifuged at 20,000 g for 20 min. This crude synaptosomal pellet was resuspended in double distilled deionized water and dispersed with the Polytron for 30 sec. The suspension was centrifuged at 8,000 g for 20 min. The supernatant, including the buffy layer was collected and was resuspended in water and centrifuged at 48,000 g for 20 min. The final pellet was suspended in 50 mM Tris-HCl buffer (pH 7.4) and stored at −70℃. Because much more tissue is required for D-2 receptor binding assays, membranes for D-2 receptor bindingassays were prepared according to Carboni *et al.* (1985) with slight modifications. In brief, brain tissue was homogenized in 50 volume ice-cold 50 mM Tris-HCl buffer (pH 7.7) with an Ultraturex homogenizer. The resulting homogenate was centrifuged twice at 48,000 g for 20 min and the final pellet was resuspended in 50 mM Tris-HCl buffer containing 0.01% (w/v) ascorbic acid and the following ions: 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$ (pH 7.4) and stored at −70℃.

[^{3}H]Sulpiride Binding

Specific binding of the D-2 dopamine receptor antagonist, [^{3}H]sulpiride, was determined following the method of Carboni *et al.* (1985) with minor modification. The frozen membranes were thawed and centrifuged at 48,000 g for 20 min to obtain a pellet. The pellet was suspended in 50 mM Tris-HCl buffer (pH 7.4) containing 0.01% (w/v) ascorbic acid and following salts (in mM): NaCl, 120; KCl, 5; $CaCl_2$, 2 and $MgCl_2$, 1.

Aliquots of 0.2 ml of the membrane preparation (0.5-1 mg protein/ml) were incubated with various concentrations (0.5-10 nM) of [^{3}H]sulpiride in a final volume of 1 ml. Nonspecific binding was determined by adding 1 μM unlabeled (−)sulpiride. Incubations were carried out for 30 min at 8℃. At the end of the incubation, the samples were filtered under reduced pressure through glass fiber filters (GF/B, Whatman) using a cell harvester. The filters were then washed twice with 5 ml of Tris-HCl buffer and, after drying, were placed into scintillation vials with 10 ml of Safety Solve. Radioactivity retained in the filters was counted by liquid scintillation spectrometry. To reduce the binding of free [^{3}H]sulpiride, the filters were presoaked in 0.1% polyethylenimine solution.

[^{3}H]SCH-23390 Binding

Specific binding of the D-1 dopamine receptor ligand, [^{3}H]SCH-23390, was determined following the method of Porceddu *et al.* (1986) with minor modification. The frozen membrane preparation was thawed and centrifuged at 48,000 g for 20 min to obtain a pellet. The pellet was suspended in 50 mM Tris-HCl (pH 7.4), containing (in mM) NaCl, 120; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 1. Aliquots (200 μl) of the membrane preparations (0.3-0.5 mg protein/ml) were incubated with various concentrations (0.05-3 nM) of [^{3}H]SCH-23390 to generate saturation curves in a final volume of 1 ml. Nonspecific binding was determined by adding 1 μM unlabeled SCH-23390. After 30 min incubation at 37℃, the reaction was terminated by rapid filtration through Whatman GF/B glass fiber filters using a cell havester. The filters were washed twice with 5 ml of ice cold buffer and then were transferred to scintillation counting vials containing 10 ml of Safety Solve and counted.

Determination of Protein Concentration

The protein content of tissue homogenates was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

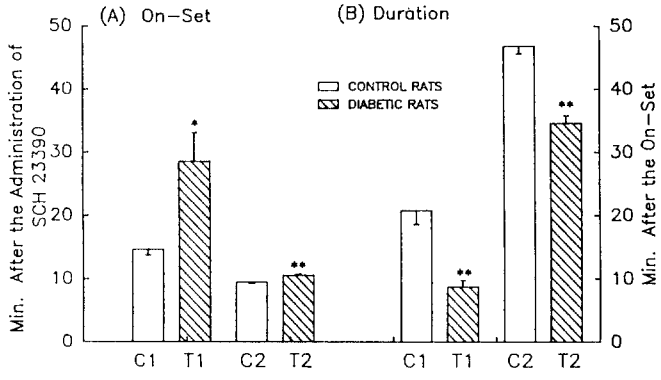

Fig. 1. SCH 23390-induced catalepsy in the STZ-induceddiabetic and control rats. The diabetic rats were induced by the i.p. injection of streptozotocin, 40 mg/kg, for three consecutive days. Twenty four hours after the last injection, the catalepsy was induced by the injection of SCH 23390 and the on-set and the ended times were recorded in individual rats. The doses of SCH 23390 were 0.05 mg/kg (C1 and T1) and 0.1 mg/kg (C2 and T2), sc, respectively. The open and the slashed bar are saline and STZ-treated groups, respectively. Values are mean± S.E. for three to five rats, sc. *p<0.05, **p<0.01 compared with the corresponding control.

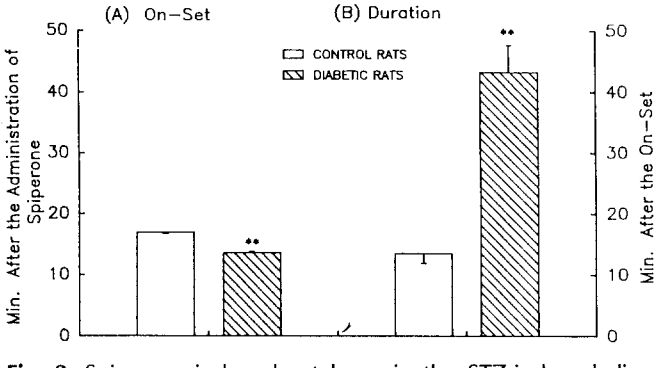

Fig. 2. Spiperone-induced catalepsy in the STZ-induced diabetic and control rats. The diabetic rats were induced by the i.p. injection of streptozotocin, 40 mg/kg, for three consecutive days. Two weeks after the last injection, the catalepsy was induced by the injection of spiperone and the on-set and the ended times were recorded in individual rats. The dose of spiperone were 0.5 mg/kg, sc. The open and the slashed bar are saline and STZ-treated groups, respectively. Values are mean± S.E. for four rats. **p<0.01 compared with the corresponding control.

Statistics

The statistical significance of differences was determined using Student's t-test. In studies of receptor characteristics, linear regression analyses, i.e., Scatchard analysis, were used to obtain all values of dissociation constants (K_d) and maximum numbers of binding sites (B_{max}), since computer-aided analyses (Munson and Rodbard, 1980) showed a single binding site in binding assays under the present conditions.

Table I. The levels of dopamine and its metabolites in the striata of STZ-induced diabetic rats

Treatments	DA	DOPAC	HVA
Control	469.4± 15.3	77.7± 2.4	29.2± 1.2
24 hr	486.1± 41.6	57.4± 2.8**	20.8± 0.7**
Control	529.5± 8.5	68.9± 1.7	22.8± 2.7
2 Weeks	618.8± 18.6*	57.1± 3.9*	13.6± 2.1

Rats were sacrificed at the indicated time after the last administration of streptozotocin. The units of DA and its metabolites are pmol/mg protein. Each value represents the mean± 1 S.E.M. of four determinations.
*p<0.05, **p<0.01 compared to the respective control values.

RESULTS

The Changes in the Behavioral Response by Dopamine Antagonists in the Hyperglycemic Rats

Figures 1 and 2 show changes in the latency to onset of and duration of catalepsy induced by the injection of either SCH-23390 or spiperone in the STZ-diabetic rats. The latency to onset of and the duration of catalepsy was dose-dependent. Twenty four hours after the last administration of STZ, the on-set of catalepsy in diabetic rats was significantly delayed as compared with that in normal rats after the injection of SCH-23390 (14.65± 0.92 min vs 28.55± 4.53 min at 0.05 mg/kg and 9.48± 0.21 min vs 10.56± 0.22 min at 0.1 mg/kg, $p<0.008$). However, the duration of catalepsy after treatment with SCH-23390 was markedly shortened (20.80± 2.20 min vs 8.73± 1.05 min at low dose and 46.85± 1.21 vs 34.60± 1.20 min at high dose) (Fig. 1). Two weeks after the last administration of STZ, the latency to onset of catalepsy in the diabetic rats was significantly decreased (16.97± 0.19 min vs 13.61± 0.22 min, $p<0.001$) and the duration of catalepsy was increased (13.48± 1.58 min vs 43.18± 3.45 min) after the injection of spiperone (Fig. 2).

Effects of STZ-Diabetes on the Dopamine Metabolism and Receptors

Table I shows the concentrations of striatal dopamine and its metabolites after the induction of STZ-diabetes. The levels of dopamine in diabetic state were significantly increased two weeks after the last administration of STZ. However, the levels of striatal dopamine metabolites, DOPAC and HVA, were significantly decreased in the diabetic rats as compared with the normal rats. The reduction of DOPAC and HVA were 26.2% and 28.8%, respectively, at 24 hr after the last administration of STZ. Even at two weeks after the last administration of STZ, the striatal DOPAC levels were still significantly decreased to 82.9% of the control level without the changes in the level of HVA.

Table II. DOPAC/DA and HVA/DA ratios in the striata of STZ-induced diabetic rats

Treatment	DOPAC/DA	HVA/DA
Control	0.166± 0.006	0.062± 0.003
24 hr	0.108± 0.009**	0.043± 0.003**
Control	0.130± 0.005	0.044± 0.003
2 Weeks	0.092± 0.005**	0.022± 0.002*

Rats were sacrificed at the indicated time after the last administration of streptozotocin. Each value represents the mean± 1 S.E.M. of four determinations.
*p<0.05, **p<0.01 compared to the respective control values.

Table III. The characteristics of D-1 and D-2 receptors in the striata of STZ-induced diabetic rats

Subtypes		K_d	B_{max}
D-1	Control	0.287± 0.013	1613.0± 34.0
	Treated	0.243± 0.010*	1676.0± 74.1
D-2	Control	1.706± 0.029	391.7± 7.1
	Treated	1.847± 0.073	437.0± 12.5*

Rats were sacrificed 24 hr after the last injection of streptozotocin. Each value represents the mean± 1 S.E.M. of four determinations, each performed in duplicate. Units of Kd and Bmax are nM and pmol/g protein, respectively.
*p<0.05 compared to the respective control values.

Furthermore, the ratios of DOPAC and HVA to DA in striata were significantly decreased at both 24 hr and two weeks after the last administration of STZ (Table II). The decreased turnover rates of DOPAC/DA and HVA/DA were 35% and 31%, respectively, 24 hr after the induction of the STZ-diabetes. Furthermore, the decreased rates of DOPAC/DA and HVA/DA were 30% and 50%, respectively, after two weeks' duration of STZ-diabetes. The activity of striatal tyrosine hydroxylase was not altered in the STZ-diabetic rats. The effects of STZ-diabetes on binding characteristics of striatal dopamine receptors are shown in Table III. The affinity of D-1 receptors was significantly increased (18 %) without any alteration in their number 24 hr after the last administration of STZ. However, maximum binding densitiy of striatal D-2 receptors was slightly but significantly increased (11%) without changes in their affinity.

DISCUSSION

The present results demonstrate that the STZ-induced diabetes mellitus alters central dopaminergic neuronal activities. We found that in this animal model of diabetes mellitus, there are alterations in striatal dopamine turnover, the binding characteristics of striatal dopamine receptors, and the latency to onset and duration of dopamine antagonist-induced catalepsy. However, striatal tyrosine hydroxylase activity was not altered.

It has been reported that locomotor and ambulatory activities are decreased in diabetic rats (Chu *et al.*, 1986;Shimomura *et al.*, 1988). Furthermore, Marshall *et al.* (1976) reported that amphetamine-induced stereotypy is reduced in diabetic rats. Some of the present results, therefore, are consistent with those of other investigators. Cataleptic behaviors in rats are related to brain areas, such as frontal cortex, striatum and nucleus accumbens (Dunstan *et al.*, 1981; Meller *et al.*, 1985; Scatton *et al.*, 1980). Cataleptic activity is also known to be mediated, at least in part, by the central dopaminergic system (Hoffman *et al.*, 1985). Therefore, the changes in the dopamine antagonists-induced cataleptic response indicate that the dopaminergic neuronal acitivity was altered in the diabetic rats. However, in the present study, the cataleptic response in diabetic rats was oppositely affected by dopamine antagonists, SCH-23390 and spiperone; the reduced response to SCH-23390 and the increased response to spiperone, respectively. It has been reported that striatal dopamine D-1 and D-2 receptors exert dual opposing role over striatal acetylcholine release (Consolo *et al.*, 1992; Stoof *et al.*, 1992) and cholinergic agonists produce the cataleptic behavior (Costall and Naylor, 1973). Therefore, the different response to the D-1 and D-2 dopamine antagonists in the diabetic rats might be due to the dual role of D-1 and D-2 receptor subtypes over cholinergic activity. However, further study on the different neuronal pathways or biochemical mechanisms in the occurrence of catalepsy are needed.

The present results also demonstrate that the striatial dopamine metabolite, DOPAC and HVA, and their ratios to DA are decreased in the hyperglycemic rats. Saller (1984) and Shimomura *et al.* (1988) have reported that striatal dopamine metabolite levels, such as DOPAC, in the chemically induced hyperglycemia rats were reduced and their ratio to DA were also decreased. Our results are consistent with those reports. In addition, the ratios of HVA to DA were also decreased. It has been reported that the ratios of DOPAC/DA and HVA/DA were closely related with the dopaminergic nervous activities (Westerink and Korf, 1976). Therefore, the reduced ratios in the diabetic animals indicate that the striatal dopaminergic activities might be decreased. It has been reported that the tyrosine hydroxylase activities in streptozotocin-treated rats were decreased in the hypothalamus (Bitar *et al.*, 1986), on the other hand norephinephrine contents in the hypothalamus were increased (Bitar and DeSouza, 1990). However, the present result did not show any difference in tyrosine hydroxylase, which indicates that dopamine synthesis is not affected in the diabetic rats.

The reason for this discrepancy was not clear, however, it might be due to the different regions examined. In view of the evidence acute administration of glucose completely suppressed the discharge of dopamine neurons (Saller and Chiodo, 1980), the decreased metabolite levels might be due to the decreased rate of DA release in striatum.

It has been reported that the binding of [^{3}H]spiperone in striatal membrane was increased in the chemically-induced diabetic rats (Lozovsky *et al.*, 1981). However, Salkovic and Lackovic (1992) reported that the maximum binding density of dopamine D-1 receptor was decreased in diabetic rats. The present results reveal the increase in the characteristics of dopamine receptor subtypes. Furthermore, the changes in the characteristics in the [^{3}H]SCH-23390 and [^{3}H]sulpiride binding sites are a little different in diabetic rats; the increased affinities and the increased maximum binding densities, respectively. It is generally agreed that the inhibition in the neuronal activities induces the supersensitivity of receptors to compensate the decreased activities. Therefore, the upregulation of dopamine receptors in the hyperglycemic rats might be due to the decreased dopaminergic nervous activities, such as the decreased turnover rate of dopamine. It has been reported that the localization of both the D-1 and D-2 receptors are on striatal cells and the D-2 receptors are also on the DA nerve terminal in striatum (Quimet *et al.*, 1984; Seeman, 1980). Thus the differential changes in the characteristic of dopamine subtypes in diabetic rats might be related to the localization of dopamine receptors in the striatum.

Although the changes in the characteristics of receptors in disease states did not always correlate with their response (Brodde and Michel, 1989) and further work in insulin independent diabetes is needed, the changes in the characteristics of dopamine receptors in the diabetes mellitus might be related to the altered response to dopaminergic agents.

In conclusion, the present study suggests that the dopaminergic nervous activities may be decreased in the hyperglycemias. Furthermore, the supersensitivity of dopamine receptors may be induced in the hyperglycemias.

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REFERENCES CITED

Barone, P., Tucci, I., Parashos, S. A. and Chase, T. N., Supersensitivity to a D-1 dopamine receptor agonist and subsensitivity to a D-2 receptor agonist following chronic D-1 receptor blockade. *Eur. J. Pharmacol.*, 149, 225-232 (1988).

Bitar, M. S. and DeSouza, E. B., Diabetes-releated changes in brain beta adrenoreceptors in rats as assessed by quantitative autoradiography: Relationship to hypothalamic norepinephrine metabolism and pituitary-gonadal hormone secretion. *J. Pharmacol. Exp. Ther.*, 254, 781-785 (1990).

Bitar, M. S., Koulu, M., Rapoport, S. I. and Linnoila, M., Diabetes-induced alterations in brain monoamine metabolism in rats. *J. Pharmacol. Exp. Ther.*, 236, 432-437 (1986).

Brodde, O.-E. and Michel, M. C., Disease states can modify both receptor number and signal transduction pathways. *Trends Pharmacol. Sci.*, 10, 383-384 (1989).

Carboni, E., Memo, M., Tanda, G. L., Carruba, M. O. and Spano, P. F., Effect of temperature and ionic environment on the specific binding of [^{3}H]sulpiride to membranes from different rat brain regions. *Neurochem. Int.*, 7, 279-284 (1985).

Carrier, O. and Aronstam, R. S., Increased muscarinic responsiveness and decreased muscarinic receptor content in ileal smooth muscle in diabetes. *J. Pharmacol. Exp. Ther.*, 254, 445-449 (1990).

Chu, P. C., Lin, M. T. and Leu, S. Y., Alterations in physiologic functions in brain monoamine content in streptozotocin-diabetic rats. *Diabetes.*, 35, 481-485 (1986).

Consolo, S., Girotti, P., Russi, G. and Di Chiara, G., Endogenous dopamine facilitates striatal *in vivo* acetylcholine release by acting on D1 receptors localized in the striatum. *J. Neurochem.*, 59, 1555-1557 (1992).

Costall, B. and Naylor, R. J., Neuroleptic and non-neuroleptic catalepsy. *Arznein-Forsch.*, 23, 674-683. (1973).

Dunstan, R., Broekkamp, C. L. and Lloyd, K. G., Involvement of caudate nucleus, amygdala or reticular formation in neuroleptic and narcotic catalepsy. *Pharmacol. Biochem. Behav.*, 14, 169-174 (1981).

Erny, R. E., Berezo, M. W. and Perlman, R. L., Activation of tyrosine 3-monooxygenase in pheochromocytoma cells by adenosine. *J. Biol. Chem.*, 256, 1335-1339 (1981).

Glowinski, J. and Iversen, L. L., Regional studies of catecholamine in the rat brain-I. The disposition of ^{3}H-norepinephrine, 3H-dopamine and 3H-DOPA in various regions of the rat. *J. Neurochem.*, 13, 655-669 (1966).

Hoffman, D. C. and Beninger, R. J., The D1 receptor antagonist SCH23390 reduces locomotor activity and rearing in rats. *Pharmacol. Biochem. Behav.*, 22, 341-342 (1985).

Horwitz, J. and Perlman, R. L., Activation of tyrosine

hydroxylase in the superior cervical ganglion by nicotinic and muscarinic agonists. *J. Neurochem.*, 43, 546-552 (1984).

Hoskins, B., and Scott, J. M., Evidence for a direct action of insulin to increase renal reabsorption of calcium and for an irreversible defect in renal ability to conserve calcium due to prolonged absence of insulin. *Diabetes.*, 33, 991-994 (1984).

Hyttel, J., SCH 23390-the first selective dopamine D-1 antagonist. *Eur. J. Pharmacol.*, 91, 153-154 (1983).

Lozovsky, D., Saller, C. F. and Kopin, I., Dopamine receptor binding is increased in diabetic rats. *Science*, 214, 1031-1033 (1981).

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193, 265-275 (1951).

Marshall, J. F., Friedman, M. I. and Heffner, T. G., Reduced anorexic and locomotor-stimulant action of D-amphetamine in alloxan-diabetic rats. *Brain Res.*, 111, 428-432 (1976).

Mayer, G. S. and Shoup, R. E., Simultaneous multiple electrode liquid chromatographical assay for catecholamines, indoleamines and metabolites in brain tissue. *J. Chromatography*, 255, 533-544 (1983).

Meller, E., Kuga, S., Friedhoff, A. J. and Goldstein, M., Selective D2 dopamine receptor agonists prevent catalepsy induced by SCH-23390, a selective D1 antagonist. *Life Sci.*, 36, 1857-1864 (1985).

Munson, P. J. and Rodbard, D., A versatile computerized approach for the characterization of ligand binding systems. *Anal. Biochem.*, 107, 220-239 (1980).

Porceddu, M. L., Giorgi, O., Ongini, E., Mele, S. and Biggio, G., ^{3}H-23390 binding sites in the rat substantia nigra: Evidence for a presynaptic localization and innervation by dopamine. *Life Sci.*, 39, 321-328 (1986).

Quimet, C. C., Miller, P. E., Hemmings, H. C., Walaas, S. I. and Greengard, P., DARPP-32, a dopamine- and adenosine 3,5-monophosphate-regulated phosphoprotein enriched in dopamine innervated brain regions. *J. Neurosci.*, 4, 111-124 (1984).

Robertson, G. S. and Robertson, H. A., D1 and D2 agonist synergism: seperate sites of action? *Trends Pharamcol. Sci.*, 8, 295-299 (1987).

Ryall, R. W., Drugs used in schizophrenia. In Ryall, R. W. ed. Mechanisms of drug action on the nervous system. Cambridge Univ. Press, N.Y. 1989 pp. 171-192.

Salkovic, M. and Lackovic, Z., Brain D1 dopamine receptor in alloxan-induced diabetes. *Diabetes*, 41, 1119-1121 (1992).

Saller, C. F., Dopaminergic activity is reduced in diabetic rats. *Neurosci. Letter*, 99, 301-306 (1984).

Saller, C. F. and Chiodo, L. A., Glucose suppresses basal firing and haloperidol-induced increases in the firing rate of central dopaminergic neurons. *Science*, 210, 1269-1271 (1980).

Saller, C. F. and Salama, A. I., D-1 and D-2 dopamine receptor blockade: Interactive effects *in vitro* and *in vivo*. *J. Pharmacol. Exp. Ther.*, 236, 714-720 (1986).

Scatton, B., Worms, P., Lloyd, K. G. and Bartholini, G., Cortical modulation of striatal function. *Brain Res.*, 232, 331-343 (1982).

Seeman, P., Brain dopamine receptors. *Pharmacol. Rev.*, 32, 229-313 (1980).

Shimomura, Y., Shimizu, H., Takahashi, M., Sato, N., Uehara, Y., Suwa, K., Kobayashi, I., Tadokoro, S. and Kobayashi, S., Changes in ambulatory activity and dopamine turnover in streptozotocin-induced diabetic rats. *Endocrinol.*, 123, 2621-2625 (1988).

Shimomura, Y., Shimizu, H., Takahashi, M., Uehara, Y., Kobayashi,I. and Kobayashi, S., Ambulatory activity and dopamine turnover in streptozotocin-induced rats. *Exp. Clin. Endocrinol.*, 95, 385-388 (1990).

Stoof, J. C., Drukarch, B., De Boer, P., Westerink, B. H. C. and Groenewegen, H. J., Regulation of the activity of striatal cholinergic neurons by dopamine. *Neuroscience*, 47, 755-770 (1992).

Stoof, J. C. and Kebabian, J., Two dopamine receptors: Biochemistry, physiology and pharmacology. *Life Sci.*, 35, 2281-2296 (1984).

Watkins, J. B. and Sherman, S. E., Long-term diabetes alters the hepatobiliary clearance of acetaminophen, bilirubin and digoxin. *J. Pharmacol. Exp. Ther.*, 260, 1337-1343 (1992).

Westerink, N. H. C. and Korf, J., Turnover of acid dopamine metabolites in striatal and mesolimbic tissue of the rat brain. *Eur. J. Pharmacol.*, 37, 249-255 (1976).

Zukins, S. R., Young, A. E. and Snyder, S. H., Gamma-aminobutyric acid binding to receptro sites in rat central nerve system. *Proc. Natl. Acad. Sci. U.S.A.* 71, 4802-4807 (1974).