# Brain D1 Dopamine Receptor in Alloxan-Induced Diabetes

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**Specific binding of [$^3$H]SCH 23390 to dopamine D1 receptors in the striatum and olfactory tubercle in 14-day alloxan-induced diabetic rats was investigated. The Scatchard analysis revealed decreased D1 receptor density in the striatum ($B_{max}$ values were 548 ± 23 fmol/mg protein for the control and 466 ± 33 fmol/mg for the diabetic rats). No change was observed in the olfactory tubercle ($B_{max}$; 299 ± 27 fmol/mg for the control and 317 ± 32 fmol/mg for the diabetic rats). Thus, specific binding of [$^3$H]SCH 23390 to striatal and olfactory tubercle membranes showed region-specific changes of brain dopamine D1 receptors in alloxan diabetic rats. *Diabetes* 41:1119–21, 1992**

In some regions of the human brain and the brains of animals with experimentally induced diabetes, diabetes mellitus is accompanied with alteration in the metabolism of dopamine (1–4). A decreased turnover rate has been the most common finding (2). An increased D2 receptor density has been observed in some brain regions (1–3). However, until now, the influence of diabetes on the affinity and density of central D1 receptors has not been investigated. Using a selective D1 receptor ligand [$^3$H]SCH 23390 (5), we here report that 14-day-old alloxan-induced diabetes in rats is accompanied with changes in the density of D1 receptors present in the striatum but not in those in the olfactory tubercle.

## RESEARCH DESIGN AND METHODS

The radioligand [$^3$H]SCH 23390 [(R)-(+)-8-chloro-2,3,4,5-tetrahydro-methyl-5-phenyl-1H-3-benzozepin-7-ol], 77 Ci/mmol, was purchased from Amersham Laboratories (Aylesbury, UK) and Aquasol was obtained from NEN (Boston, MA). Ketanserin was a gift from Janssen, Beerse, Belgium. All other chemicals were purchased from Sigma (St. Louis, MO).

Male Wistar rats (Department of Pharmacology, Medical School of Zagreb, Croatia), weighing 200–250 g, were group housed and given standard food pellets and water ad libitum. Diabetes was induced by a single i.v. injection of alloxan monohydrate (80 mg/kg) dissolved in saline. Forty-eight-hours after alloxan administration, diabetes was verified by glucosuria and blood glucose concentration (>20 mM, $n = 9–12$); both values were further checked once a week and immediately before decapitation. Glucosuria was verified by reagent strips (Rapignost, Pliva, Zagreb, Croatia) and blood glucose concentration by reagent strips and Glucomat apparatus (TRS, Zagreb, Croatia). Immediately before decapitation, mean values of blood glucose concentration per experiment were in the range from 22.22 to 26.41 mM and 5.33 to 7.09 mM in the diabetic and control group, respectively. Animals were decapitated 14 days after alloxan injection. From the removed brain, the striatum and olfactory tubercle were dissected on ice and stored at −22°C for 24 h before the binding assay. Experiments were repeated five times.

**Radioligand binding assay.** The tissue was homogenized (Ultra-turrax T25, Janke & Kunkel, IKA Labortechnik, Stanfen, Germany) in 100 vol (wt/vol) of ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25°C). The homogenate was centrifuged twice for 10 min at 20,000 × $g$ at 4°C after the pellet was resuspended in fresh 50 mM Tris-HCl buffer. The final pellet was resuspended in 50 mM Tris-HCl buffer (pH 7.7 at 25°C) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 0.1% ascorbic acid. The radioligand [$^3$H]SCH 23390 (in concentration ranging from 0.06 to 6.00 nM) in 200 μl aliquots was added to triplicate tubes containing 200 μL of membrane preparations and 200 μL of 50 nM ketanserin. Specific

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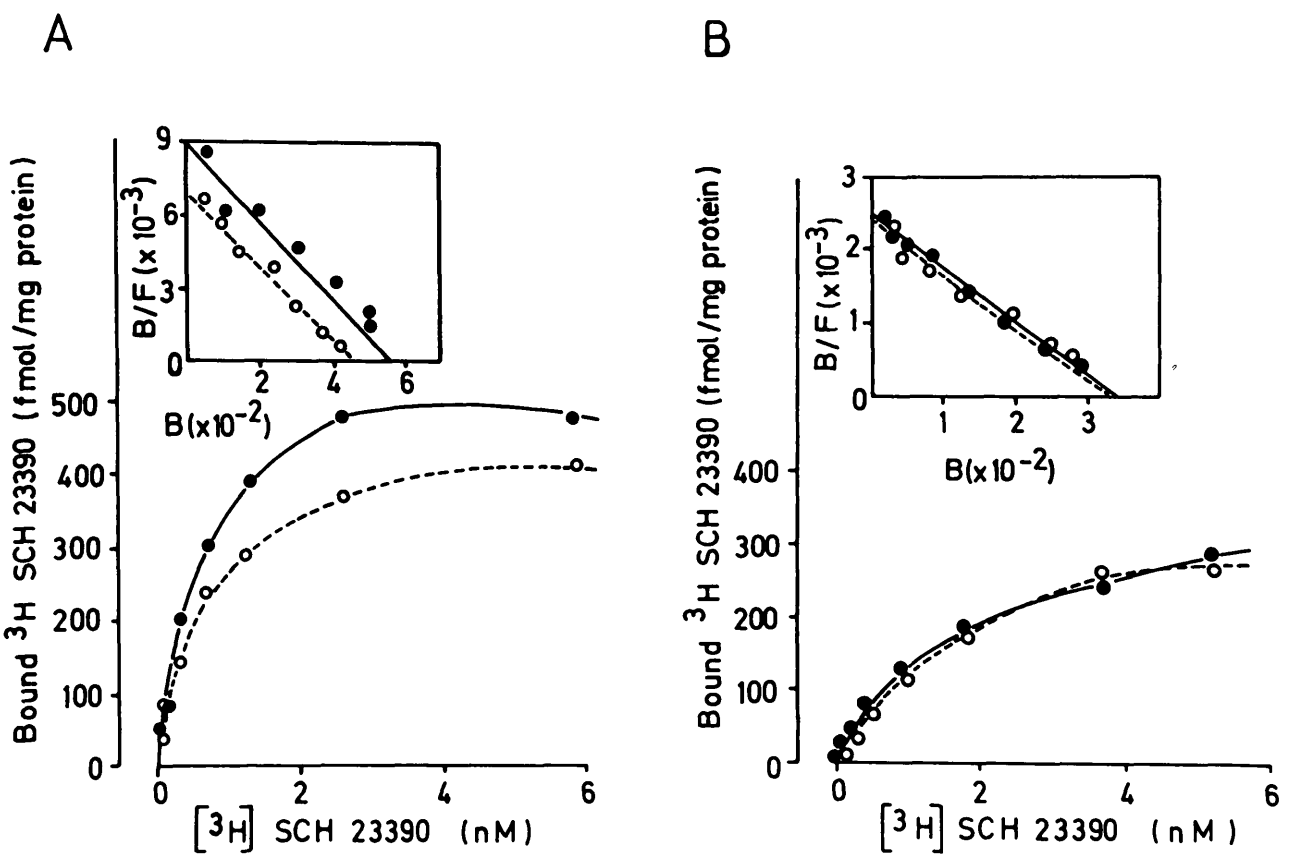


**FIG. 1. Saturation curves and Scatchard plot of [$^3$H]SCH 23390 binding to the rat striatal (*A*) and olfactory tubercle (*B*) membranes in 14-day-old alloxan-induced diabetes. The values are taken from a typical experiment (repeated 5 times) performed in triplicate. ● Control and ○ Diabetic group. Values of $K_d$ (nM) and $B_{max}$ (fmol/mg protein) expressed as means ± SE, for the control and the diabetic group, respectively, are *A*: $K_d$ 0.96 ± 0.07 and 0.99 ± 0.09, $B_{max}$ 548 ± 23 fmol/mg and 466 ± 33 fmol/mg, $P < 0.05$ according to analysis of $\chi^2$ test. *B*: $K_d$ 1.01 ± 0.14 and 1.08 ± 0.17, $B_{max}$ 299 ± 27 fmol/mg and 317 ± 32 fmol/mg.**

binding was defined as the difference in binding in the absence and in the presence of 200 μl of 1 μM SCH 23390. The final assay volume was 1000 μl, whereas the final protein concentration (6) ranged between 200 and 300 μg/ml. Incubation was performed for 15 min at 37°C and was terminated by rapid filtration under reduced pressure over Whatman GF/B glass-fiber filters (Maidstone, England) and three washes with 5 ml of cold 50 mM Tris-HCl buffer. Radioactivity was measured by liquid scintillation in a Beckman LC counter (LC 1701, Beckman, Palo Alto, CA) after 3.5 ml of Aquasol had been added.

**Statistics.** The data were expressed as means ± SE ($n = 5$ experiments) and analyzed using $\chi^2$ test and the significance level of $P < 0.05$.

## RESULTS

Saturable, specific binding of [$^3$H]SCH 23390 was observed in all the tissues investigated (Fig. 1*A* and *B*). Nonspecific binding accounts for 10–25% of the total binding. To determine possible changes in D1 receptor density and affinity, the $B_{max}$ (fmol/mg protein) and $K_d$ (nM) values were calculated using Scatchard analysis.

In all five experiments performed, the $K_d$ values of [$^3$H]SCH 23390 binding to the striatal tissue of the control and diabetic rats did not differ (0.96 ± 0.07 nM for the control and 0.99 ± 0.09 nM for the diabetic group) (Fig. 1*A*). In contrast to that, the $B_{max}$ values in the striatum decreased in all five experiments; 466 ± 33 fmol/mg in the diabetic group vs. 548 ± 23 fmol/mg in the control group ($P < 0.05$). Figure 1*A* shows a typical experiment.

We also conducted a preliminary experiment with striatal homogenates of streptozocin-induced diabetic rats (streptozocin, a gift of Upjohn, Puurs, Belgium, was given in a dose of 100 mg/kg i.p.) and observed similar results; decreased $B_{max}$ values (513 fmol/kg and 367 fmol/kg for the control and the diabetic group, respectively) with no significant changes in the $K_d$ values (0.94 and 1.09 nM for the control and the diabetic group, respectively).

Under the same experimental conditions neither $K_d$ (1.01 ± 0.14 nM for the control and 1.08 ± 0.17 nM for the diabetic group) nor $B_{max}$ values (299 ± 27 fmol/kg for the control and 317 ± 32 fmol/kg for the diabetic group) were found to differ in the olfactory tubercle of 14-day diabetic rats (Fig. 1*B*).

## DISCUSSION

Diabetes mellitus is often accompanied with emotional, behavioral, and mood disturbances or some centrally mediated neurological complications (7), the pathophysiology of which is still unclear, but might be related to

brain dopaminergic system dysfunctions already described by several laboratories (1–4). In this respect, investigations of central dopamine receptors in diabetes mellitus could be particularly interesting.

Dopamine D2 receptors exploiting [$^3$H]spiroperidol binding to some regions of diabetic rat brain (1–3) have already been studied. For example, a 35% increase was found in the $B_{max}$ of specific [$^3$H]spiroperidol binding to striatal membranes persisting after 4–6 weeks of diabetes (1,2). This could be accounted for by the fact that most of central disturbances in diabetes, whose pathophysiology is related to striatal dopaminergic neurons (like locomotor or ambulatory activity (8)) are thought to be mediated through D2 receptors. Although a recent discovery of dopamine receptor D3 subtype, which binds spiroperidol with similar affinity as D2 subtype (9), has made the interpretation of D2 receptors research less specific, there are obviously significant alterations in D2/D3 receptor binding sites. In experiments presented here, we found that experimental, alloxan-induced diabetes mellitus in rat is accompanied with alterations in central D1 receptor subtype, as well.

The binding of [$^3$H]SCH 23390, assumed to be a specific D1 receptor ligand (5), to the striatal and olfactory tubercle membranes of alloxan-induced diabetic rats was studied. Experiments were repeated five times, and each binding assay was done in triplicate.

Affinity of [$^3$H]SCH 23390 binding to D1 receptors in the striatum did not change during the 14-day course of diabetes (Fig. 1*A*). Contrary to that, the $B_{max}$ values showed a decrease of D1 receptor density (548 fmol/mg protein for the control and 466 fmol/mg for the diabetic group, $P < 0.05$ (Fig. 1*A*). Similar results, i.e., decreased $B_{max}$ values, were observed also in a preliminary experiment on striatal homogenates of streptozocin-induced diabetic rats. Thus, it seems to us that the effect is related to diabetes and not to alloxan itself.

The study of the olfactory tubercle, the region rich in D1 receptors, but unexplored in diabetes, gave a different picture of D1 receptor density. Contrary to the values in the striatum, the $B_{max}$ values of [$^3$H]SCH 23390 binding in this region were unchanged in 14-day-old diabetes (299 fmol/mg protein for the control and 317 fmol/mg for the diabetic group).

It is an old hypothesis that brain monoamines (and consequently their receptors) in diabetes mellitus are altered as a result of changes in the availability of precursor amino acids (10). Observed changes in dopamine metabolism (4) and dopamine receptors (reported herein) in different brain areas, as well as the production of alterations in brain monoamines similar to those described in diabetes (11), by direct intracerebroventricular administration of diabetogenic drugs, do not support this hypothesis. Recently, however, an apparent functional imbalance between Gs-protein- and Gi/Go-protein-mediated transduction mechanisms with an increased efficacy of Gs activity, probably as a result of the loss of Gi/Go inhibitory functions, has been found in the striatum and other tissues of diabetic animals (12–14). Possible, but only speculative, explanation could be that the decrease in D1 (reported here) and the increase in D2 (1–3) receptor density in the striatum during alloxan- and streptozocin-induced diabetes might be somehow related to alterations in G protein functions. Although a possible mechanism is still unknown, it could be a small piece within a large diabetes-dopamine receptor mosaic. Additional explanation could be related to the discovery of insulin (which does not eliminate G-protein imbalance because insulin-receptor transduction system, too, might involve G-like proteins; 15) and insulin receptors, its region-specific distribution in the brain (16) and in particular to an insulin-dopamine interaction (17), the mechanism of which remains to be elucidated.

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