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Report

Interactions of 7-[3-(4-[2,3-Dimethylphenyl]piperazinyl)-propoxy]-2(1H)-quinolinone (OPC-4392) with ^{3}H-Spiperone and ^{3}H-SCH 23390 Binding in Rat Striatum: Effects of Lesions

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7-[3-(4-[2,3-Dimethylphenyl]piperazinyl)propoxy]-2(1H)-quinolinone (OPC-4392), a presynaptic dopamine autoreceptor agonist and postsynaptic D-2 receptor antagonist (Yasuda *et al., Life Sci.* 42:1941–1954, 1988), was studied for its binding characteristics at ^{3}H-SCH 23390-labeled dopamine D-1 receptors and ^{3}H-spiperone-labeled dopamine D-2 receptors in rat striatum. The binding affinity of OPC-4392 for ^{3}H-spiperone-labeled D-2 receptors was 500 times higher than for ^{3}H-SCH 23390-labeled D-1 receptors. 6-Hydroxydopamine lesions of striatum and high-frequency current lesions of medial forebrain bundle did not affect the competition of OPC-4392 for ^{3}H-spiperone binding. Kainic acid lesions of striatum significantly changed the one-site model fit to a two-site model fit of the competition curve of OPC-4392 for ^{3}H-spiperone binding, suggesting that OPC-4392 competed with ^{3}H-spiperone binding differently for postsynaptic dopamine D-2 receptors and for presynaptic dopamine autoreceptors.

KEY WORDS: OPC-4392; binding; lesions; dopamine receptors; rat striatum.

INTRODUCTION

Behavioral, biochemical, and electrophysiological evidence has been provided for the existence of dopamine receptors located on dopamine neurons (presynaptic dopamine autoreceptors) (1–5). The results obtained from radioreceptor binding and autoradiography experiments have demonstrated that the dopamine presynaptic autoreceptor is of the D-2 type (6,7). A new dopaminergic agent, 7-[3-(4-[2,3-dimethylphenyl]piperazinyl)propoxy]-2(1H)-quinolinone (OPC-4392), has recently been reported to act as an agonist at presynaptic dopamine autoreceptors and as an agonist at postsynaptic dopamine D-2 receptors according to a series of behavioral and biochemical tests (8). In this study, we investigated the interactions of OPC-4392 with ^{3}H-spiperone and ^{3}H-SCH 23390 binding in rat striatal membranes. We also describe the effects of unilateral 6-hydroxydopamine and kainic acid lesions of the striatum, and high-frequency current lesions of the medial forebrain bundle, on the competition of OPC-4392 for ^{3}H-spiperone binding. Further, the effect of guanine nucleotide (GTP) on the competition of OPC-4392 for ^{3}H-spiperone binding to striatal membranes was investigated.

MATERIALS AND METHODS

Materials

^{3}H-Spiperone (21.4 Ci/mmol) and ^{3}H-SCH 23390 (66 Ci/mmol) were purchased from NEN (Boston, MA). The other compounds were obtained from the following sources: 6-hydroxydopamine and apomorphine, Sigma (St. Louis, MO); (+)3-PPP, RBI (Natic, MA); (±)3-PPP, gift from Mr. T. Kikuchi; Haloperidol, Dainippon (Tokyo, Japan); SCH 23390, Schering Plough (Bloomfield, NJ); guanosin-5′-triphosphate (GTP), Boehringer Mannheim (West Germany); and kainic acid, Nakarai (Kyoto, Japan). OPC-4392 (Fig. 1) was synthesized by Laboratories of New Drug Research, Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd. (Japan).

Animal Surgeries

Male Wistar rats (150–170 g) were anesthetized with 35 mg/kg i.p. of Somnopentyl and then placed on a stereotaxic apparatus. 6-Hydroxydopamine (8 μm in 3 μl saline containing 2 μg ascorbate) or kainic acid (2 μg in 1 μl saline, buffered to pH 6.5) was injected with a 5-μl microsyringe into the right striatum (coordinates, A 7.9, R 2.6, V +1.1). The left side was injected with the vehicle (saline or saline with ascorbate). The high-frequency current lesions of medial

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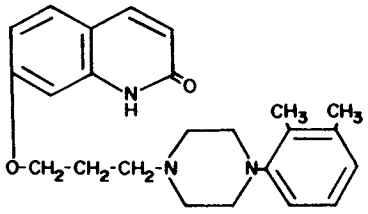

Fig. 1. Chemical structure of OPC-4392.

forebrain bundle were performed by solid-state electrosurgery (Suntec SLS-40W; setting—output, 5; model, coag; time, 60 sec; coordinates, A 4.5, R 1.5, V −3.0). After 3 weeks the rats were tested for efficacy of the lesions by administering i.p. 2 mg/kg apomorphine. Only those animals exhibiting clear and continuous turning behavior contralateral to the side of 6-hydroxydopamine and high-frequency current lesions or ipsilateral to the side of kainic acid lesions were used in the binding studies.

Membrane Preparations and Binding Assays

The striatal membrane preparations were obtained from Wistar male rats (200–250 g). Briefly, the striata were homogenized in 100 vol (wet weight/volume) ice-cold 50 m*M* Tris–HCl buffer containing 120 m*M* NaCl, 5 m*M* KCl, 2 m*M* $CaCl_2$, 1 m*M* $MgCl_2$ pH 7.4. The homogenate was centrifuged at 50,000*g* for 10 min at 4°C. The resulting pellet was suspended in an identical volume of the above buffer. The suspension was centrifuged again under the same conditions as above. The resulting pellet was resuspended in assay buffer.

Competition binding experiments were performed according to the methods described by Creese and Hess (9) and Billard *et al.* (10). Striatal membranes (0.15 mg protein per tube) were incubated for 15 min at 37°C in a final volume of 1.0 ml 50 m*M* Tris–HCl assay buffer (pH 7.4) containing 120 m*M* NaCl, 5 m*M* KCl, 2 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, 1 m*M* EDTA, different concentrations of unlabeled drug, and 0.3 n*M* ^{3}H-spiperone or ^{3}H-SCH 23390. Ketanserin (50 n*M*) was added to the ^{3}H-spiperone assay system to mask serotonin-2 receptors. The incubation was terminated by rapid filtration through Whatman GF/B filters with three 5-ml rinses with ice-cold 50 m*M* Tris–HCl buffer. (+)Butaclamol (2 μ*M*) was used to define the nonspecific binding for both of ^{3}H-spiperone and ^{3}H-SCH 23390 binding assays.

Data Analysis

Data were analyzed by a computer-assisted nonlinear least-squares curve-fitting program. Data were fitted to single- and multiple-site models and tested for statistical significance of differences. The model that best described the data at the 0.05 level of significance was chosen.

RESULTS AND DISCUSSION

The rank order of potency of various ligands in competition with ^{3}H-spiperone binding to dopamine D-2 receptors was haloperidol > OPC-4392 > apomorphine > SCH 23390 > (±)3-PPP > (+)3-PPP (Fig. 2A). OPC-4392 competed for ^{3}H-spiperone binding with 500 times higher affinity than for

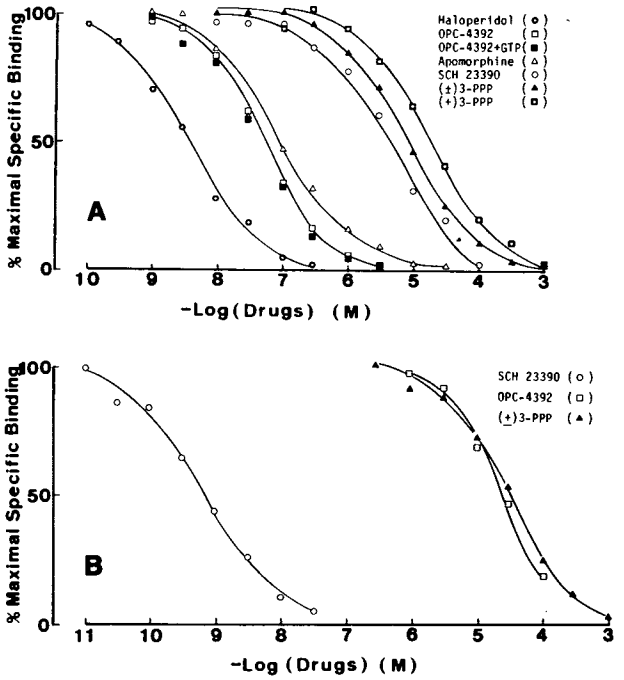

Fig. 2. Inhibition of ^{3}H-spiperone binding to D-2 receptor (A) and ^{3}H-SCH 23390 binding to D-1 receptor (B) by OPC-4392 and other dopaminergic drugs in rat striatum. Each point represents the mean value of three or four independent experiments, each carried out in triplicate. The standard error of each mean value was less than 20%. In A, the IC_{50} values were 3.7, 48, 44, 83, 5670, 8530, and >10,000 n*M* for haloperidol, OPC-4392, OPC-4392 + GTP (0.1 m*M*), apomorphine, SCH 23390 (±)3-PPP, and (+)3-PPP, respectively. The corresponding Hill coefficients were 0.74, 0.92, 0.84, 0.72, 0.71, 0.83, and 0.80. In B, the IC_{50} values were 0.75, >20,000, and >20,000 n*M* for SCH 23390, OPC-4392, and (±)3-PPP, respectively. The corresponding Hill coefficients were 0.72, 1.00, and 0.80. The computer-assisted nonlinear least- squares analysis showed that the best fit was a one-site model for all of the ligands studied.

^{3}H-SCH 23390 binding (Figs. 2A and B), indicating that OPC-4392 was selective for dopamine D-2 receptors over D-1 receptors in the striatum. This result is consistent with our previous finding that OPC-4392 caused only slight inhibition of dopamine-sensitive adenylate cyclase activity (K_i = 860 n*M*) (8). The competition curves of OPC-4392 for ^{3}H-spiperone binding to striatal membranes was best described by a one binding component. The competition was not affected by the addition of 0.1 m*M* GTP (Fig. 2A). These results suggested that the binding of OPC-4392 to ^{3}H-spiperone-labeled dopamine D_2 receptors did not induce the formation of the ternary complex of ligand–receptor–guanine nucleotide binding protein. These phenomena are commonly associated with the competition of dopamine antagonists for ^{3}H-spiperone binding to striatal membranes.

The following experiments were designed to investigate differences in OPC-4392 competition for ^{3}H-spiperone binding to postsynaptic D_2 receptors and presynaptic dopamine receptors in the striatum. As previously reported, both 6-hydroxydopamine lesions of striatum and high-frequency current lesions of medial forebrain bundle resulted in a removal of the dopaminergic input of the striatum (11–13), so that following the lesions, OPC-4392 might compete with

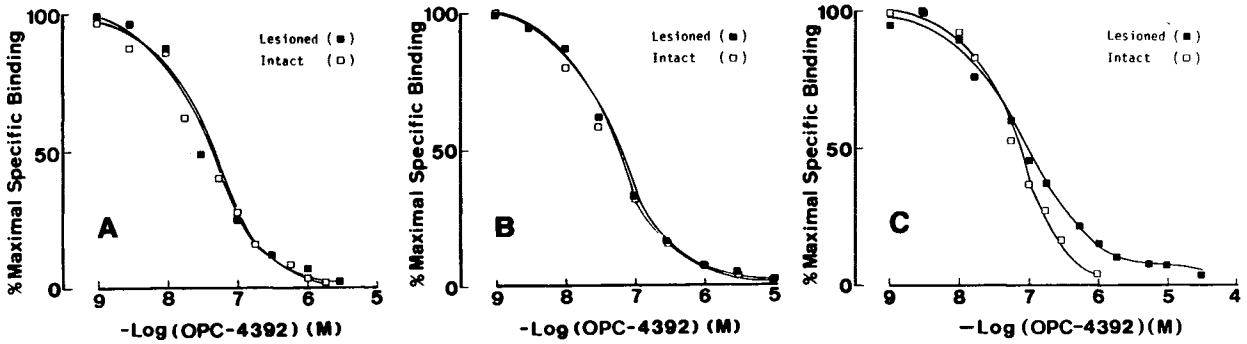

Fig. 3. Effects of unilateral 6-hydroxydopamine lesions of striatum (A), high-frequency current lesions of medial forebrain bundle (B), and kainic acid lesions of striatum (C) on inhibition of ^{3}H-spiperone binding to striatal membranes by OPC-4392. Each point represents mean value of three or four independent experiments, each carried out in triplicate. (A) IC_{50}'s—46 ± 8 n*M* for lesioned side and 51 ± 7 n*M* for intact side; Hill coefficients—0.91 ± 0.08 for lesioned side and 0.89 ± 0.09 for intact side. (B) IC_{50}'s—48 ± 3.5 n*M* for lesioned side and 42 ± 9.5 n*M* for intact side; Hill coefficients—1.03 ± 0.06 for lesioned side and 0.96 ± 0.04 for intact side. (C) The computer-assisted analysis yielded a significant two-site fit resolution as compared with the one-site fit for the data of lesioned side (R_H, 74 ± 9%; K_H, 45 ± 0.8 n*M*; R_L, 26 ± 8%; K_L, 651 ± 275 n*M*; $P < 0.05$). For intact side, IC_{50} was 45 ± 3 n*M* and Hill coefficient was 0.92 ± 0.07.

^{3}H-spiperone binding mainly for postsynaptic D-2 receptors. As shown in Figs. 3A and B, unilateral 6-hydroxydopamine lesions of striatum and high-frequency current lesions of medial forebrain bundle had no effect on the OPC-4392 competition for ^{3}H-spiperone binding to striatal membranes. The competition curves of OPC-4392 in the lesioned side were best described by a model with one binding component as in the control. Kainic acid treatment was reported to cause degeneration of neurons with cell bodies near the injection site but to spare axons terminating in or passing through the area (14). Accordingly, kainic acid lesions of striatum may selectively damage the postsynaptic receptor. As shown in Fig. 3C, the competition curve of OPC-4392 for ^{3}H-spiperone binding to striatal membranes in kainic acid-treated side was best described by a two-site components, while a one-site model fit was still sufficient for the OPC-4392 competition curve in the contralateral intact side. These results suggest that OPC-4392 binding to ^{3}H-spiperone sites differed for postsynaptic dopamine D-2 receptors and for presynaptic dopamine autoreceptors. Accordingly, OPC-4392 might be a valuable tool to further explore the differences in pharmacology and biochemistry between postsynaptic dopamine D_2 receptors and presynaptic dopamine receptors in the striatum.

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