Biochemical Pharmacology, Vol. 38, No. 12, pp. 2019–2025, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

INTERACTION OF PERMANENTLY CHARGED CHLORPROMAZINE AND DOPAMINE ANALOGS WITH THE STRIATAL D-1 DOPAMINERGIC RECEPTOR

RAYE ANN WALLACE, LANE WALLACE, MARK HARROLD, DUANE MILLER and NORMAN J. URETSKY*

Divisions of Pharmacology and Medicinal Chemistry, College of Pharmacy, The Ohio State University, Columbus, OH 43210, U.S.A.

(*Received* 4 *June* 1988; *accepted* 31 *October* 1988)

Abstract—Although a structural feature common to all dopaminergic agonists and antagonists is a side-chain basic amino group, it is unclear whether this moiety binds to the D-1 dopamine (DA) receptor in the charged or uncharged form. To obtain information on this point, we synthesized permanently charged dimethylsulfonium and quaternary ammonium analogs of chlorpromazine and DA and determined whether these compounds can bind to the D-1 receptor by measuring their abilities to inhibit the binding of SCH 23390, a D-1 receptor antagonist. Chlorpromazine and the dimethylsulfonium and trimethylammonium analogs of chlorpromazine were found to inhibit the binding of [^{3}H]SCH 22390, which was maximally inhibited to the same extent by all three compounds. In addition, inhibition curves for the compounds fit a one-site binding model, indicating binding to a single class of sites. However, while the permanently charged chlorpromazine analogs were able to inhibit [^{3}H]SCH-23390 binding, they were considerably less potent than chlorpromazine. DA and dimethyl DA were also able to inhibit [^{3}H]SCH 23390 binding. However, the permanently charged dimethylsulfonium and trimethylammonium analogs of DA were ineffective in inhibiting [^{3}H]SCH 23390 binding. In addition, the permanently uncharged methylsulfide analog did not inhibit binding. These studies show that permanently charged analogs of chlorpromazine can bind to the striatal D-1 receptor, which is consistent with an anionic recognition site on the D-1 receptor that interacts with antagonists in the cationic form. In addition, it appears that a nitrogen atom is not required for binding to the D-1 receptor, since the sulfonium analog of chlorpromazine bound to the receptor to the same extent as chlorpromazine. However, since the permanently charged or uncharged analogs of DA did not bind to the D-1 receptor, it is still unclear as to whether the charged form of a dopaminergic agonist can bind. The lower potency or ineffectiveness of the permanently charged analogs compared to the parent amines (chlorpromazine, DA, dimethyl DA) in binding to the D-1 receptor may reflect the inability of the permanently charged analogs to undergo hydrogen binding with the anionic site of the receptor.

It is generally accepted that dopamine (DA) receptors can be classified into two categories depending upon the nature of their association with the enzyme, adenylate cyclase. One population of receptors, the D-1 DA receptors, is associated with adenylate cyclase in a stimulatory manner, while the other population, the D-2 DA receptors, is not associated with adenylate cyclase or inhibits this enzyme [1]. Recent studies have indicated that activation of both D-1 and D-2 DA receptors is necessary for the expression of dopaminergic mediated behavioral effects [2, 3].

Studies assessing the relative importance of D-1 and D-2 receptors in mediating dopaminergic effects have been made possible by the development of selective D-1 and D-2 agonists and antagonists. However, while D-1 and D-2 selective ligands have been developed, the specific structural requirements necessary for their interaction with D-1 or D-2 DA receptors remain unresolved. A structural feature common to all D-1 and D-2 DA agonists and antagonists is the presence of a basic amino group in the molecule. In previous studies [4] we have shown that structural analogs of DA and chlorpromazine (CPZ) in which the side chain amino group has been replaced by a dimethylsulfonium group can interact with the striatal D-2 DA receptor and exert dopaminergic agonist and antagonist effects respectively. Recently, a sulpiride analog in which the amino group was replaced with a monoethylsulfonium group was also shown to interact with the D-2 DA receptor and produce antagonist effects. These results suggest that an amino group is not essential for interaction with the striatal D-2 DA receptor [4]. Furthermore, since the dimethylsulfonium CPZ and DA analogs and the monoethylsulfonium sulpiride analog have a permanent positive charge in the side chain, the results also show that compounds with a positive charge can interact with the D-2 DA receptor and exert functional activity similar to that of the parent amines [4]. In contrast, the permanently uncharged analogs of DA have been found to be inactive as DA agonists, and while they do bind to the D-2 receptor, their binding characteristics are different from those of DA or the permanently charged analogs of DA [5]. In recent studies it was found that an uncharged sulfide analog of sulpiride was inactive as a D-2 receptor antagonist and did not bind to the D-2 receptor (Harrold *et al.*, unpublished observations). These results are consistent with the

* Correspondence: Dr Norman J. Uretsky, The Ohio State University, College of Pharmacy, 500 West 12th Ave., Columbus, OH 43210.

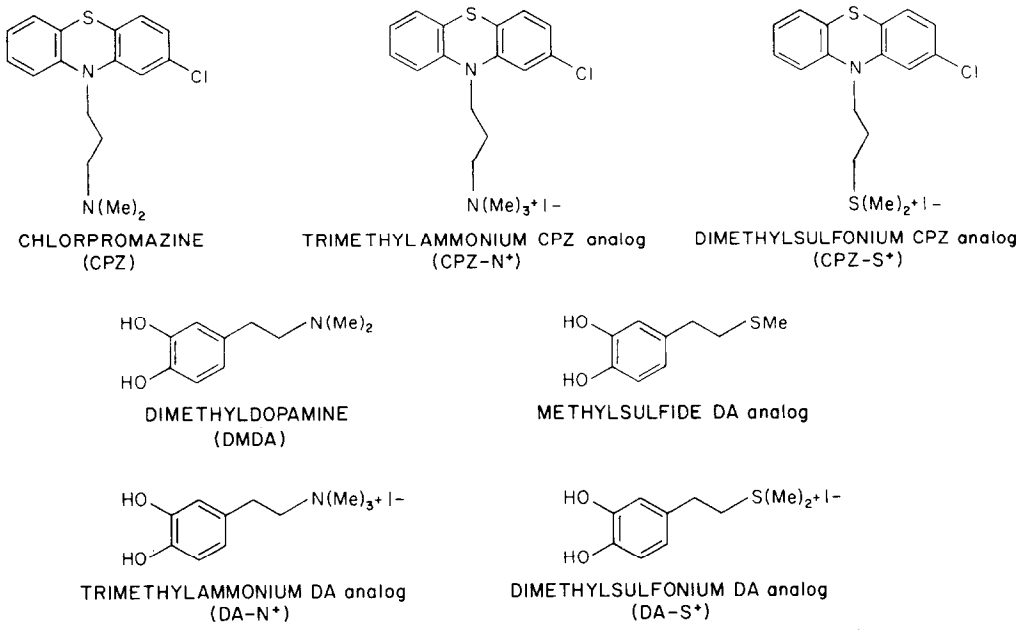

Fig. 1. Chemical structures of chlorpromazine, dimethyldopamine and the permanently charged and uncharged analogs.

presence of an anionic site on the D-2 DA receptor.

The importance of a positive charge on the amino group in the interaction of dopaminergic drugs with the D-1 DA receptor is unknown. Therefore, the purpose of this study was to determine whether structural analogs of CPZ and DA in which the side chain amino group is replaced by a dimethylsulfonium group can interact with the D-1 DA receptor (Fig. 1). This was accomplished by measuring the abilities of these compounds to inhibit the binding of the D-1 selective antagonist, [^{3}H]SCH 23390 [6]. Since the dimethylsulfonium analogs possess a permanent positive charge, we also measured the abilities of the trimethylammonium analog of CPZ and DA (Fig. 1) to inhibit [^{3}H]SCH 23390 binding to determine if compounds with a positively charged amino group can bind to the striatal D-1 DA receptor. The results of our studies show that the dimethylsulfonium analog of CPZ inhibited the binding of [^{3}H]SCH 23390, suggesting that an amino group is not essential for binding to the striatal D-1 DA receptor. In addition, our studies also showed that analogs of CPZ with a permanent positive charge bound to the D-1 receptor.

MATERIALS AND METHODS

Materials. [^{3}H]SCH 23390 (70.0 Ci/mmol) was obtained from New England Nuclear (Boston, MA). DA, dimethyldopamine (DMDA), CPZ and pargyline were obtained from the Sigma Chemical Co. (St Louis, MO). Cinanserin and fluphenazine (Prolixin injection) were obtained from E. R. Squibb & Sons, Inc. (Princeton, NJ). Quinpirole (LY 171555) was obtained from Eli Lilly & Co. (Indianapolis, IN) and (+)- and (−)-SCH 23390 were obtained from Research Biochemicals (Wayland, MA). SKF 38393 was obtained from Smith, Kline & French Laboratories (Philadelphia, PA). The trimethylammonium and dimethysulfonium analogs of DA and CPZ were synthesized in our laboratory.

Preparation of striatal homogenates. Male Sprague–Dawley rats (Harlan Sprague Dawley, Inc., Indianapolis, IN), 300–400 g, were killed by decapitation. The brains were removed, and the striata were dissected, weighed and placed in 50 vol. of ice-cold buffer (50 mM Tris-base, 2 mM $MgSO_4$, pH 7.7, at 25°). The striatal tissue was homogenized (9 complete strokes) using a Potter–Elvehjem glass homogenizer fitted with a Teflon pestle. After homogenization, the tissue suspension was centrifuged for 10 min at 48,000 *g*. The supernatant fraction was discarded and the pellet was resuspended in 50 vol. of buffer (same as above) and centrifuged again for 10 min at 48,000 *g*. The pellet was then resuspended in 200 vol. of ice-cold assay buffer (50 mM Tris-base, 1 mM $MgSO_4$, 125 mM NaCl, 5 mM KCl, 1.25 mM $CaCl_2$, 1 mM ascorbic acid, 0.1 μM cinanserin, 10 μM pargyline, pH 7.7, at 25°) resulting in a final concentration of 5 mg original tissue wet weight/ml buffer. For some of the binding studies, the assay buffer consisted of: 50 mM Tris-base, 5 mM $MgSO_4$, 0.5 mM EDTA, 0.02% ascorbic acid, 10 μM pargyline and 0.1 μM cinanserin, designated as buffer B. The tissue homogenate was stored on ice until addition to the incubation tubes.

[^{3}H]SCH 23390 binding assays. The buffer used in the assays was the same as the buffer in which the tissue was finally suspended as described above. Binding assays were done in duplicate in disposable glass test tubes (16 × 125 mm). For saturation assays, the tubes received in order: [^{3}H]SCH 23390 (diluted and added in a volume to give a final concentration of 0.01 to 10 nM), 0.95 ml fluphenazine (to give a

final concentration of 1 μM) added to some samples to determine nonspecific binding, assay buffer (sufficient to bring the total assay volume to 5 ml) and 1.0 ml striatal homogenate (final concentration of 1 mg original wet tissue weight/ml). Specific binding of [^{3}H]SCH 23390 was defined as the difference between total [^{3}H]SCH 23390 bound and [^{3}H]SCH 23390 bound in the presence of 1 μM fluphenazine. For competition assays, the tubes received in order: [^{3}H]SCH 23390 (added to give a final concentration of 0.25 nM), various concentrations of cold competitor, assay buffer (sufficient to yield a final assay volume of 5 ml) and 1.0 ml of striatal homogenate (final concentration of 1 mg original tissue wet weight/ml). All assays were carried out at room temperature (23–25°) [7]. The tubes were incubated for 100 min, a time at which equilibrium had been established. The reaction was terminated by separation of the free radioligand from bound by rapid vacuum filtration (Whatman B glass fiber filters) using a 12-well cell harvester (Brandel, Gaithersburg, MD). The filters were washed with 20 ml (4 × 5 ml washes) of assay buffer at room temperature; the duration of the washing was approximately 30 sec. The filters were then transferred to liquid scintillation vials (20 ml), and 10 ml of scintillation fluid (Formula 963, New England Nuclear) was immediately added. The vials were then shaken for 30 min in a mechanical shaker after which time the bound radioactivity was counted in a Beckman LS 6800 liquid scintillation counter at 40% efficiency.

Analysis of data. All binding data were analyzed using an iterative nonlinear least squares curve-fitting program. For [^{3}H]SCH 23390 saturation studies the data were fit to a model assuming either one ligand and one binding site or one ligand and two binding sites. The equilibrium dissociation constant for [^{3}H]SCH 23390 was derived from the analysis of the [^{3}H]SCH 23390 saturation studies and was used in the subsequent analysis of the [^{3}H]SCH 23390 competition studies. For the [^{3}H]SCH 23390 competition studies the data were fit to a model assuming either two ligands and one binding site or two ligands and two binding sites. From these analyses the apparent equilibrium dissociation constants of the competing drugs were determined.

To determine whether a one-site or two-site model more appropriately described the data, the generalized form of the logistic function was initially fit to the binding data [8]. This analysis yields a slope factor that describes the steepness of the curve and represents the slope of the logit-log plot when the concentration of the cold competing drug is expressed in terms of natural logarithms. When the slope factor equals one, the logistic equation becomes identical to the law of mass action equation which describes the interaction of one binding site with one ligand (saturation experiments) or with two competing ligands (competition experiments). Therefore, binding curves with slope factors equal to one were assumed to represent the case in which ligands interact with one class of binding sites. Binding curves with slope factors significantly less than one, as determined by Student's *t*-test, were considered justification to further analyze the data using

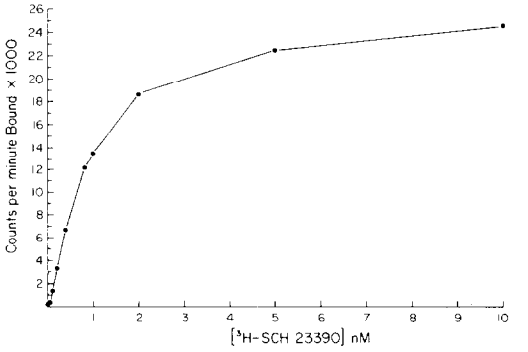

Fig. 2. [^{3}H]SCH 23390 saturation binding curve. Y-axis represents specific counts per minute bound. Each value is the mean of three determinations.

the model which describes interactions with two classes of binding sites. A partial F-statistic, used to determine whether the two-site model fit the data better than the one-site model, was calculated from equation

$$F = \frac{\frac{SS_1 - SS_2}{df_1 - df_2}}{\frac{SS}{df_2}}$$

where SS_1, and df_1 and SS_2 and df_2 represent the residual sum of squares and degrees of freedom associated with the one-site and two-site model respectively [9]. Only when the two-site binding model resulted in a significant reduction in the residual sum of squares, as determined by the partial F-test, was the binding data considered to represent the binding of the ligand to two classes of receptors.

Student's *t*-test was used to determine whether a significant difference existed between two binding dissociation constants. ANOVA and Duncan's multiple-range test were used to determine whether significant differences existed among the binding dissociation constants when comparing groups of three or more. Before statistical analyses, the binding dissociation constants were transformed to negative logarithms in order to obtain data which are normally distributed. The level of significance employed for all statistical tests was $P < 0.05$.

RESULTS

Binding characteristics of [^{3}H]SCH 23390. The binding of [^{3}H]SCH 23390 was saturable and to a single population of binding sites (Fig. 2). The equilibrium binding dissociation constant (K_D) was 1.1 ± 0.1 nM, and the maximum density of receptors (B_{max}) was 62.5 ± 3.9 pmol/g wet weight (N = 3). Specific binding represented 95% of total binding.

[^{3}H]SCH 23390 exhibited binding characteristics consistent with a D-1 receptor profile. Thus, SKF 38393, a selective D-1 agonist, potently inhibited the binding of [^{3}H]SCH 23390 with an apparent equilibrium binding inhibitory dissociation constant (K_i) of 42 ± 2.9 nM (Fig. 3). In contrast, quinpirole, a selective D-2 agonist, negligibly

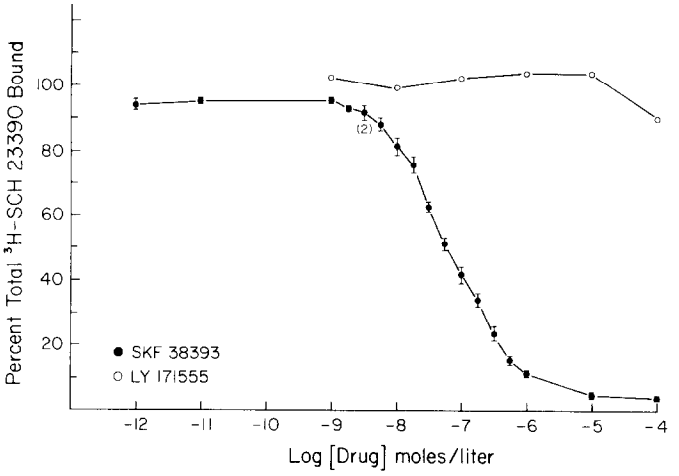

Fig. 3. Inhibition of total [³H]SCH 23390 binding (0.25 nM) by various concentrations of SKF 38393 and quinpirole (LY 171555). For SKF 38393, each point is the mean ± SE of three determinations, except for one point (indicated in parentheses) which is the mean ± the range of two determinations. For quinpirole, each point represents one determination (done in duplicate).

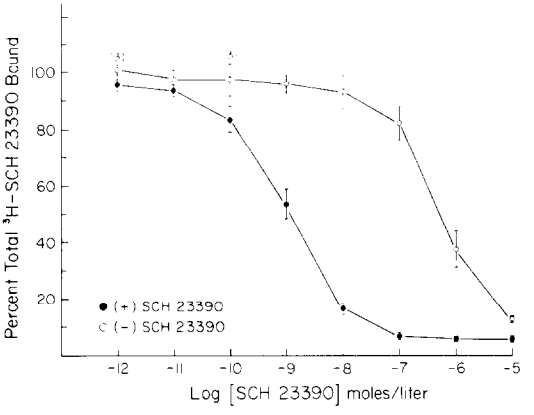

Fig. 4. Inhibition of total [³H]SCH 23390 binding by the (+)- and (−)-isomers of SCH 23390. Each point is the mean ± SE (N = 4 except where indicated).

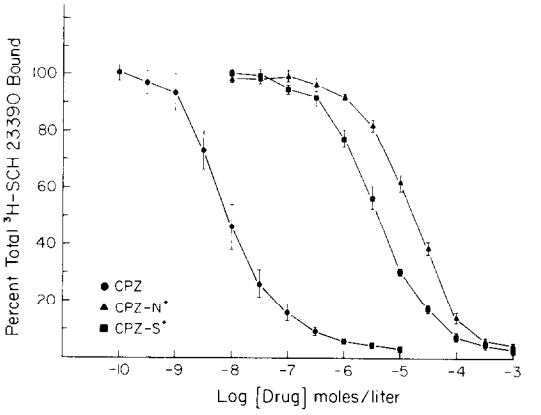

Fig. 5. Inhibition of total [³H]SCH 23390 binding (0.25 nM) by CPZ, CPZ-S^+ and CPZ-N^+. Each point is the mean ± SE of three determinations.

inhibited [³H]SCH 23390 binding with only a 10% inhibition of total [³H]SCH 23390 binding at the highest concentration tested (0.1 mM) (Fig. 3). In addition, the binding of [³H]SCH 23390 was stereoselective since the (+)-isomer of SCH 23390 was 460-fold more potent in inhibiting [³H]SCH 23390 binding than the (−)-isomer of SCH 23390 (Fig. 4).

Effects of CPZ, the trimethylammonium analog of CPZ (CPZ-N^+) and the dimethylsulfonium analog of CPZ (CPZ-S^+) on [³H]SCH 23390 binding. CPZ was found to inhibit [³H]SCH 23390 binding in a concentration-dependent manner with an apparent equilibrium binding dissociation constant (K_i) of 6.2 ± 2.0 nM and a maximum inhibition of 95% (Fig. 5). The CPZ/[³H]SCH 23390 inhibition curve was steep (slope factor not significantly different from one), indicating that the binding of CPZ is to a single class of sites. Similarly, the trimethylammonium (CPZ-N^+) and dimethylsulfonium (CPZ-S^+) analogs of CPZ (Fig. 1) were found to inhibit 95% of total [³H]SCH 23390 binding in a concentration-dependent manner with steep inhibition curves indicative of binding to a single population of sites (Fig. 5). The K_i values for inhibiting [³H]SCH 23390 binding were 12.3 ± 1.6 μM for CPZ-N^+ and 2.75 ± 0.37 μM for CPZ-S^+. Therefore, the order of potency for inhibiting [³H]SCH 23390 binding was CPZ ≫ CPZ-S^+ > CPZ-N^+.

Effects of DA, dimethyldopamine (DMDA), the trimethylammonium analog of DA (DA-N^+), the dimethylsulfonium analog of DA (DA-S^+) and the monomethylsulfide analog of DA on [³H]SCH 23390 binding. Both DA and DMDA effectively inhibited [³H]SCH 23390 binding in a concentration-dependent manner (Fig. 6). The apparent equilibrium binding dissociation constants (K_i) for DA and DMDA were 2.73 ± 0.83 and 14.1 ± 1.6 μM respectively. The K_i for DA was significantly less than the K_i for DMDA, indicating that DA was more potent in inhibiting [³H]SCH 23390 binding

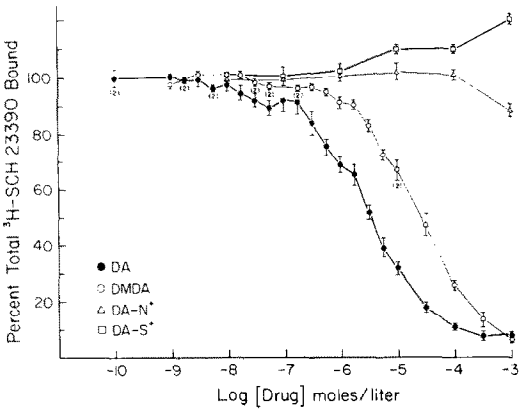

Fig. 6. Inhibition of total [³H]SCH 223390 binding (0.25 nM) by DA, the dimethyl DA analog (DMDA), the dimethylsulfonium analog (DA-S⁺), and the trimethylammonium analog (DA-N⁺). For DA, DMDA and DA-S⁺ each point is the mean ± SE of three determinations except where indicated. For DA-N⁺, each point is the mean ± range of two determinations.

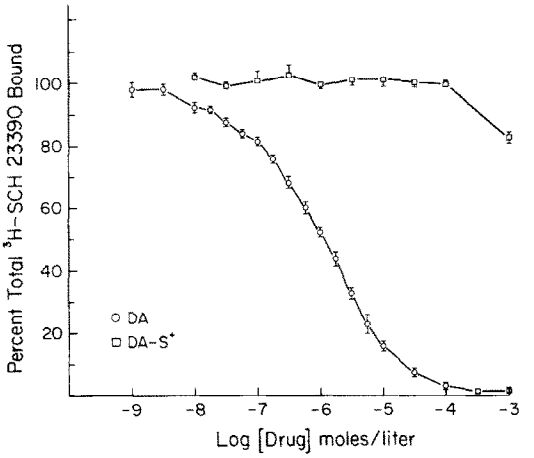

Fig. 7. Inhibition of total [³H]SCH 23390 binding (0.25 nM) by DA and the dimethylsulfonium analog (DA-S⁺) in the presence of buffer B. See Materials and Methods for a description of buffer B. For DA, each point is the mean ± SE of three determinations. For DA-S⁺, each point is the mean ± range of two determinations.

than DMDA. The permanently charged trimethylammonium analog of DA did not inhibit [³H]SCH 23390 binding except at a concentration of 1 mM at which concentration the inhibition was approximately 15% (Fig. 6). The permanently charged dimethylsulfonium analog did not inhibit [³H]SCH 23390 binding at any concentration tested. Instead, the total binding of [³H]SCH 23390 was somewhat elevated (10–20%) in the presence of a 0.01 to 1 mM concentration of the dimethylsulfonium analog (Fig. 6). The permanently uncharged monomethylsulfide analog of DA also did not appear to inhibit [³H]SCH 23390 binding except at the highest concentration tested, 1 mM (data not shown).

The DA/[³H]SCH 23390 inhibition curves could not be resolved into two binding sites, representing the high- and low-affinity states of the D-1 receptor. Thus, the [³H]SCH 23390 inhibition curves for DA were adequately described assuming a one-site binding model with the fit of the inhibition curves not being statistically improved by assuming a two-site binding model. In the literature, reported values for the proportion of D-1 sites to which DA binds with high affinity vary from 10 to 46% [10–12]. Frequently, the distinction between high- and low-affinity D-1 binding is not made, and an overall K_i is reported [6, 7, 12]. The K_i of DA in the present study (2.73 ± 0.83 μM) closely agrees with the K_i reported for the low-affinity D-1 binding site [10–12], suggesting that we may be primarily measuring DA binding to the low-affinity state of the D-1 receptor. Therefore, it could be argued that the permanently charged or uncharged analogs may bind primarily to the high-affinity state of the D-1 receptor which would not be observed under the present assay conditions in which only low-affinity binding is measured.

We therefore determined the inhibitory effects of DA on [³H]SCH 23390 binding using assay conditions which are more optimal for the expression of high-affinity D-1 binding. In this study buffer B (see Materials and Methods) was used since it has been reported that, in the presence of this buffer, 46% of DA binding sites are in the high-affinity state [13, 14]. Using this buffer we found that the K_D and B_{max} for [³H]SCH 23390 binding were 0.18 nM and 56 pmol/g wet weight (N = 2) respectively. In addition, approximately 23% of DA binding sites represented the high-affinity state of the D-1 receptor with K_i values for the high- and low-affinity states of 12.9 ± 8.2 and 676 ± 67 nM respectively. However, even under these conditions in which high-affinity D-1 binding was observed, the permanently charged DA-S⁺ analog was still ineffective in inhibiting [³H]SCH 23390 binding except at the highest concentration tested (1 mM) (Fig. 7). Using this buffer system, no increase in [³H]SCH 23390 binding was observed in the presence of the DA-S⁺ analog in contrast to what was noted using the standard buffer system. In addition, the permanently charged DA-N⁺ analog and the permanently uncharged monomethylsulfide analog of DA also did not inhibit [³H]SCH 23390 binding until a concentration of 1 mM was tested (data not shown).

DISCUSSION

These results show that structural analogs of CPZ, in which the tertiary amine group has been replaced by either a permanently charged dimethylsulfonium (CPZ-S⁺) or trimethylammonium (CPZ-N⁺) group, effectively inhibited the binding of [³H]SCH 23390 to D-1 DA receptors in rat striatal membranes. In addition, the binding characteristics of the permanently charged CPZ analogs were similar to those of the parent compound, CPZ. Thus, the binding of [³H]SCH 23390 was maximally inhibited to the same extent by CPZ, CPZ-N⁺ and CPZ-S⁺. Additionally, the [³H]SCH 23390 inhibition curves of CPZ, CPZ-N⁺ and CPZ-S⁺ all fit a one-site binding model, indicating binding to a single class of receptor sites. Therefore, a major finding of the present study is

that compounds containing a permanent positive charge can bind to the striatal D-1 DA receptor which is consistent with the existence of an anionic site at the D-1 DA receptor. The presence of an anionic binding site on the D-1 DA receptor has also been suggested by previous investigators [13, 14] who found that [^{3}H]SCH 23390 binding could be almost totally abolished by *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), a compound which is thought to irreversibly interact with anionic groups such as ionized carboxyl moieties. In addition, since the dimethylsulfonium analog of CPZ exhibited binding characteristics similar to those of CPZ and CPZ-N^+, it appears that a nitrogen atom is not required for binding to the striatal D-1 DA receptor.

While permanently charged analogs of CPZ were able to inhibit [^{3}H]SCH 23390 binding to the same extent as CPZ, they were less potent. This result suggests that the presence of a permanent positive charge may lead to a decrease in the affinity of a compound for the D-1 receptor. It has been suggested that the affinity of a drug for a receptor is positively influenced by the degree of fat solubility of the drug [15, 16]. The argument is that the concentration of drug at the receptor will be higher for drugs which are more fat soluble due to their greater ability to associate with the lipophilic components in the vicinity of the receptor recognition site [16]. Therefore, the presence of a permanent positive charge may lead to a decrease in the lipophilicity of the compound which may, in turn, result in a decreased ability of the compound to interact with the membrane in the vicinity of the receptor, thus leading to a lower concentration of drug at the receptor site than would be expected with a compound which can also exist in the more lipophilic uncharged form.

It is also possible that lower potencies of the permanently charged CPZ analogs in binding to the D-1 receptor may arise from a decreased ability of these compounds to bind as tightly to an anionic recognition site when compared to the parent amine, CPZ. The permanently charged CPZ analogs can associate with an anionic site via an ionic interaction between the positive and negative charges. Since CPZ contains a proton on the side chain amine group when it is in the charged form, CPZ is, therefore, capable of interacting with an anionic site via hydrogen-bond formation in addition to an ionic interaction. The combination of ionic and hydrogen bonds has twice the bond strength of either bond alone [17]. Since CPZ is capable of associating with the anionic site of the receptor with twice the bond strength of the permanently charged CPZ analogs, the affinity of CPZ would be expected to be higher than that of the permanently charged analogs of CPZ. Therefore, the lower potencies of the permanently charged CPZ analogs for the D-1 receptor may not be due to the presence of a permanent positive charge, *per se*, but may instead arise from the inability of these compounds to donate a proton and engage in hydrogen-bond formation with an anionic recognition site.

In addition to CPZ-N^+ and CPZ-S^+ being less potent than CPZ in inhibiting [^{3}H]SCH 23390 binding, CPZ-N^+ was less potent than CPZ-S^+. The trimethylammonium analog of CPZ has three methyl groups bound to the nitrogen, whereas the dimethylsulfonium CPZ analog has two methyl groups bound to the sulfur. The presence of an additional methyl group in CPZ-N^+ may sterically hinder the analog from associating with the receptor as easily as the CPZ-S^+ analog and, thus, result in a decreased affinity of CPZ-N^+ for the D-1 DA receptor. These results agree with previous studies [4] in which we have shown that the permanently charged CPZ analogs bind to the striatal D-2 DA receptor with the same order of potency as exhibited for the D-1 DA receptor.

In addition to determining the abilities of CPZ and permanently charged CPZ analogs to inhibit [^{3}H]SCH 23390 binding, we also determined the abilities of DA, DMDA, permanently charged analogs of DA in which the side chain amino group has been replaced with a dimethylsulfonium (SA-S^+) or trimethylammonium (DA-N^+) group, and a permanently uncharged monomethylsulfide DA analog (DA-S) to inhibit [^{3}H]SCH 23390 binding. Both DA and DMDA were found to effectively inhibit [^{3}H]SCH 22390 binding in a concentration-dependent manner; however, DMDA was less potent than DA. This result is in contrast to our observations regarding the striatal D-2 DA receptor for which DA and DMDA had equal affinity [4] and suggests that the addition of two methyl groups to the DA molecule results in a decreased affinity for the D-1, but not the D-2, DA receptor.

Although both DA and DMDA were able to inhibit 95% of total [^{3}H]SCH 23390 binding, the permanently charged dimethylsulfonium and trimethylammonium DA analogs and the permanently uncharged monomethylsulfide analog were inactive in inhibiting [^{3}H]SCH 23390 binding unless very high concentrations were used (1 mM). The ineffectiveness of the permanently charged DA analogs in binding to the D-1 receptor would not appear to be the result of additional side chain methyl groups since DMDA, which has the same number of side chain methyl groups as the dimethylsulfonium analog, is able to effectively bind to the D-1 receptor. This observation, however, is similar to that of our previous studies with the striatal D-2 DA receptor in which the affinity of the permanently charged DA analogs for the striatal D-2 receptor was much lower than that of DA or DMDA [4].

While the reasons for the ineffective binding of the permanently charged analogs to the D-1 receptor are unclear, the permanently charged analogs of DA, like the permanently charged analogs of CPZ, lack a proton on the side chain amino or sulfonium group and would also be expected to be less fat soluble than DA or DMDA at physiological pH. Therefore, as suggested for the positively charged analogs of CPZ, it is possible that the ineffectiveness of the permanently charged DA analogs in binding to the D-1 receptor may be related to their inability to form hydrogen bonds with an anionic site on the receptor and/or to interact with lipophilic components in the vicinity of the receptor.

REFERENCES

1. Kebabian JW and Calne DB, Multiple receptors for

dopamine. *Nature* **277**: 93–96, 1979.
2. Braun AR and Chase TN, Obligatory D-1/D-2 receptor interaction in the generation of dopamine agonist related behaviors. *Eur J Pharmacol* **131**: 301–306, 1986.
3. Walters JR, Bergstrom DA, Carlsson JH, Chase TN and Braun AR, D_1 dopamine receptor activation required for postsynaptic expression of D_2 agonist effects. *Science* **236**: 719–722, 1987.
4. Wallace RA, Farooqui T, Wallace L, Ares J, Chang YA, Miller D and Uretsky N, Interaction of permanently charged analogs of dopamine with the D-2 dopaminergic receptor. *Biochem Pharmacol* **36**: 3903–3910, 1987.
5. Wallace RA, Farooqui T, Wallace L, Ares J, Chang YA, Miller D and Uretsky N, Interaction of permanently uncharged dopamine analogs with the D-2 dopaminergic receptor. *Biochem Pharmacol* **37**: 2077–2084, 1988.
6. Billard W, Ruperto V, Crosby G, Iorio LC and Barnett A, Characterization of the binding of ^{3}H-SCH 23390, a selective D-1 receptor antagonist ligand, in rat striatum. *Life Sci* **35**: 1885–1893, 1984.
7. Schulz DW, Stanford EJ, Wyrick SW and Mailman RB, Binding of [^{3}H]SCH-23390 in rat brain: Regional distribution and effects of assay conditions and GTP suggest interactions at a D-1-like dopamine receptor. *J Neurochem* **45**: 1601–1611, 1985.
8. DeLean A, Munson PJ and Rodbard D, Simultaneous analysis of families of sigmoidal curves: Application to bioassay, radioligand assay and physiological dose–response curves. *Am J Physiol* **235**: E97–E102, 1978.
9. Munson PJ and Rodbard D, Ligand: A versatile computerized approach for characterization of ligand-binding systems. *Anal Biochem* **107**: 220–239, 1980.
10. Hyttel J and Arnt J, Characterization of binding of [^{3}H]SCH 23390 to dopamine D-1 receptors. Correlation to other D-1 and D-2 measures and effect of selective lesions. *J Neural Transm* **68**: 171–189, 1987.
11. Seeman P, Ulpian C, Grigoriadis D, Pri-Bar I and Buchman O, Conversion of dopamine D-1 receptors from high to low affinity for dopamine. *Biochem Pharmacol* **34**: 151–154, 1985.
12. Anderson PH, Gronvald FC and Jansen JA, A comparison between dopamine-stimulated adenylate cyclase and [^{3}H]SCH 23390 binding in rat striatum. *Life Sci* **37**: 1971–1983, 1985.
13. Hess EJ and Creese I, Biochemical characterization of dopamine receptors. In: *Dopamine Receptors* (Eds. Venter JC and Harrison LC), pp. 1–27. Alan R. Liss, New York, 1987.
14. Hess EJ, Battaglia G, Norman AB and Creese I, Differential modification of striatal D_1 dopamine receptors and effector moieties by *N*-ethyoxycarbonyl-2-ethoxy-1,2-dihydroquinoline *in vivo* and *in vitro*. *Mol Pharmacol* **31**: 50–57, 1987.
15. Seeman P, Brain dopamine receptors. *Pharmacol Rev* **3**: 229–313, 1980.
16. Seeman P, Neuroleptics (antipsychotics). In: *Principles of Medical Pharmacology* (Eds. Kalant H, Roschlau WHE and Sellers EM), pp. 321–326. University of Toronto Press, Toronto, 1985.
17. Zimmerman JJ and Feldman S, Physical–chemical properties and biological action. In: *Principles of Medicinal Chemistry* (Ed. Foye WO), pp. 11–51. Lea & Febiger, Philadelphia, PA, 1981.