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INTERACTION OF PERMANENTLY CHARGED ANALOGS OF DOPAMINE WITH THE D-2 DOPAMINERGIC RECEPTOR

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Abstract—Dopamine can exist in both charged and uncharged forms at physiological pH. At present it is unclear which of these forms is responsible for dopaminergic agonist activity. The purpose of this study was to determine whether permanently charged structural analogs of dopamine containing either a nitrogen, sulfur, or selenium atom in the side chain can bind to and activate the D-2 dopamine receptor. Binding to and activation of the D-2 dopamine receptor were measured by determining the abilities of the permanently charged dopamine analogs to inhibit [^{3}H]spiperone binding to striatal homogenates and to inhibit K^+-stimulated [^{3}H]acetylcholine release from striatal slices respectively. The quaternary ammonium, dimethylsulfonium and dimethylselenonium analogs of dopamine were all found to inhibit [^{3}H]spiperone binding to the same extent and in a manner qualitatively similar to the parent amines, dopamine and dimethyldopamine. Thus, [^{3}H]spiperone inhibition curves for dopamine, dimethyldopamine and the permanently charged dopamine analogs were generally shallow and fit best to a two-site binding model as indicated by computer-assisted analyses. The addition of 125 mM NaCl to the incubation medium resulted in a significant decrease in the proportion of high affinity binding sites for both the permanently charged analogs and the parent amines. Similarly, the permanently charged dopamine analogs were found to maximally inhibit the K^+-stimulated release of [^{3}H]acetylcholine to the same extent as dopamine and dimethyldopamine. However, the permanently charged analogs were less potent in inhibiting both [^{3}H]spiperone binding and K^+-stimulated [^{3}H]acetylcholine release than dopamine and dimethyldopamine. These results show that dopamine analogs possessing a permanent positive charge in the side chain can bind to and activate the D-2 dopamine receptor. The lower potencies of the permanently charged analogs in binding to and activation of the D-2 dopamine receptor suggest that, while the ability of a compound to exist in an uncharged form is not a requirement, both charged and uncharged forms of the agonist molecule appear to play a role in D-2 dopamine agonist activity.

An abnormality of central dopaminergic neurotransmission has been implicated in several disorders of which Parkinson's disease and schizophrenia are the best known [1]. Dopaminergic agonists such as L-DOPA and bromocriptine are used in the treatment of Parkinson's disease, whereas dopaminergic antagonists such as chlorpromazine are used in the treatment of schizophrenia [2, 3]. The use of presently available dopaminergic agonists and antagonists is associated with adverse effects such as psychiatric reactions and dyskinesias. The cause of these adverse reactions is not clear, but they appear to involve, in part, dopamine receptors. It is possible that the dopamine receptors mediating the therapeutic effects are different from those mediating the adverse effects. One approach that may lead to the development of dopaminergic drugs with more specific actions and fewer adverse effects is to determine which parts of the dopamine molecule are important in producing specific dopaminergic effects. The specific structural requirements for binding to and activation of dopamine receptors have yet to be resolved [4]. The two portions of the dopamine molecule readily accessible to structural modifications are the catechol nucleus and the ethylamine side chain. Although there are exceptions, activation of the D-1 receptor (associated with adenylate cyclase in a stimulatory manner) generally requires the presence of a catechol nucleus [5]. However, the catechol nucleus does not appear to be required for binding to and activation of the D-2 receptor (not associated with adenylate cyclase in a stimulatory manner) [5]. For D-2 agonists it appears that the presence of a pyrrole (e.g. ergolines) or pyrazole (e.g. LY 171555: quinpirole) ring system is sufficient to activate D-2 receptors [6].

Less is known concerning the structural requirements of the ethylamine side chain [4]. It is thought that the optimal spacing between the aromatic ring and the basic amino group of dopamine is a distance equivalent to two methylene groups in an extended conformation [6]. Other considerations concerning the side chain center around the role of the nitrogen atom and whether it is required for binding to and activation of the dopamine receptor. Previous studies have indicated that, when the nitrogen atom of dopamine is replaced with a sulfur atom (producing the dimethylsulfonium analog of dopamine), dopamine agonist activity is still retained, suggesting that the

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amine group is not necessary [7, 8]. Another consideration is whether it is the charged or uncharged form of the nitrogen which is optimal for binding to and activation of the dopamine receptor. Dopamine can exist in both the charged and uncharged forms at physiological pH, and it is unclear which form is responsible for dopaminergic activity. An early study reported that the dopamine antagonist, butaclamol, would exist primarily in the uncharged form at physiological pH based on the pK_a of the side chain nitrogen, suggesting that dopamine agonists and antagonists might interact with the dopamine receptor in the uncharged form [9]. However, a subsequent study contended that the earlier reported pK_a of butaclamol was too low and, therefore, butaclamol would exist mainly in the charged form at physiological pH [10]. However, the problem of studying amines in which the charged and uncharged molecules are in equilibrium is that, although the equilibrium may favor the presence of one form, the possible physiological significance of the other form cannot be disregarded. One way to circumvent this problem is to study molecules that cannot exist in both the charged and uncharged forms. Therefore, we have chosen to examine the abilities of various permanently charged structural analogs of dopamine that contain either a nitrogen, sulfur or selenium atom in the side chain to bind to (as measured by the ability to inhibit [^{3}H]spiperone binding) and activate (as measured by the ability to inhibit K^+-stimulated [^{3}H]acetylcholine release) the D-2 dopamine receptor. The addition of NaCl has been shown to alter classical dopamine agonist binding to D-2 dopaminergic receptors by converting sites of high affinity to those of low affinity [11, 12]. Consequently, binding studies were carried out in the presence and absence of NaCl, and the binding of the permanently charged analogs was compared to that of dopamine and dimethyldopamine. Our findings indicate that a variety of permanently charged analogs can bind to and activate the D-2 dopamine receptor in the same manner as that of classical dopamine agonists and support our previous observations that the nitrogen atom is not an absolute requirement for activation of this receptor.

MATERIALS AND METHODS

Materials. [^{3}H]Spiperone (23.2 Ci/mmol) and [^{3}H]choline (80.0 Ci/mmol) were obtained from New England Nuclear (Boston, MA). Dopamine and dimethyldopamine were obtained from the Sigma Chemical Co. (St. Louis, MO). Cinanserin and (+)- and (−)-butaclamol were obtained from E. R. Squibb & Sons, Inc. (Princeton, NJ) and Research Biochemicals (Wayland, MA) respectively. The quaternary ammonium, dimethylsulfonium and dimethylselenonium analogs of dopamine 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 (nine 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 centrifugred 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 per ml buffer. For some of the binding studies, NaCl was omitted from the buffer. The tissue homogenate was stored on ice until addition to the incubation tubes.

[^{3}H]*Spiperone 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]spiperone (diluted and added in such a volume as to give a final concentration of 0.01 to 1 nM); 50 μl (+)-butaclamol (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]spiperone was defined as the difference between total [^{3}H]spiperone bound and [^{3}H]spiperone bound in the presence of 1 μM (+)-butaclamol. For competition assays, the tubes received in order: [^{3}H]spiperone (added to give a final concentration of 0.1 nM); various concentrations of cold competitor; assay buffer (with or without 125 mM NaCl) sufficient to yield a final assay volume of 5 ml; and 1 ml of striatal homogenate (with a final concentration of 1 mg original tissue wet weight/ml). Cinanserin (0.1 μM) was included in the assay buffer to eliminate the serotonergic component of [^{3}H]spiperone binding. This concentration (0.1 μM) of cinanserin has been reported previously to saturate S-2 serotonergic sites without affecting [^{3}H]spiperone binding to D-2 dopaminergic sites [13].

All assays were carried out at room temperature (23–25°) [13, 14]. The tubes were incubated for 100 min, a time at which equilibrium had been established. The reaction was terminated by separation of the free from bound radioligand 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 mixture (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.

Measurement of the K^+*-induced release of* [^{3}H]*acetylcholine from striatal slices.* Male Swiss–Webster mice (Harlan Sprague Dawley, Inc.) were injected with reserpine (5 mg/kg) and α-methyl-*p*-

tyrosine (250 mg/kg) 20 and 2 hr, respectively, before decapitation. The brains were removed, and the striatal tissue rostral to the anterior commissures was dissected [15]. The tissue was cut into 0.6 mm × 0.6 mm slices using a McIlwain tissue chopper (Brinkmann Instruments, Westbury, NY) and dispersed into a Krebs–Ringer bicarbonate medium. The medium contained (mM): NaCl, 118; KCl, 4.8; $CaCl_2$ 1.3; $MgSO_4$, 1.2; $NaHCO_3$; 25; KH_2PO_4, 1.2; ascorbic acid, 0.6; disodium EDTA, 0.03; and glucose, 11. The medium was stored on ice, bubbled with a 95% O_2–5% CO_2 mixture, and adjusted to a pH of 7.2 with NaOH. The slices were incubated for 20 min with [^{3}H]choline at a final concentration of 0.1 μM, a low concentration that favors the selective uptake of choline into cholinergic neurons through a high affinity uptake system. After incubation, the slices were rinsed with cold medium and transferred to a plastic tube with nylon mesh (15 mm in diameter) attached to one end. This tube was placed into a water jacketed tissue chamber maintained at 37°, and the slices were superfused with normal medium at a constant rate of 0.5 ml/min for 45 min. The superfusion medium and the subsequent incubation medium contained hemicholinium (10 μM) and α-methyl-p-tyrosine (250 mM). At the end of the superfusion, the tube containing the slices was removed from the tissue chamber, and two slices were placed in each of twelve tubes with nylon mesh attachments. The slices in each tube were then transferred at 5 min intervals into six different 10-ml beakers, which contained 3 ml of fresh medium at 37°. The first four beakers contained normal medium, while the fifth and sixth beakers contained medium in which the concentration of K^+ was increased to 13.8 mM (the concentration of sodium was reduce to maintain isotonicity). The dopaminergic agonists, when present, were added to the high K^+-medium. After the tubes containing the slices were removed from the last beaker, the slices were homogenized in 0.4 N perchloric acid. The radioactivity in the medium that remained in the beakers and the perchloric acid extracts was determined by liquid scintillation counting. The tritium that was released into the medium by the high K^+ was not further characterized. Several previous studies have demonstrated that in the presence of phyostigmine, an inhibitor of acetylcholinesterase, radioactive acetylcholine is the main radioactive constituent of the medium [16–21]. In the present study, physostigmine was not added to the medium since it can result in high extracellular levels of acetylcholine which has been shown to inhibit the depolarization-induced release of acetylcholine [21, 22]. Under the conditions of the present study, the K^+-induced release of tritium was completely dependent on the presence of calcium ions in the medium (data not shown).

The amount of tritium released from the tissue into the medium in each 5 min incubation period is expressed as a percentage of the total tritium content of the tissue at the start of the incubation period (fractional release × 100). This was calculated by correcting the tissue content of tritium for the tritium released into the medium. The K^+-evoked increase of tritium release is the mean percentage release of tritium obtained when the slices were incubated in the beakers with high K^+ medium above the baseline of spontaneous release. The latter is the percentage fractional release obtained during the incubation of slices in normal medium preceding their incubation in high K^+ medium.

Analysis of data. All binding data were analyzed using an iterative nonlinear least squares curve-fitting program. For [^{3}H]spiperone 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]spiperone was derived from the analysis of the [^{3}H]spiperone saturation studies and was used in the subsequent analysis of the [^{3}H]spiperone competition studies. For the [^{3}H]spiperone 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 [23]. 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 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 the following equation:

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

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 models respectively [24]. 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 if significant changes in affinity or in the proportion of high affinity sites occurred when 125 mM NaCl was added to the incubation medium. ANOVA and Duncan's multiple-range test were used to determine whether significant differences existed among the binding dis-

sociation constants for the different dopamine analogs tested. Before statistical analyses, the data were transformed by converting the binding dissociation constants to negative logarithms and the proportion of high affinity sites to the arcsine of the square-root of the proportion in order to obtain data which are normally distributed.

The linear portions of the concentration–response curves for the inhibition of K^+-stimulated [^{3}H]acetylcholine release were analyzed using linear regression for grouped data where for each independent variable (agonist concentration) there are multiple dependent variables (percent inhibition of K^+-stimulated [^{3}H]acetylcholine release). The half-maximal effective concentration (EC_{50}) and the 95% confidence limits for the EC_{50} values were calculated for each curve. Curves were considered to be significantly different if their confidence limits did not overlap.

The level of significance employed for all statistical tests was $P < 0.05$.

RESULTS

Binding properties of [^{3}H]*spiperone in the presence and absence of* NaCl. To characterize the binding properties of various dopamine agonists, the abilities of these compounds to compete for [^{3}H]spiperone binding sites were determined in the presence and absence of 125 mM NaCl. The analysis of these data requires that the binding parameters of the radioligand be determined for each condition. Therefore, saturation experiments of [^{3}H]spiperone binding were done in the presence and absence of 125 mM NaCl.

Under both conditions, [^{3}H]spiperone binding was found to be saturable, of high affinity, and fit best to a one-site model indicating binding to a single class of receptors. The K_d of [^{3}H]spiperone in the presence of 125 mM NaCl was significantly lower than the K_d of [^{3}H]spiperone in the absence of NaCl, indicating that [^{3}H]spiperone has a higher affinity for the D-2 dopamine receptor in the presence of NaCl (Table 1). These differences in affinities of [^{3}H]spiperone binding were taken into consideration when calculating the binding parameters of competing dopamine agonists in subsequent experiments. The B_{max} for [^{3}H]spiperone binding was the same in the presence and absence of NaCl. Additionally, the binding of [^{3}H]spiperone was stereoselective since it was inhibited more effectively by (+)- than by (−)-butaclamol (Fig. 1). Specific binding represented 80–90% of total binding.

Table 1. Binding parameters of [^{3}H]spiperone in the presence and absence of 125 mM NaCl

NaCl (mM)	K_d (pM)	B_{max} (pmoles/g tissue)
0	75 ± 15	24.2 ± 2.1
125	31 ± 8*	25.3 ± 0.7

K_d is the equilibrium binding dissociation constant.
* $P < 0.05$.

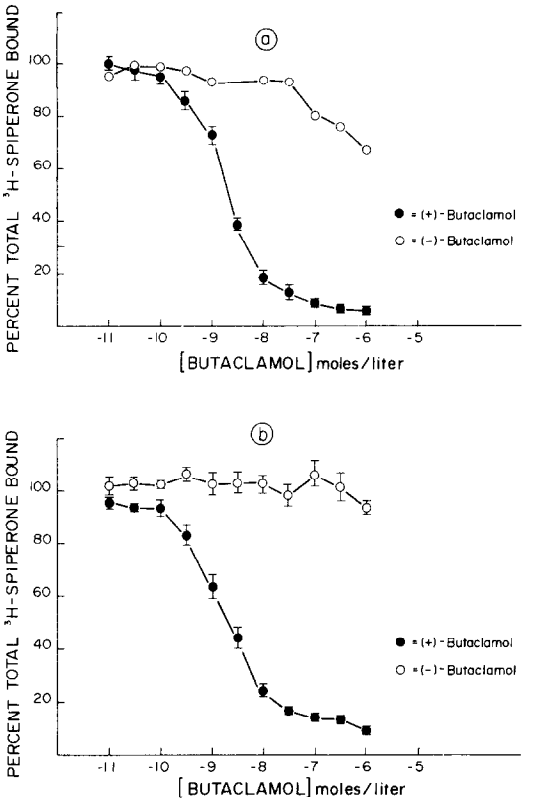

Fig. 1. Inhibition of total [^{3}H]spiperone binding (0.1 nM) by (+)- or (−)-butaclamol in the presence (a) and absence (b) of 125 mM NaCl. Each point represents the mean ± SEM of three separate determinations done in duplicate with the exception of (−)-butaclamol in the presence of NaCl which represents one determination done in duplicate.

Binding properties of dopamine and dimethyldopamine in the presence and absence of NaCl. The binding properties of dopamine and dimethyldopamine were determined in competition assays with [^{3}H]spiperone in the presence and absence of 125 mM NaCl. Under both conditions, the inhibition curves for dopamine and dimethyldopamine were shallow (Figs. 2 and 3). Analyses of the data indicated that the slope factors were significantly less than one and that the inhibition curves fit best to a model in which the amines bind to two sites (Table 2). In NaCl-free incubation medium, the high affinity sites for dopamine and dimethyldopamine accounted for 60 ± 3 and 51 ± 10% of the binding sites respectively. The addition of 125 mM NaCl to the medium resulted in a significant decrease in the proportion of high affinity binding sites, which were now 24 ± 1 and 26 ± 5% of total sites for dopamine and dimethyldopamine respectively. NaCl also increased the dissociation constants of dimethyldopamine for both the (K_H) and low affinity (K_L) sites (Table 2). In contrast, the addition of NaCl increased only the K_L for dopamine; the K_H was not changed significantly. In NaCl-containing medium, the K_H for dopamine was significantly lower than that for dimethyldopamine, whereas no significant difference occurred in NaCl-free medium. The K_L for dopamine

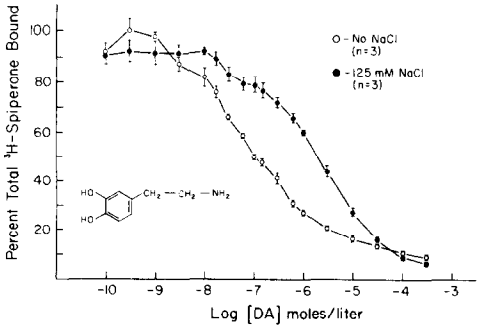

Fig. 2. Dopamine (DA)/[³H]spiperone (0.1 nM) competition curves in the presence and absence of 125 mM NaCl. Values are means ± SEM.

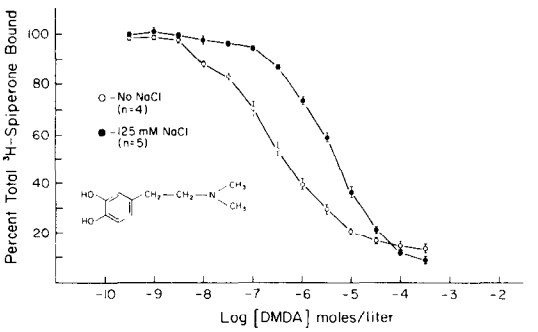

Fig. 3. Dimethyldopamine (DMDA)/[³H]spiperone (0.1 nM) competition curves in the presence and absence of 125 mM NaCl. Values are means ± SEM.

was not significantly different from the K_L for dimethyldopamine in either the presence or absence of NaCl (Table 2).

Binding properties of permanently charged dopamine analogs in the presence and absence of NaCl. The abilities of the quaternary ammonium, dimethylsulfonium and dimethylselenonium analogs of dopamine to compete for [³H]spiperone binding sites were determined in order to compare the binding properties of permanently charged dopamine analogs with those of dopamine and dimethyldopamine. All three permanently charged compounds were found to inhibit [³H]spiperone binding in both the presence and absence of NaCl (Figs. 4–6). Under both conditions the inhibition curves for the dimethylselenonium and quaternary ammonium analogs were shallow, and the slope factors were significantly less than one. Statistical analyses indicated that they fit better to a two binding site model than a one site model in both the absence and presence of NaCl (Table 2). In the absence of NaCl, the dimethylsulfonium analog also displayed a shallow inhibition curve which was best modelled to two sites. However, in the presence of 125 mM NaCl, the dimethylsulfonium inhibition curve was shallow with a slope factor less than one, but it did not fit a two-site model significantly better than a one-site model. In NaCl-free medium, the high affinity binding sites for the dimethylsulfonium, dimethyselenonium, and quaternary ammonium analogs represented 55 ± 7, 62 ± 10 and 58 ± 5% of the binding sites respectively. The addition of 125 mM NaCl to the medium reduced the proportion of high affinity sites to 30 ± 6% (dimethylselenonium analog) and 10 ± 1% (quaternary ammonium analog), while a high affinity binding component could no longer be detected for the dimethylsulfonium analog. NaCl also produced changes in the equilibrium binding dissociation constants; this usually involved an increase for most of the drugs tested (Table 2).

Inhibition of potassium-stimulated [³H]*acetylcholine release from striatal slices.* Dopamine, dimethyldopamine and the permanently charged analogs of dopamine all inhibited the K^+-stimulated release of [³H]acetylcholine in a concentration-dependent manner. In addition, all compounds maximally inhibited [³H]acetylcholine release to a similar extent. Table 3 presents the EC_{50} values and 95% confidence limits for these studies. The order of potency for inhibition of the K^+-evoked stimulation of [³H]acetylcholine release was: dimethyldopamine > dopamine > dimethylselenonium analog = quaternary ammonium analog > dimethylsulfonium analog.

Table 2. Summary of the apparent equilibrium binding dissociation constants for dopamine (DA), dimethyldopamine (DMDA) and the permanently charged dopamine analogs in NaCl-free and NaCl-containing medium

	No NaCl			125 mM NaCl		
	K_H (μM)	K_L (μM)	%H	K_H (μM)	K_L (μM)	%H
DA	0.010 ± 0.002*	0.354 ± 0.040*	60 ± 3	0.011 ± 0.001*,†	0.958 ± 0.003*	24 ± 1
DMDA	0.025 ± 0.007*	0.724 ± 0.173*	51 ± 10	0.118 ± 0.023	1.73 ± 0.13*	26 ± 5
DA-N^+	2.63 ± 0.52‡	70.6 ± 10.7	58 ± 5	0.344 ± 0.158	160 ± 74	10 ± 1
DA-S^+	1.28 ± 0.28	76.9 ± 36.5	55 ± 7	ND	46.8 ± 7.4	0
DA-Se^+	0.541 ± 0.151	22.9 ± 5.8	62 ± 10	4.45 ± 1.13§	152 ± 99	30 ± 6

Quaternary ammonium analog (DA-N^+), dimethylsulfonium analog (DA-S^+), dimethylselenonium analog (DA-Se^+). K_H and K_L represent the high and low affinity dissociation constants respectively; %H represents the percentage of high affinity binding sites. ND = not detected. Each value is the mean ± SEM of three to nine experiments.

* $P < 0.05$ when compared to all permanently charged analogs.

† $P < 0.05$ when compared to dimethyldopamine.

‡ $P < 0.05$ when compared to the dimethylselenonium analog.

§ $P < 0.05$ when compared to dimethyldopamine and the quaternary ammonium analog.

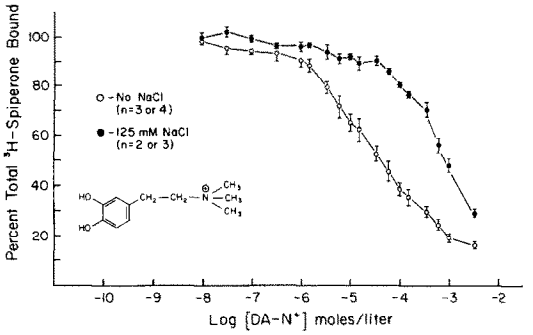

Fig. 4. Inhibition of total [³H]spiperone binding (0.1 nM) by the quaternary ammonium dopamine analog (DA-N⁺). Values are means ± SEM.

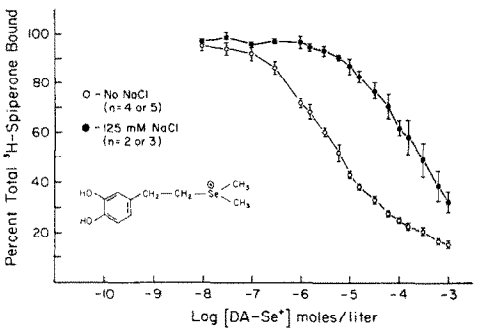

Fig. 6. Inhibition of total [³H]spiperone binding (0.1 nM) by the dimethylselenonium dopamine analog (DA-Se⁺). Values are means ± SEM.

DISCUSSION

The major findings of these studies are that permanently charged analogs of dopamine, in which the side chain nitrogen is replaced by another atom, can inhibit [³H]spiperone binding to striatal membranes and exert dopaminergic agonist activity. Thus, the permanently charged analogs of dopamine were shown to inhibit effectively the binding of [³H]spiperone to the D-2 dopamine receptor. In addition, the binding characteristics of the permanently charged analogs were similar to those of the amines, dopamine and dimethyldopamine, which can exist in both charged and uncharged forms. Thus, the binding of [³H]spiperone was maximally inhibited to the same extent by both the permanently charged analogs and the amines. Furthermore, in the absence of NaCl, the [³H]spiperone inhibition curves for both the amines and the permanently charged analogs fit a two-site model significantly better than a one-site model with the proportion of high affinity sites being similar for all compounds tested. The addition of NaCl to the incubation medium decreased the proportion of high affinity sites for all compounds tested. These results are in agreement with previous studies of the striatal D-2 dopamine receptor using [³H]spiperone which have demonstrated that dopaminergic agonists (unlike dopaminergic antagonists) bind to high and low affinity states of the D-2 dopamine receptor. In addition, it has been shown that the presence of NaCl produces a marked reduction in the proportion of high affinity sites, without changing the total number of binding sites, suggesting a conversion of sites of high affinity to those of low affinity [11, 12]. Therefore, the abilities of the permanently charged analogs of dopamine to bind to the high and low affinity sites and the reduction in the proportion of high affinity sites in the presence of NaCl suggest that the permanently charged molecules bind to the D-2 dopamine receptor in the same way as known dopaminergic agonists [11], including dopamine and dimethyldopamine (Figs. 2 and 3).

In the presence of NaCl, the permanently charged dimethylsulfonium analog of dopamine seemed to bind to a single class of sites, whereas in the absence of NaCl the binding of this analog was best described by assuming two classes of sites. This latter observation differs from that of Seeman *et al.* [25], who reported that this compound did not distinguish between the two forms of the D-2 receptor. This discrepancy could be explained by the different type of tissue and animal species used by Seeman *et al.* [25], which was porcine anterior pituitary gland. The ability of the dimethylsulfonium analog of dopamine to bind to both high and low affinity states of the D-2 receptor from striatal membranes is in agreement with our results using other dopamine analogs with a permanent positive charge.

In addition to binding to the D-2 dopaminergic receptor, the permanently charged analogs of dopamine were also found to activate the dopaminergic

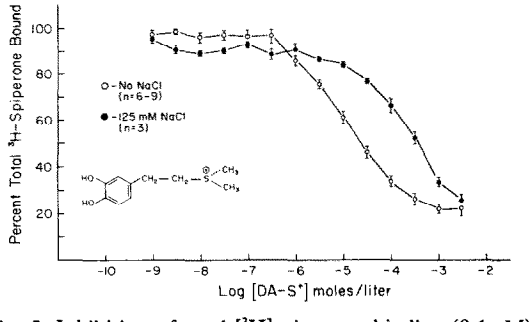

Fig. 5. Inhibition of total [³H]spiperone binding (0.1 nM) by the dimethylsulfonium dopamine analog (DA-S⁺). Values are means ± SEM.

Table 3. EC_{50} and 95% confidence limits for the inhibition of the K^+-evoked release of [³H]acetylcholine from straital slices for dopamine (DA), dimethyldopamine (DMDA), and the permanently charged analogs of dopamine

Drug	EC_{50} (μM)	95% Confidence limits (μM)
DMDA	0.06	0.03– 0.12
DA	1.93	1.13– 3.28
DA-N⁺	7.49	3.83–14.7
DA-Se⁺	8.99	5.94–13.6
DA-S⁺	22.7	16.8 –30.7

Quaternary ammonium analog (DA-N⁺), dimethylselenonium analog (DA-Se⁺), dimethylsulfonium analog (DA-S⁺).

receptor regulating the depolarization-induced release of [^{3}H]acetylcholine from striatal slices. Thus, the permanently charged analogs significantly inhibited the K^+-evoked release of [^{3}H]acetylcholine in a manner similar to the amines, dopamine and dimethyldopamine. The inhibition of the K^+-evoked release of [^{3}H]acetylcholine by all compounds tested appears to be due to the direct activation of the dopaminergic receptor and not to the release of endogenous dopamine since it is observed in striatal slices treated with reserpine and α-methyl-*p*-tyrosine. This treatment has been shown previously to antagonize completely the inhibition of the K^+-evoked release of [^{3}H]acetylcholine by amphetamine, a compound that produces its effects by releasing endogenous dopamine [8]. Additionally, the inhibitory effects of the permanently charged analogs appear to be mediated by activation of the D-2 dopaminergic receptor subtype since they have been shown to be antagonized by sulpiride, a selective D-2 dopaminergic antagonist [26].

In some instances the relative affinities for binding to the D-2 receptor were not in complete agreement with the relative potencies in inhibiting the K^+-evoked release of [^{3}H]acetylcholine from striatal slices. In the presence of NaCl, the affinities of dopamine and dimethyldopamine for the D-2 site were similar; however, dimethyldopamine was more potent than dopamine in activating the D-2 receptor in striatal slices. Similarly, the quaternary ammonium and dimethylsulfonium analogs had similar affinities for binding to the D-2 receptor; yet, the quaternary ammonium analog was more potent than the dimethylsulfonium analog in inhibiting the K^+-evoked release of [^{3}H]acetylcholine. These results suggest that factors other than binding may play an important role in the inhibition of the K^+-evoked release of [^{3}H]acetylcholine from the slices. These factors may include the affinity of the agonists for uptake sites in the dopaminergic nerve terminal since their uptake into the nerve terminal would be expected to remove these compounds from the vicinity of the dopaminergic receptor. In addition, the abilities of these compounds to activate dopaminergic D-1 receptors in the slice preparation would also be expected to affect the inhibition of [^{3}H]acetylcholine release since it has been shown recently that D-1 receptor agonists can antagonize the D-2 receptor mediated inhibition of the K^+-evoked release of [^{3}H]acetylcholine [27]. Nonetheless, the findings that permanently charged dopamine analogs can inhibit both [^{3}H]spiperone binding and [^{3}H]acetylcholine release indicate that, while dopamine is present in both charged and uncharged forms at physiological pH, dopamine agonists do not have to exist in an uncharged form in order to bind to and activate the D-2 dopamine receptor.

While the binding characteristics of the permanently charged analogs were similar to those of dopamine and dimethyldopamine, the binding dissociation constants (K_H and K_L) of dopamine and dimethyldopamine were significantly lower than those of the permanently charged analogs of dopamine (Table 2). This was true for binding determined in either the presence or absence of NaCl. These observations indicate that dopamine and dimethyldopamine have a greater affinity for both the high and low affinity states of the D-2 receptor than do dopamine analogs in which the side chain is permanently charged. This conclusion is consistent with the observation that the permanently charged analogs were less potent agonists in inhibiting the K^+-evoked release of [^{3}H]acetylcholine from striatal slices. Since dopamine and dimethyldopamine can exist in both uncharged and charged molecular forms, the greater binding potencies of these amines suggest that both the charged and uncharged forms play a role in determining affinity and agonist activity for the D-2 receptor.

In summary, these studies show that dopamine analogs with a permanent positive charge can bind to the D-2 dopamine receptor and exert agonist activity, suggesting the presence of an anionic site at the D-2 dopamine receptor. In addition, the amine group of the dopamine molecule was not required for agonist action. However, the permanently charged analogs were less potent in binding and agonist activity that the parent primary or tertiary amines that can exist in the charged and uncharged forms. This suggests that both forms of the agonist molecule play a role in D-2 dopamine agonist activity.

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REFERENCES

1. P. Seeman, *Pharmac. Rev.* **32**, 229 (1981).
2. J. R. Bianchine, in *The Pharmacological Basis of Therapeutics* (Eds. A. G. Gilman, L. S. Goodman and A. Gilman), 6th Edn, p. 475. Macmillan, New York (1980).
3. R. J. Baldessarini, in *The Pharmacological Basis of Therapeutics* (Eds. A. G. Gilman, L. S. Goodman and A. Gilman), 6th Edn, p. 391. Macmillan, New York (1980).
4. D. Miller, *Fedn Proc.* **37**, 2394 (1978).
5. Y. Itoh, M. E. Goldman and J. W. Kebabian, *Eur. J. Pharmac.* **108**, 99 (1985).
6. C. Kaiser and T. Jain, *Mednl Res. Rev.* **5**, 145 (1985).
7. R. Boldry, Y. Chang, D. D. Miller and N. J. Uretsky, *Pharmac. Biochem. Behav.* **24**, 223 (1986).
8. B. Turowski, M. Szkrybalo, K. Anderson, D. Miller and N. Uretsky, *Biochem. Pharmac.* **33**, 2371 (1984).
9. A. H. Philipp, L. G. Humber and K. Voith, *J. med. Chem.* **22**, 768 (1979).
10. F. A. Chrzanowski, B. A. McGrogan and B. E. Maryanoff, *J. med. Chem.* **28**, 399 (1985).
11. D. Grigoriodis and P. Seeman, *J. Neurochem.* **44**, 1925 (1985).
12. M. Watanabe, S. R. George and P. Seeman, *J. Neurochem.* **45**, 1842 (1985).
13. M. W. Hamblin, S. E. Leff and I. Creese, *Biochem. Pharmac.* **33**, 877 (1984).
14. R. G. Mackenzie and M. J. Zigmond, *Eur. J. Pharmac.* **113**, 159 (1985).
15. J. Glowinski and L. L. Iversen, *J. Neurochem.* **13**, 655 (1966).
16. J. Richter and R. Marchbanks, *J. Neurochem.* **18**, 691 (1971).
17. D. Greewal and J. Quastel, *Biochem. J.* **132**, 1 (1973).
18. P. Molenaar, V. Nicholson and R. Polak, *Br. J. Pharmac.* **47**, 97 (1973).
19. G. Somogyi and J. C. Szerb, *J. Neurochem.* **19**, 2667 (1973).

20. A. Mulder, H. Yamamura, M. J. Kuhar and S. H. Snyder, *Brain Res.* **70**, 372 (1974).
21. P. Hadhazy and J. C. Szerb, *Brain Res.* **123**, 311 (1977).
22. J. Szerb and G. Somogyi, *Nature New. Biol.* **241**, 121 (1973).
23. A. DeLean, P. J. Munson and D. Rodbard, *Am. J. Physiol.* **235**, E92 (1978).
24. P. J. Munson and D. Rodbard, *Analyt. Biochem.* **107**, 220 (1980).
25. P. Seeman, M. Watanabe, D. Grigoriadis, J. L. Tedesco, S. R. George, U. Svensson, J. Lars, G. Nilsson and J. L. Neumeyer, *Molec. Pharmac.* **28**, 391 (1985).
26. B. Sabol, R. Boldry, T. Farooqui, Y. A. Chang, D. Miller and N. Uretsky, *Biochem. Pharmac.* **36**, 1679 (1987).
27. J. M. Gorell, B. Czarnecki and S. Hubbell, *Life Sci.* **38** 2247 (1986).