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

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**Abstract**—The purpose of this study was to determine if structural analogs of dopamine in which the side chain nitrogen has been replaced by a permanently uncharged monomethylsulfide, monomethylselenide or sulfoxide group are capable of binding to the striatal D-2 dopamine receptor and acting as agonists at this receptor. All the permanently uncharged dopamine analogs were found to bind to the D-2 dopamine receptor as evidenced by their abilities to inhibit significantly [$^3$H]spiperone binding to striatal homogenates. However, the inhibition of [$^3$H]spiperone binding by the uncharged dopamine analogs was incomplete and was almost abolished by the addition of NaCl (125 mM) to the incubation medium or by the addition of dopamine or quinpirole at a concentration that saturates the high-affinity state of the D-2 dopamine receptor. These effects of NaCl, dopamine and quinpirole suggest that the uncharged dopamine analogs bind primarily to the high-affinity state of the D-2 dopamine receptor. Whether the uncharged monomethylsulfide and sulfoxide analogs could function as dopamine agonists at the striatal D-2 dopamine receptor was assessed by determining the abilities of these compounds to inhibit the $K^+$-evoked release of [$^3$H]acetylcholine from striatal slices. Both the monomethylsulfide and sulfoxide analogs inhibited the $K^+$-evoked release of [$^3$H]acetylcholine, but this inhibitory effect does not appear to be due to the activation of the D-2 dopamine receptor since it was not reversed by the selective D-2 dopamine antagonist, sulpiride. Additionally, the uncharged monomethylsulfide and sulfoxide dopamine analogs were found to antagonize the ability of apomorphine to inhibit the $K^+$-evoked release of [$^3$H]acetylcholine, but this antagonistic effect does not appear to be due to the reversible blockade of the D-2 dopamine receptor since it was not reduced by increasing the concentration of apomorphine. Therefore, while the permanently uncharged analogs of dopamine appear to bind to the high-affinity state of the D-2 dopamine receptor, they are not dopamine agonists or antagonists at the striatal D-2 dopamine receptor involved in regulating the release of acetylcholine. These results suggest that a positive charge may be a requirement for the activation of the striatal D-2 dopamine receptor.

The specific structural elements of the ethylamine side chain of dopamine necessary for agonist activity have yet to be determined. One unresolved issue is whether the presence of a positive charge in the ethylamine side chain is optimal for interacting with the dopamine receptor. The side chain nitrogen of dopamine can exist in both charged and uncharged forms at physiological pH. Based on the $pK_a$ of the side chain amine group (8.93), the equilibrium between the charged and uncharged forms of dopamine at pH 7.4 is in favor of the charged form. An equilibrium in favor of the charged form of dopamine, though, does not preclude the existence and possible physiological significance of the uncharged form. It has been suggested that the orientation of the nitrogen electron pair is important in the interaction of agonists with the dopamine receptor [1]. If the nitrogen electron pair were to interact with the dopamine receptor, it follows that the side chain nitrogen of dopamine would be in the uncharged molecular form. However, whether it is the charged or uncharged form of dopamine which is responsible for binding to, and activation of, dopamine receptors is still an unresolved issue.

In a previous study we synthesized structural analogs of dopamine that contained a permanent positive charge in the ethylamine side chain and determined their abilities to act as dopaminergic agonists and to bind to the D-2 dopamine receptor [2]. The analogs that were synthesized contained either a permanently charged trimethylammonium, dimethylsulfonium or dimethylselenonium group in place of the primary amine of dopamine. These permanently charged analogs of dopamine were found to bind to the D-2 receptor in a manner similar to conventional dopamine agonists and to exert D-2 dopaminergic activity at the dopamine receptor that inhibits the depolarization-evoked release of [$^3$H]acetylcholine. These results support the hypothesis that it is the positively charged form of the dopamine agonist molecule that interacts with the D-2 dopamine receptor, in part, by binding to an anionic recognition site. Therefore, if the ability to interact with an anionic site on the dopamine D-2

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receptor is a requirement for the expression of agonist activity, then permanently uncharged analogs of dopamine would not be expected to bind to the D-2 dopamine receptor or produce dopamine agonist activity. To test this hypothesis, we have now synthesized three permanently uncharged dopamine analogs which are similar in structure to the permanently charged dopamine analogs. These are the monomethylsulfide, monomethylselenide and the sulfoxide derivatives (see Figs. 3–5). To determine whether these uncharged compounds can bind to the D-2 dopamine receptor and/or exert D-2 agonist activity, we examined their effects on [$^3$H]spiperone binding to rat striatal membranes and on the $K^+$-evoked release of [$^3$H]acetylcholine from striatal slices.

Our studies show that, although permanently uncharged dopamine analogs inhibited [$^3$H]spiperone binding, they did not possess activity at the striatal D-2 dopamine receptor characteristic of typical dopamine agonists.

## 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, U.S.A.). Dopamine and dimethyldopamine were obtained from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Cinanserin and (+)- and (−)-butaclamol were obtained from E. R. Squibb & Sons, Inc. (Princeton, NJ, U.S.A.) and Research Biochemicals (Wayland, MA, U.S.A.) respectively. The monomethylsulfide, monomethylselenide and sulfoxide analogs of dopamine were synthesized in our laboratories.

*Preparation of drug solutions.* All drugs were freshly prepared. The monomethylsulfide, monomethylselenide and sulfoxide analogs of dopamine were dissolved in a small amount of solvent which consisted of 2 parts 95% ethanol, 2 parts dimethyl sulfoxide (DMSO) and 6 parts PEG 400 and then diluted with buffer. The concentration of solvent used had no effect on either [$^3$H]spiperone binding or the $K^+$-evoked release of [$^3$H]acetylcholine. Butaclamol was dissolved in a small amount of concentrated acetic acid and then diluted with hot 0.1% ascorbic acid. All other drugs were dissolved in buffer.

*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 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, 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 aq.) 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 [3].

All assays were carried out at room temperature (23–25°) [3, 4]. 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, U.S.A.). 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 added immediately. 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 [5]. The tissue was cut into 0.6 mm × 0.6 mm using a McIlwain tissue chopper (Brinkman 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 $\mu$M. This low concentration was used because it 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 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 $\mu$M) and $\alpha$-methyl-*p*-tyrosine (250 $\mu$M). 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 tubes were then transferred at 5-min intervals into six successive 10-ml beakers, each of 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 reduced to maintain isotonicity). To test for dopamine agonist activity, the compounds were added to the high $K^+$ medium. To test for dopamine antagonist activity, the compounds were added to the normal medium (beakers 3 and 4) as well as to the high $K^+$ medium (beakers 5 and 6) while apomorphine was added only to the high $K^+$ medium. After the last incubation, the tubes containing the slices were removed from the beaker, and 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 characterized further. Several previous studies have demonstrated that in the presence of physostigmine, an inhibitor of acetylcholinesterase, radioactive acetylcholine is the main radioactive constituent of the medium [6, 7]. 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 [7, 8]. 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 in 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 of slices incubated in normal medium (beaker 4) which precedes 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 [9]. 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 [10]. 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 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 fractional release of [$^3$H]acetylcholine obtained for the different treatment groups. Before statistical analyses, the data were transformed by converting the proportion of high-affinity sites and the [$^3$H]acetylcholine fractional release data to the arcsine of the square-root

of the proportion in order to obtain data which are normally distributed.

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

## RESULTS

*Effects of dopamine and dimethyldopamine on [$^3$H]spiperone binding in the absence and presence of* NaCl. [$^3$H]Spiperone binding was found to be saturable, stereoselective and fit best to a one-site binding model (data not shown). Specific binding represented 80–90% of total binding. In the presence of 125 mM NaCl, the equilibrium binding dissociation constant ($K_d$) and $B_{max}$ of [$^3$H]spiperone binding were 31 ± 8 pM and 25.3 ± 7 pmol/g tissue respectively. In the absence of NaCl, the $K_d$ and $B_{max}$ of [$^3$H]spiperone binding were 75 ± 15 pM and 24.2 ± 2.1 pmol/g tissue respectively.

In NaCl-free medium, dopamine (Fig. 1) and dimethyldopamine (Fig. 2) maximally inhibited total [$^3$H]spiperone binding by 90% which corresponds to the complete inhibition of specific [$^3$H]spiperone binding. The inhibition curves were found to fit best to a two-site binding model which is believed to represent the high- and low-affinity states of the D-2 dopamine receptor [11]. For both compounds, binding to the high-affinity site represented approximately 50–60% of total binding which is similar to that reported for dopaminergic agonists by Grigoriadis and Seeman [12]. In the absence of NaCl, the binding dissociation constant of dopamine for the high-affinity site ($K_H$) was 9.7 ± 2.0 nM (SEM, N = 3) and the binding dissociation constant for the low-affinity site ($K_L$) was 354 ± 40 nM (SEM, N = 3) (Fig. 1). The binding dissociation constant of dimethyldopamine for the high-affinity site ($K_H$) was 24.6 ± 7.2 nM (SEM, N = 4) and that for the low-affinity site ($K_L$) was 720 ± 170 nM (SEM, N = 4) (Fig. 2). The addition of 125 nM NaCl to the incubation medium produced a marked decrease in the proportion of high-affinity binding sites for dopamine (60% to 24%) and dimethyldopamine (51% to 26%), but did not change the maximum inhibitory effect of these compounds (Figs. 1 and 2). This effect of NaCl has been interpreted as a conversion of a high-affinity state of the receptor to a low-affinity state [12]. In the presence of NaCl, the $K_H$ for dopamine was 11.4 ± 1.1 nM (SEM, N = 3) and the $K_L$ was

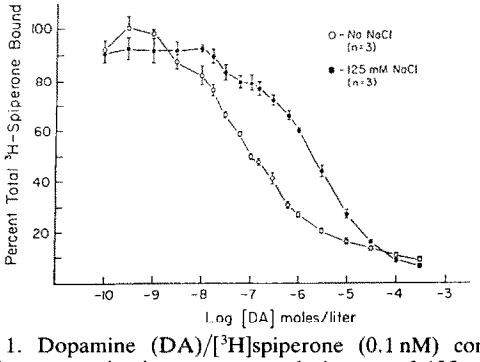


Fig. 1. Dopamine (DA)/[$^3$H]spiperone (0.1 nM) competition curves in the presence and absence of 125 mM NaCl. Each value is the mean ± SEM.

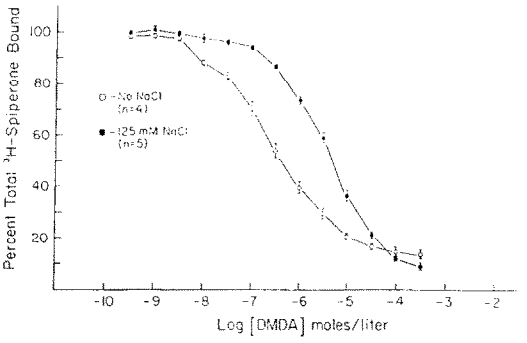


Fig. 2. Dimethyldopamine (DMDA)/[$^3$H]spiperone (0.1 nM) competition curves in the presence and absence of 125 mM NaCl. Each value is the mean ± SEM.

958 ± 3 nM (SEM, N = 3) (Fig. 1). The $K_H$ for dimethyldopamine was 117.5 ± 22.7 nM (SEM, N = 5) and the $K_L$ was 1.73 ± 0.13 μM (SEM, N = 5) (Fig. 2).

*Effect of the permanently uncharged dopamine analogs on [$^3$H]spiperone binding in the absence and presence of* NaCl. In the absence of NaCl, all three permanently uncharged compounds inhibited [$^3$H]spiperone binding (Figs. 3–5). The methysulfide and methylselenide analogs produced a maximum inhibition of total [$^3$H]spiperone binding of 60%, whereas the sulfoxide analog produced a maximum inhibition of 40%. Computer-assisted analysis indicates that the inhibition curves for the three uncharged analogs, unlike the curves for dopamine and dimethyldopamine, did not fit a two-site binding model better than a one-site model. Very high concentrations of all three uncharged analogs (1 mM and above) were found to increase, rather than decrease, [$^3$H]spiperone binding. This effect, which may be related to nonspecific membrane changes produced by these lipid soluble drugs, was not examined further because the large amounts of drug required for this study were not available. The apparent equilibrium binding dissociation constants ($K_i$) values for the methylselenide, methylsulfide and sulfoxide analogs were 3.8 ± 1.4 μM (SEM) (N = 3), 16.1 ± 9.1 μM (SEM) (N = 3) and 32.7 ± 2.0 μM (SEM) (N = 3) respectively.

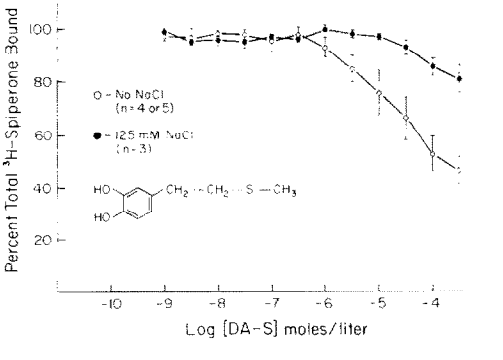


Fig. 3. Inhibition of total [$^3$H]spiperone binding by the uncharged monomethylsulfide dopamine analog (DA-S) in the presence and absence of 125 mM NaCl. Each value is the mean ± SEM.

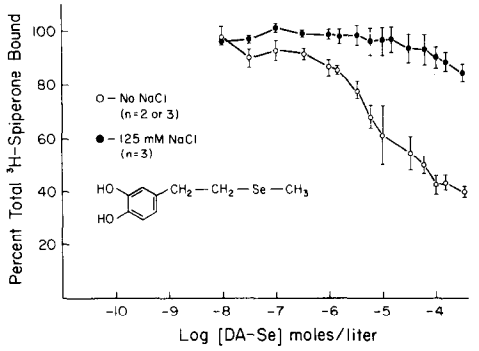


Fig. 4. Inhibition of total [³H]spiperone binding by the uncharged monomethylselenide dopamine analog (DA-Se) in the presence and absence of 125 mM NaCl. Values are means ± SEM (N = 3) or range (N = 2).

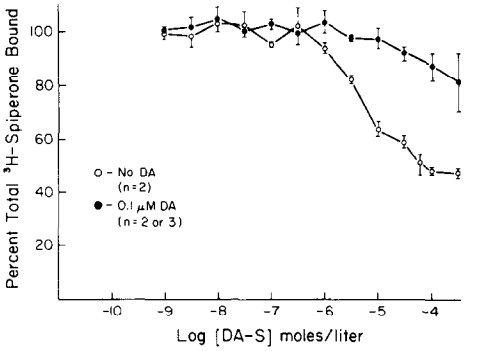


Fig. 6. Inhibition of total [³H]spiperone binding by the uncharged monomethylsulfide dopamine analog (DA-S) in the presence and absence of 0.1 μM dopamine in NaCl-free medium. Values are means ± SEM (N = 3) or range (N = 2).

The addition of 125 mM NaCl to the incubation medium almost abolished the inhibitory effects of the uncharged analogs on [³H]spiperone binding (Figs. 3–5). Thus, in the presence of NaCl, the maximum inhibition of total [³H]spiperone binding for the methylsulfide analog was only 20% at a concentration of 0.3 mM, whereas that for the methylselenide and the sulfoxide analogs was even less. These results show that the binding of the permanently uncharged analogs to the D-2 dopaminergic receptor resembles the high-affinity binding of typical dopamine agonists in that both are reduced markedly by the addition of NaCl to the incubation medium. This effect of NaCl on the binding properties of the uncharged analogs suggests that the uncharged analogs, in the absence of NaCl, may bind predominantly to the high-affinity state of the D-2 receptor.

*Effects of dopamine and quinpirole on the inhibition of* [³H]*spiperone binding produced by the permanently uncharged methylsulfide analog of dopamine.* To test further whether the permanently uncharged methylsulfide analog binds to the high-affinity state of the D-2 dopamine receptor, we determined whether the methylsulfide analog could inhibit [³H]spiperone binding in the presence of 1 μM dopamine in NaCl-free medium. This concentration of dopamine is ten times greater than the $K_i$ of dopamine for the high-affinity sites and 1/7 the $K_i$ of dopamine for the low-affinity sites and would, therefore, be expected to saturate the high-affinity sites while having minimal effect on the low-affinity sites. This prediction was confirmed in studies in which dopamine at 1 μM was found to inhibit [³H]spiperone binding by 50% which would be expected since, as shown in Fig. 1, high-affinity binding accounts for 50% of total binding. Furthermore, in the presence of 1 μM dopamine, dimethyldopamine, a drug which binds to both high- and low-affinity sites, inhibited [³H]spiperone binding, but the inhibition curve modelled best to a single class of sites with a $K_i$ of $5 \times 10^{-7}$ M which is similar to the $K_i$ of dimethyldopamine for the low-affinity site in NaCl-free medium, $7.2 \pm 1.7 \times 10^{-7}$ M. Figure 6 shows that dopamine at a concentration of 1 μM almost abolished the ability of the uncharged methylsulfide analog to inhibit [³H]spiperone binding in NaCl-free medium, suggesting that the uncharged methylsulfide analog binds predominantly to the high-affinity state of the receptor.

Additionally the effect of quinpirole, a selective D-2 dopamine receptor agonist, on the inhibition of [³H]spiperone binding produced by the permanently uncharged methylsulfide analog was determined.

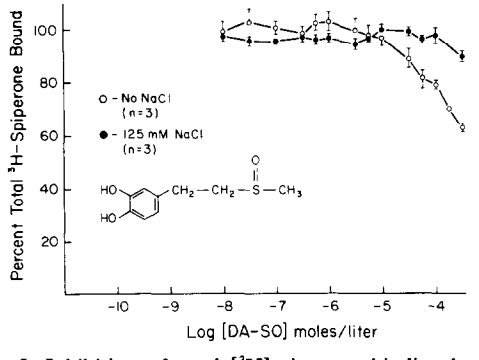


Fig. 5. Inhibition of total [³H]spiperone binding by the uncharged sulfoxide dopamine analog (DA-SO) in the presence and absence of 125 mM NaCl. Each value is the mean ± SEM.

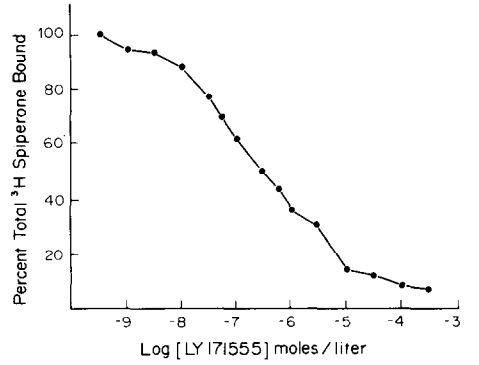


Fig. 7. Inhibition of total [³H]spiperone binding by LY 171555 (quinpirole) in NaCl-free medium.

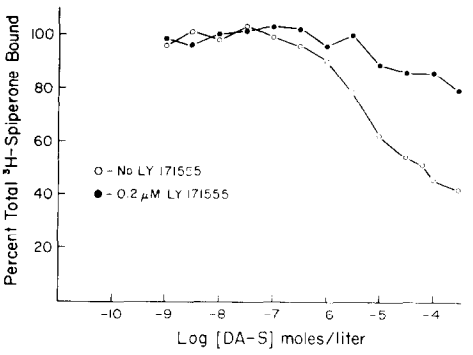


Fig. 8. Inhibition of total [$^3$H]spiperone binding by the uncharged monomethylsulfide dopamine analog in the presence and absence of 0.2 $\mu$M LY 171555 (quinpirole) in NaCl-free medium.

Similar to other typical D-2 dopamine receptor agonists, quinpirole inhibited [$^3$H]spiperone binding in NaCl-free medium in a biphasic manner (Fig. 7). The $K_i$ values for binding to the high- and low-affinity sites were 0.02 and 1.4 $\mu$M respectively. Consequently, a concentration of 0.2 $\mu$M, which is ten times higher than the $K_i$ for the high-affinity site and 1/7 the $K_i$ for the low-affinity site was used to selectively inhibit the binding of [$^3$H]spiperone to high-affinity agonist sites. In the presence of 0.2 $\mu$M quinpirole, dimethyldopamine inhibited [$^3$H]spiperone binding with a $K_i$ of $5.8 \times 10^{-7}$ M which is similar to the $K_L$ of dimethyldopamine in NaCl-free medium, $7.2 \pm 1.7 \times 10^{-7}$ M. Similar to the effect observed with 1 $\mu$M dopamine, the addition of 0.2 $\mu$M quinpirole to the medium was found to almost abolish the inhibitory effect of the uncharged sulfide analog on [$^3$H]spiperone binding in NaCl-free medium (Fig. 8), further supporting the concept that the binding of the uncharged sulfide analog in NaCl-free medium is predominantly to the high-affinity state of the D-2 dopamine receptor.

*Effect of the permanently uncharged analogs of dopamine on the potassium-evoked release of [$^3$H]acetylcholine from mouse striatal slices.* To determine whether the permanently uncharged analogs of dopamine could act as agonists on D-2 dopaminergic receptors, we determined whether these compounds could inhibit the $K^+$-evoked release of [$^3$H]acetylcholine from striatal slices. The uncharged sulfide (3 mM) and sulfoxide (1 mM) analogs of dopamine were found to inhibit significantly the $K^+$-evoked release of [$^3$H]acetylcholine by approximately 35% (Fig. 9). However, the inhibition produced by the sulfide and sulfoxide analogs was not reversed by 2 $\mu$M sulpiride, a D-2 selective antagonist (Fig. 9). In contrast, dopamine (10 $\mu$M) produced a 70% inhibition of the $K^+$-evoked release of [$^3$H]acetylcholine which was reversed completely by 2 $\mu$M sulpiride (Fig. 9). These results suggest that the inhibition of the $K^+$-evoked release of [$^3$H]acetylcholine produced by the uncharged analogs is not due to a specific action on D-2 dopamine receptors.

The ability of the uncharged methylsulfide dopamine analog to antagonize the apomorphine-induced inhibition of the $K^+$-evoked release of [$^3$H]acetylcholine was also determined. Apomorphine (0.3 $\mu$M) produced a 65% inhibition in the $K^+$-evoked release of [$^3$H]acetylcholine, an effect which was partially antagonized by the uncharged sulfide dopamine analog (1 mM) and completely antagonized by sulpiride (2 $\mu$M) (Fig. 10). Increasing the concentration of apomorphine to 100 $\mu$M resulted in an 80% inhibition of the $K^+$-evoked release of

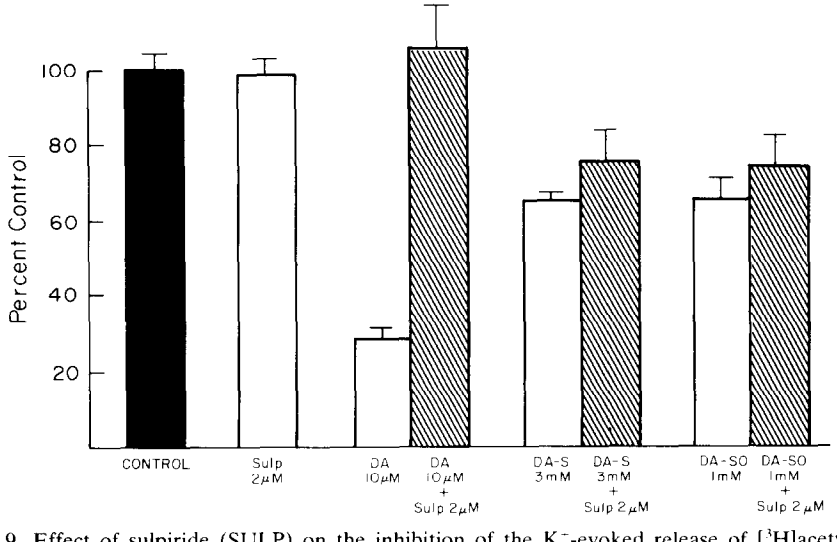


Fig. 9. Effect of sulpiride (SULP) on the inhibition of the $K^+$-evoked release of [$^3$H]acetylcholine produced by dopamine (DA), the uncharged monomethylsulfide dopamine analog (DA-S) and the sulfoxide dopamine analog (DA-SO). Differences according to Duncan's Multiple Range test, $P < 0.05$: values for DA, DA-S and DA-SO groups were significantly less than control; values for DA + Sulp group were significantly greater than DA alone; values for DA-S + Sulp and DA-SO + Sulp were not significantly different from those of the DA-S group or DA-SO group respectively. Each value is the mean ± SEM of 4–18 determinations. The $K^+$-evoked release (net fractional release × 100) of [$^3$H]acetylcholine for controls was 18.30 ± 0.76% (N = 18).

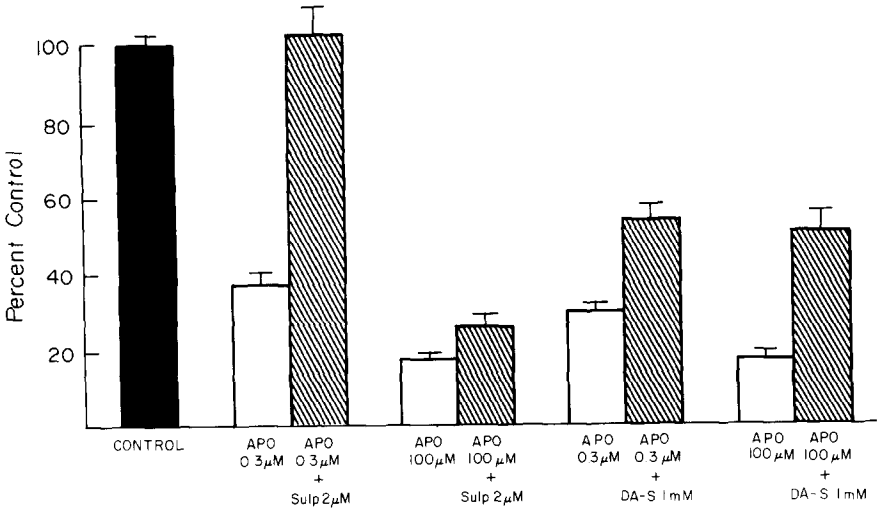


Fig. 10. Effect of increasing apomorphine (APO) concentration on the abilities of sulpiride (Sulp) and the uncharged monomethylsulfide analog of dopamine (DA-S) to antagonize the APO-induced inhibition of the $K^+$-evoked release of [$^3$H]acetylcholine. Differences according to Duncan's Multiple Range test, $P < 0.05$: values for all groups containing APO (0.3 and 100 $\mu$M) were significantly less than control; values for both APO (0.3) + Sulp and APO (100) + Sulp were significantly greater than those of APO (0.3) and APO (100), respectively; values for APO (0.3) + DA-S and APO (100) + DA-S were significantly greater than APO (0.3) and APO (100) respectively. Each value is the mean ± SEM of 4–22 determinations. The $K^+$-evoked release (net fractional release × 100) of [$^3$H]acetylcholine for controls was 17.80 ± 0.49% (N = 22).

[$^3$H]acetylcholine, but this effect was reversed only slightly by 2 $\mu$M sulpiride (Fig. 10). Thus, the antagonist action of sulpiride was reduced markedly when the concentration of apomorphine was raised to 100 $\mu$M. In contrast, there was no difference in the ability of the uncharged methylsulfide dopamine analog to antagonize the inhibitory effects of 0.3 and 100 $\mu$M apomorphine (Fig. 10), suggesting that the uncharged methylsulfide analog of dopamine is not reversibly blocking the effect of apomorphine on the D-2 dopamine receptor.

## DISCUSSION

The results of this study demonstrate that the permanently uncharged methylsulfide, methylselenide and sulfoxide analogs of dopamine were able to inhibit effectively [$^3$H]spiperone binding to D-2 dopamine receptors in rat striatal membranes in the absence of NaCl. However, the binding properties of the uncharged dopamine analogs were different from those of dopamine and dimethyldopamine. In the absence of NaCl, the permanently uncharged dopamine analogs maximally inhibited total [$^3$H]spiperone binding by 40–60%, and the inhibition curves were best described by a one-site binding model. In contrast, the maximum inhibition of total [$^3$H]spiperone binding observed with dopamine and dimethyldopamine was 90%, and the inhibition curves were best described by a two-site binding model in which the receptor exists in both high- and low-affinity states. Thus, these studies show that the uncharged dopamine analogs can bind to the D-2 site in NaCl-free medium, but that the binding is not characteristic of typical dopamine agonists.

Since the binding of the uncharged analogs is best described by a one-site binding model, these compounds may be binding predominantly to either the high- or low-affinity state of the D-2 receptor. The addition of NaCl to the incubation medium has been shown to convert high-affinity binding sites for dopamine and dimethyldopamine to low-affinity binding sites [12] (Figs 1 and 2). Consequently, we determined the effect of NaCl on the inhibition of [$^3$H]spiperone binding produced by the uncharged analogs. The inhibitory effect of all uncharged analogs on [$^3$H]spiperone binding was abolished almost totally in the presence of NaCl, suggesting that the uncharged analogs of dopamine bind primarily to the high-affinity state of the D-2 receptor. Additionally, the binding of the uncharged dopamine analogs in the presence of concentrations of dopamine or quinpirole which preferentially saturate the high-affinity site was also almost totally abolished. These observations suggest that the binding of the uncharged analogs is predominantly to the high-affinity state of the D-2 receptor.

In addition to measuring the abilities of the uncharged analogs to bind to the D-2 dopamine receptor, we also assessed the potential D-2 agonist and antagonist activities of the uncharged dopamine analogs by determining their abilities to inhibit the $K^+$-induced release of [$^3$H]acetylcholine from striatal slices and to antagonize the inhibitory activity of the dopamine agonist, apomorphine, on this system. In these studies the sulfide and sulfoxide analogs were able to produce a small, but significant, inhibition of the $K^+$-evoked release of [$^3$H]acetylcholine, but only at concentrations of 3 and 1 mM respectively. The inhibition produced by these uncharged analogs does not appear to be due to the activation of dopamine

receptors since it was not antagonized by the D-2 antagonist, sulpiride, which was shown to completely antagonize the inhibition produced by dopamine and apomorphine (Fig. 9). The uncharged dopamine analogs were also able to partially antagonize the ability of apomorphine to decrease the $K^+$-evoked release of [$^3$H]acetylcholine (Fig. 10). However, the antagonistic effects of the uncharged analogs were not reversed by increasing the concentration of apomorphine, suggesting that the abilities of the sulfide and sulfoxide analogs to antagonize the effects of apomorphine are not due to the reversible blockade of the D-2 dopamine receptor. Thus, the uncharged dopamine analogs are neither specific dopamine agonists nor dopamine antagonists of the dopamine receptor regulating the release of acetylcholine.

In summary, these studies show that permanently uncharged analogs of dopamine bound primarily to the high-affinity state of the D-2 dopamine receptor but did not possess D-2 dopamine agonist activity. In contrast, previous studies have shown that permanently charged analogs of dopamine bind to both high- and low-affinity states of the D-2 dopamine receptor and are agonists at this site [2, 13]. These results suggest that the ability to bind to the low-affinity state of the D-2 dopamine receptor may be an important determinant of agonist activity. Furthermore, the observation that binding to the low-affinity state occurred with permanently charged and not permanently uncharged analogs suggests that low-affinity binding may involve an interaction between a positively charged group on the drug molecule and an anionic site on the D-2 dopamine receptor. Therefore, the presence of a positive charge, as in the case of the permanently charged dopamine analogs, or the capacity to produce a positive charge, as in the case of dopamine or dimethyldopamine, may be a requirement for activation of the D-2 dopamine receptor and subsequent agonist activity.

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