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Dopamine D_1 and D_2 Receptors Are Sensitive to the Cationic Form of Apomorphine

PHILIP SEEMAN and H.-C. GUAN

Department of Pharmacology, University of Toronto, Toronto, Canada M5S 1A8

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SUMMARY

The protonated form of apomorphine was found to be active at the high affinity state of dopamine D_1 and D_2 receptors in canine striatum, since pH reduction from 7.4 to 6.4 enhanced the apomorphine potency 3-fold at both these sites without affecting the potency of the permanently charged apomorphine methiodide. It was estimated that the protonated form of apomorphine was approximately 20-fold more potent than the uncharged form of apomorphine at both the D_1 and the D_2 receptors.

Since most dopamine agonists are tertiary amines (1), they exist in solution in both the charged and uncharged forms. It has not yet been established which of these two forms of the agonist is active at dopamine receptors.

Molecular models for dopamine agonists interacting with dopamine receptors have generally assumed (2) that the active species is charged (i.e., protonated). However, only indirect evidence hitherto supports this assumption. First, since the pK_a for dopamine is 8.8 (at 25°) or 8.3 (37°) (3, 4), approximately 90% of the dopamine *in vivo* would be in the charged form. Second, permanently charged sulfonium and selenonium congeners of dopamine have agonist activity (5–8). However, since the absolute potencies of these congeners are much reduced in comparison to the nitrogen-containing congeners (2, 7), it remained to be established which form of dopamine agonist was more active at dopamine receptors.

The present results suggest that the charged form of apomorphine, a typical dopamine agonist, is the more active species at dopamine D_1 and D_2 receptors. This is based on [^{3}H]SCH 23390/apomorphine and [^{3}H]spiperone/apomorphine competition experiments at different pH.

Materials and Methods

Tissue preparation. The tissues were striata from canine frozen brain (Pel-Freeze Biologicals, Rogers, AR). The striata were suspended to yield 4 mg of tissue/ml of buffer (50 mM Tris-HCl, pH 7.4 at 20°, 5 mM KCl, 1.5 mM $CaCl_2$, and 4 mM $MgCl_2$). NaCl was not present in these experiments in order to retain the agonist high affinity state of the dopamine receptors (9). The suspension was homogenized with a Brinkmann Polytron (PT-10 probe) for 20 sec at setting 6 (maximum setting, 10). The homogenate was washed twice by centrifugation at 28,000 × *g*, and the final pellet was resuspended at 4 mg of tissue/ml of buffer at pH 6.4 or 7.4.

Saturation experiments using [^{3}H]SCH 23390 and [^{3}H]spiperone. The densities of dopamine D_1 or D_2 receptors were measured at different pH by saturating the tissue with increasing concentrations of [^{3}H]SCH 23390 (85 Ci/mmol; New England Nuclear, Boston, MA) or of [^{3}H]spiperone (75–98 Ci/mmol; Amersham, U.K.), as follows. Glass test tubes (12 × 75 mm) in triplicate received aliquots in the following order: 0.25 ml of buffer (pH 6.4 or 7.4 at 20°), 0.25 ml of [^{3}H]SCH 23390 or [^{3}H]spiperone (12 final concentrations ranging from 10 pM to 5000 pM for [^{3}H]SCH 23390 or from 10 pM to 2000 pM for [^{3}H]spiperone in buffer at pH 6.4 or 7.4), and 0.5 ml of tissue homogenate, bringing the final total volume to 1 ml. In order to determine nonspecific binding to the tissue, the buffer aliquot in half the tubes contained (+)-butaclamol (Research Biochemicals Inc., Wayland, MA) such that the final concentration was 1 μM. The tubes were incubated at room temperature (20°) for 90 min, at which time the samples were filtered through a glass fiber filter mat (Skatron No. 7034, Sterling, VA), using a 12-well cell harvester (Skatron, Lier, Norway) and a vacuum of 400–500 mm Hg. The filtered samples were rinsed for 20 sec with 7 ml of wash buffer (50 mM Tris-HCl, pH 7.4, for all of the experiments). The filter circles were placed in liquid scintillation mini-vials along with 4 ml of scintillation fluid (Ready-Solv, Beckman Instruments, Fullerton, CA), shaken overnight (100 shakes/min), and finally monitored for tritium in a Packard 4660 scintillation spectrometer at 55% efficiency. Specific binding was defined as that inhibited by the presence of 1 μM (+)-butaclamol. The density of [^{3}H]SCH 23390- or [^{3}H]spiperone-binding sites (B_{max}) and the dissociation constant, K_D, were obtained by Scatchard analysis (10).

Competition experiments using [^{3}H]SCH 23390 or [^{3}H]spiperone. The competition between apomorphine (Merck Frosst Laboratories, Montreal, Canada) and either [^{3}H]SCH 23390 or [^{3}H]spiperone was tested as follows. Glass test tubes (12 × 75 ml) in triplicate received the following: 0.25 ml of buffer (pH 6.4 or 7.4 at 20°), 0.5 ml of competing drug, 0.25 ml of either [^{3}H]SCH 23390 or [^{3}H]spiperone

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ABBREVIATION: SCH 23390, *R*-(+)-8-chloro-3-methyl-5-phenyl-7-ol-benzazepine.

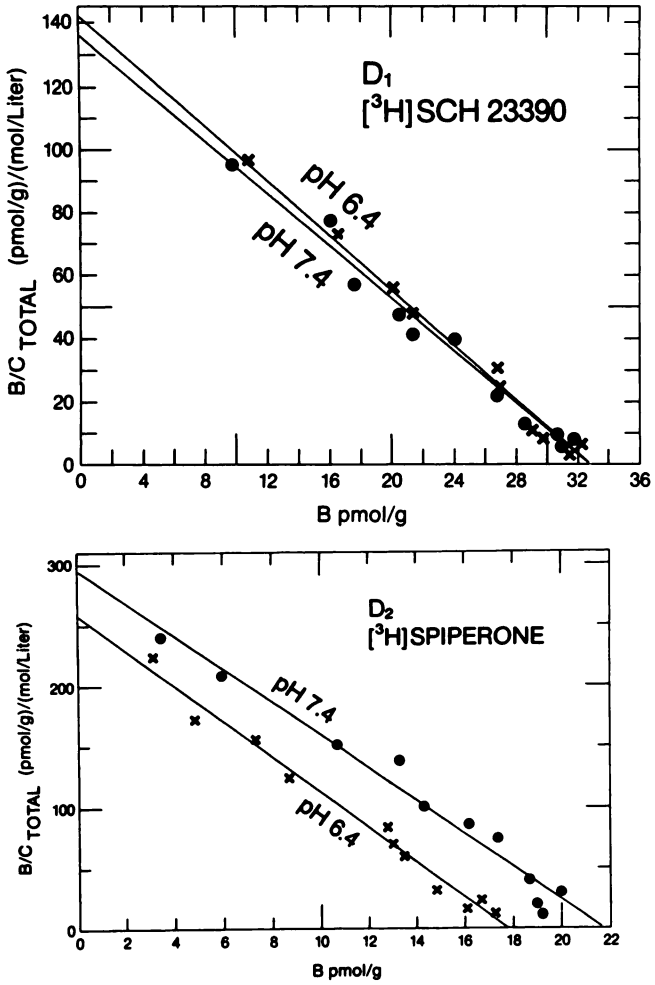

Fig. 1. *Top:* Saturation of D_1 receptors (canine brain striatal homogenate) by [^{3}H]SCH 23390 at pH 7.4 and 6.4, was analyzed using Scatchard analysis. The dissociation constant, K_D, was unaffected by pH. *Bottom:* Saturation of D_2 receptors by [^{3}H]spiperone at pH 7.4 and 6.4, as analyzed using Scatchard analysis. The dissociation constant was unaffected by pH.

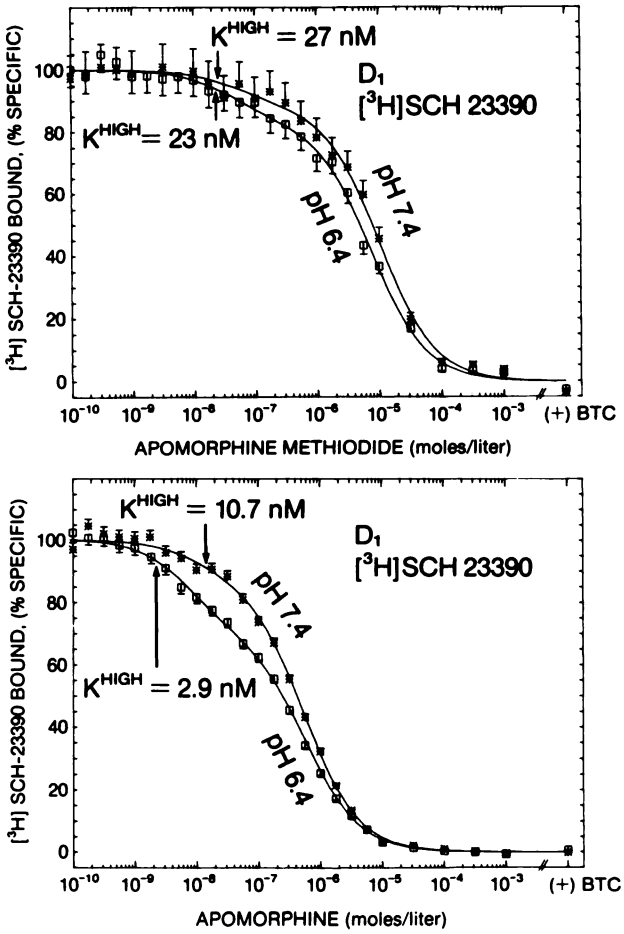

Fig. 2. D_1 receptors and pH. *Top:* The potency of the quaternary apomorphine, apomorphine methiodide, to inhibit the binding of 150 pM [^{3}H]SCH 23390 was the same (25 ± 2 nM) at pH 6.4 and 7.4. *Bottom:* Data averaged from three independent experiments for the competition between apomorphine and 150 pM [^{3}H]SCH 23390. The potency of apomorphine at D_1^{High} was enhanced 3-fold (from 10.7 nM to 2.9 nM) upon lowering the pH. The total binding of [^{3}H]SCH 23390 was approximately 2000 dpm/filter at both pH values.

(final concentration of 150 pM at pH 6.4 or 7.4), and 0.5 ml of tissue homogenate, bringing the final volume to 1.5 ml. The tubes were incubated at room temperature (20°) for 90 min and filtered and monitored for tritium as above. The competition data for specific binding were analyzed using the LIGAND program (see Ref. 2). The program provided two statistical criteria to judge whether a two-site fit was better than a one-site fit, or whether a three-site fit was better than a two-site fit. The competition between [^{3}H]SCH 23390 or [^{3}H] spiperone and apomorphine methiodide (generously donated by Professor J. L. Neumeyer, Northeastern University, Boston, MA) was also examined.

Results

The D_1 receptor. The dissociation constant, K_D, of [^{3}H] SCH 23390 at the D_1 receptor remained the same upon lowering the pH from 7.4 to 6.4, as illustrated in Fig. 1 (*top*). The K_D for [^{3}H]SCH 23390 at pH 7.4 was 241 pM, while that at pH 6.4 was 229 pM.

The competition data between [^{3}H]SCH 23390 and apomorphine methiodide were also virtually identical at pH 6.4 and 7.4, with the dissociation constant, K^{High}, for apomorphine methiodide being 25 ± 2 nM for the high affinity state of D_1 (i.e., D_1^{High}) at both pH values (Fig. 2, *top*).

For apomorphine itself, however, lowering the pH from 7.4 to 6.4 consistently enhanced the potency of this agonist at the D_1 receptor. The apomorphine K^{High} value at the D_1 receptor averaged (±SE) 10.7 ± 2 nM ($N = 3$) at pH 7.4 and 2.9 ± 0.7 nM ($N = 3$) at pH 6.4 (Fig. 2, *bottom*). At pH 7.4 approximately 20% of the apomorphine-displaceable binding of [^{3}H]SCH 23390 was at the high affinity state of D_1, whereas at pH 6.4 this value was about 40% (Fig. 2, *bottom*).

The D_2 receptor. The dissociation constant, K_D, of [^{3}H] spiperone at the D_2 receptor remained the same upon lowering the pH from 7.4 to 6.4, as shown in Fig. 1 (*bottom*). The K_D for [^{3}H]spiperone was 74 pM at pH 7.4 and 69 pM at pH 6.4.

The [^{3}H]spiperone/apomorphine methiodide competition data were also identical at pH 6.4 and 7.4, with the dissociation constant, K^{High}, for apomorphine methiodide being 101 pM for the high affinity state of D_2 (i.e., D_2^{High}) at both pH values (Fig. 3, *top*).

For apomorphine itself, however, lowering the pH from 7.4 to 6.4 consistently enhanced the potency at D_2. The apomorphine K^{High} at D_2 averaged 4.1 ± 1.5 nM ($N = 3$) at pH 7.4 and

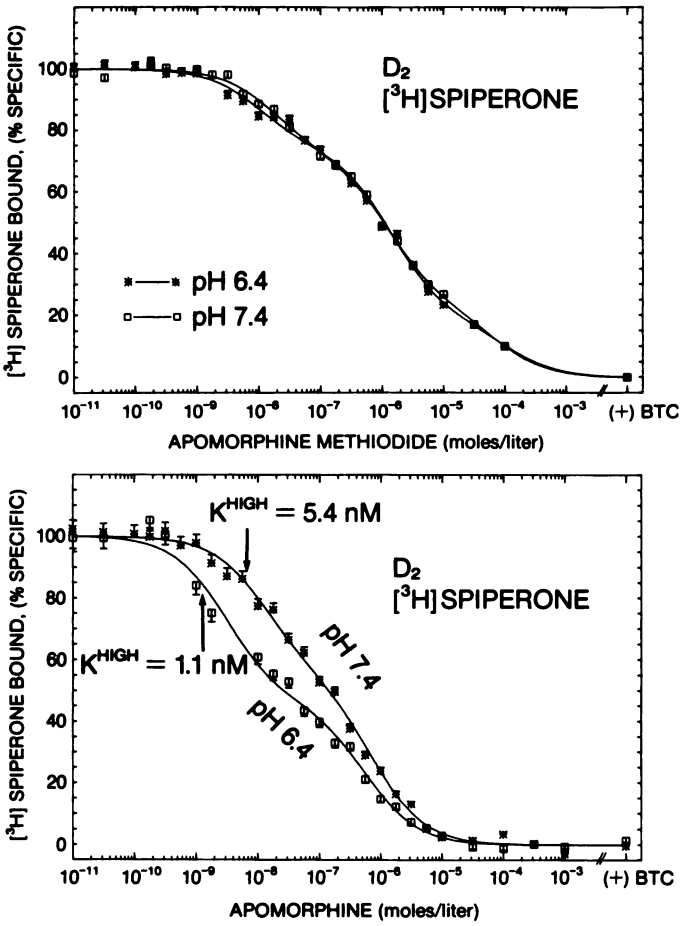

Fig. 3. D_2 receptors and pH. *Top:* The potency of apomorphine methiodide to inhibit the binding of 150 pM [^{3}H]spiperone was identical at pH 6.4 and 7.4. *Bottom:* A representative experiment for the competition between apomorphine and 150 pM [^{3}H]spiperone at two different pH values. The K^{High} was 1.1 nM (54% of the binding sites) at pH 6.4, but was 5.4 nM (45% of the binding sites) at pH 7.4. The total binding of [^{3}H]spiperone was 2000 dpm/filter at both pH values.

1.2 ± 0.3 nM ($N = 3$) at pH 6.4. The proportion of high affinity states recognized by apomorphine was 50% at both pH 6.4 and 7.4 (Fig. 3, *bottom*).

Discussion

The main finding for both the D_1 and D_2 receptors is that the apomorphine K^{High} decreased by a factor of 3 upon lowering the pH from 7.4 to 6.4, whereas there was no effect on the K^{High} value for apomorphine methiodide. Since the average pK value for apomorphine is 7.3 at 25° (3, 11), approximately 88% of the apomorphine molecules will be protonated at pH 6.4, with only about 46% protonated at pH 7.4. In other words, raising the pH from 6.4 to 7.4 lowered the concentration of charged apomorphine molecules about 2-fold, while raising the concentration of uncharged apomorphine about 4-fold. The corresponding 3-fold rise in K^{High} for apomorphine, indicating a reduced affinity of apomorphine at pH 7.4 (compared to pH 6.4), supports the idea that it is the charged species of apomorphine that is active at both dopamine receptors, particularly at the functional high affinity state (12).

It is possible to obtain a crude estimate of the relative potencies of the charged and uncharged forms of apomorphine at each of the two dopamine receptors by using a modification of an approach taken by Barlow and Winter (13). Thus, in the case of D_1, the apomorphine K^{High} was 10.7 nM at pH 7.4 (where 46% of the apomorphine molecules were protonated) and 2.9 nM at pH 6.4 (where about 88% of apomorphine was protonated), whereas the value for apomorphine methiodide was the same at both pH values (Fig. 2). Extrapolating to 0% protonated apomorphine, as was done by Barlow and Winter (13), results in a value of 19 nM for K^{High}; extrapolating to 100% protonated apomorphine yields a K^{High} value of 0.7 nM. Thus, the protonated form of apomorphine is approximately 19 nM/0.7 nM or 27-fold more potent than the uncharged form of apomorphine at the D_1 receptor.

A similar calculation for the data of apomorphine acting at the D_2 receptor indicates that the protonated form of apomorphine is approximately 19-fold more potent than the uncharged form of apomorphine.

Although dopamine D_2 receptor antagonists (neuroleptics) are also membrane active in the cationic form (14–17), similar to local anesthetics (16, 18–22), it remains to be established whether or not the protonated antagonists are the predominantly active form at dopamine receptors.

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Send reprint requests to: Dr. Philip Seeman, Department of Pharmacology, Medical Sciences Building, University of Toronto, Toronto, Canada M5S 1A8.