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ANATOMICAL AND AFFINITY STATE COMPARISONS BETWEEN DOPAMINE D_1 AND D_2 RECEPTORS IN THE RAT CENTRAL NERVOUS SYSTEM

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Abstract—The anatomical distributions and affinity states of dopamine D_1 and D_2 receptors were compared in the rat central nervous system using quantitative autoradiography. [^{3}H]SCH23390 and [^{3}H]spiperone (in the presence of 100 nM mianserin) were used to label the D_1 and D_2 receptors, respectively. The densities of D_1 and D_2 receptors displayed a positive correlation among 21 brain regions (Pearson correlation coefficient, $r = 0.80$, $P < 0.001$).

The affinity states for the D_1 and D_2 receptors were found to be quite different from each other, and different from the results obtained by others using homogenate preparations. Both the D_1 and D_2 receptors were best modeled using a two-state model. In the absence of exogenous guanine nucleotides and using the nonselective agonist dopamine as the competitor, the D_1 receptor was primarily in a low affinity agonist state ($R_H = 21 \pm 6\%$), whereas the D_2 receptor was primarily in the high affinity agonist state ($R_H = 77 \pm 3\%$). In the presence of 10 μM guanylyl-imidodiphosphate or guanosine-5′-*O*-(2-thiophosphate) both the D_1 and the D_2 receptor were completely in a low affinity agonist state ($R_L = 100\%$). These affinity states were found both in the nucleus accumbens and olfactory tubercle using dopamine as the competitor and in the striatum using selective D_1 or D_2 agonists as competitors.

Receptor occupancy of the D_2 receptor with either an agonist or antagonist did not alter the affinity states of the D_1 receptor, and conversely, receptor occupancy of the D_1 receptor did not alter the affinity states of the D_2 receptor.

The correlation between densities of D_1 and D_2 receptors provides an anatomical framework for evaluating behavioral and electrophysiological evidence of an interaction between the two dopamine receptor subtypes. This interaction does not appear to be due to a sharing or coupling of G-proteins in such a way that binding to one dopamine receptor subtype alters the affinity state of the other receptor subtype. The differences between dopamine receptor distributions described by labeled agonists and antagonists may be due in part to differences in their affinity states. The low proportion of high affinity state D_1 receptors may explain some of the difficulties in assigning specific behavioral roles to the D_1 receptor.

Dopamine (DA) receptors have been classified into two subtypes designated D_1 and D_2,[31,62] with different biochemical,[31] pharmacological,[9,17,63] anatomical,[17] and behavioral profiles.[3,4,6,23] The continued development and application of selective D_1 and D_2 agonists and antagonists have enabled a better understanding of the roles of each DA receptor subtype in the function of the CNS.[32]

Whereas the parathyroid gland contains only D_1 receptors[5,15] and the pituitary gland contains only D_2 receptors,[60] the majority of the structures in the CNS contain both types of DA receptor subtypes.[10,13,18,22,40,53–56] The existence of a generalized functional interaction between the two types of DA receptor subtypes has not been determined. Areas with both high and low densities of the receptor subtypes have been reported, but detailed quantitative comparisons under similar binding conditions have not been performed. The distributions of both D_1 and D_2 receptors have been studied using labeled selective agonists and antagonists.[2,10,13,16a,18,20a,21,40,45,58,69] Differences between the distributions and densities using an agonist and an antagonist have been described,[16a] but the reason for these differences are incompletely known. Agonist affinity states of the receptors may play a role. Regional differences in affinity states or differences in affinity states between receptor subtypes make it difficult to compare the regional distributions and densities of receptors and receptor subtypes.

Recent behavioral[3,4,6,14,23,37,41,52,57] and electrophysiological[16,70] studies have suggested that the D_1 receptor interacts with the D_2 receptor. These studies have suggested that the D_1 receptor may play a

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Abbreviations: B_{max}, maximal number of binding sites; CV 205-502, *N*,*N*-diethyl-*N*′-[(3α,4aα,10β)-1,2,3,4,4a,5,10,10a-octahydro-6-hydroxy-1-propyl-3-benzo[9]quinolinyl]sulfamide; DA, dopamine; GDP-βS, guanosine-5′-*O*-(2-thiophosphate); GmP-PnP, guanylyl-imidodiphosphate; 5-HT, 5-hydroxytryptamine (serotonin); K_D, equilibrium dissociation rate constant; K_H, high affinity rate constant; K_I, inhibitory rate constant; K_L, low affinity rate constant; LY 171555, 4,4a,5,6,7,8,8a,9-octahydro-5-*n*-propyl-2H-pyrazolo-3,4-*g*-quinoline; N-0437, (*N*-propyl-*N*-2-thienylethyl-amino)-5-hydroxytetralin; NMDA, *N*-methyl-D-aspartate; R_H, high affinity agonist state; R_L, low affinity agonist state; SCH 23390, (R)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol; SKF 38393, 2,3,4,5-tetrahydro-7,8-di-hydroxy-1-phenyl-1H-3-benzazepine; TCP, *N*-(1-[2-thienyl]cyclohexy)3,4-piperidine.

"synergistic", "permissive", or "modulatory" role in the functions of the D_2 receptor in mediating certain behaviors or the tonic single unit activity of cells in the CNS. Since all of these studies relied on the systemic administration of drugs, the sites and mechanisms of this interaction remain unknown. A comparative study between the anatomical distributions and densities of the D_1 and D_2 receptor might help to locate regions where interactions might occur and suggest an underlying mechanism for such interactions.

Analogous to β-adrenergic receptors, DA receptors, when binding an agonist, have been shown to recognize two affinity states designated R_H (high affinity) and R_L (low affinity).[22,24,26,28,74] The role of affinity states in the behavioral and biochemical functions of DA receptors remains unresolved. The possibility of an allosteric interaction between D_1 and D_2 receptors has not been demonstrated. A comparison between the affinity states of the D_1 and D_2 receptors might help to determine their contributions to different behaviors mediated by selective and nonselective drugs. We report the results of detailed quantitative comparisons of the CNS distributions and agonist affinity states of the D_1 and D_2 receptors.

EXPERIMENTAL PROCEDURES

Tissue preparation

Brains were obtained from male Sprague–Dawley rats, weighing 175–225 g, after rapid decapitation. The brains were frozen on tissue pedestals with Lipshaw embedding matrix using crushed dry ice. The brains were warmed to −20°C and coronal sections 14 or 20 μm thick were cut on a Lipshaw cryostat microtome. The 20-μm-thick sections were used for distribution studies, while the 14-μm-thick sections were used for competition studies. The sections were thaw-mounted onto gelatin coated slides and were then dehydrated on a warming plate at 30°C and stored at −20°C until used in assays.

Receptor assays

Both the D_1 and D_2 receptor assays were performed in the same buffer, consisting of 25 mM Tris–HCl (pH 7.5) containing 100 mM NaCl, 1 mM $MgCl_2$, 1 μm pargyline, and 0.001% ascorbate, as previously described.[53,55] Briefly, slides were warmed to room temperature for 1 h then incubated with tritiated ligand at room temperature for either 120 (D_2 assay) or 150 (D_1 assay) min. After the incubation, the slides were washed for 10 min in cold (4°C) buffer, dipped in distilled water for 3 sec, and dried using a stream of cool air.

The D_1 receptor was assayed using [^{3}H]SCH 23390. The details of this autoradiographic assay have been described elsewhere.[18,55] All incubations were done at a concentration of [^{3}H]SCH 23390 equal to the equilibrium dissociation rate constant (K_D) (0.57 nM) in rat striatum. Specific binding was determined by subtracting the amount bound in the presence of 1 μM *cis*-flupentixol (nonspecific binding) from the total amount bound. Specific binding represented 98–99% of the total binding in the striatum. Under these conditions, [^{3}H]SCH 23390 binds only to the D_1 receptor.[55]

The D_2 receptor was assayed using [^{3}H]spiperone (spiroperidol). The properties of this autoradiographic assay have been described elsewhere.[2,13,54–56] All incubations were done at a concentration of [^{3}H]spiperone equal to the K_D (0.25 nM) in rat striatum and included 100 nM mianserin to block the binding of [^{3}H]spiperone to the serotonin (5-HT) receptor.[2,53,55] Specific binding was determined by subtracting the amount bound in the presence of 25 μM DA (nonspecific binding) from the total amount bound. Specific binding represented 85% of total binding in the striatum. Under these conditions, dopamine does not displace the binding of [^{3}H]spiperone to other sites to which it binds.[27,56]

Competition studies were carried out using sections containing the rostral striatum, rostral nucleus accumbens and olfactory tubercle. A competition curve was derived from 24 consecutive 14-μm-thick sections, of which two sections in the middle of the series were incubated only with tritiated ligand to yield total binding, two other sections in the middle of the series were incubated with tritiated ligand plus the blank to yield nonspecific binding, and the remaining 20 sections were incubated with tritiated ligand and decreasing concentrations of competitor. Three competition curves were performed in each animal and two animals were used in each experiment. Some competition curves were derived from sections incubated in the presence of 10 μM of a nonmetabolizable guanine nucleotide. Additional competition curves were derived from sections incubated in fixed concentrations of a selective D_1 (SKF 38393) or D_2 (LY 171555, quinpirole) agonist or a selective D_1 (SCH 23390) or D_2 (sulpiride) antagonist as listed in the results. Quantitative autoradiography was used to determine the amount of binding in various brain regions for all competitions.

Data analysis

Best-fit least squares iterative curve fitting was performed using the Lundon II software package (Lundon, Inc., Cleveland, OH) to analyse all competition curves. Each curve was first analysed as a one-site fit, and subsequently analysed as a two-site fit. A two-site fit was selected only if the F-test comparing the sum of squares for errors for the two-site fit to that for the one-site fit indicated that the sum of squares for errors was significantly reduced using the more complex (two-site) model ($P < 0.05$).[36] All curves reported (having one or two sites) had an insignificant runs test ($P > 0.05$) supporting the goodness of the fit.[8,36] All curves were constrained by having the dissociation constant for the tritiated ligand remain fixed and equal to its K_D for both one- and two-site fits. The K_Ds for the [^{3}H]SCH 23390 and [^{3}H]spiperone binding to the D_1 and D_2 receptors, respectively, were determined from saturation experiments done independently and indicated binding to single sites.[55]

Autoradiography

Dried slides were placed in an x-ray cassette with ^{14}C plastic standards previously calibrated with ^{3}H brain paste sections.[48,50] The sections and standards were apposed to LKB Ultrofilm-^{3}H at 4°C for either 7–10 (D_1 receptor) or 14–21 (D_2 receptor) days. The LKB Ultrofilm-^{3}H was developed in Kodak D19 for 3 min at room temperature and fixed in Kodak rapid fix for 3.5 min.

All binding data, in units of fmol/mg protein, were determined directly from film densities in regions of interest using a video based densitometer[56] and fourth degree polynomial curve fitting to the standards. Regions of interest were outlined bilaterally with a mouse controlled cursor, sampled, and the template was stored for use on subsequent consecutive sections. Sections through the striatum were analysed for medial to lateral gradients in binding by generating receptor density versus distance histograms.

Four rats were used to study the distribution of D_1 and D_2 receptors in different regions of the CNS. Each rat had adjacent sections throughout the forebrain assayed for the density of D_1 and D_2 receptors. The same 21 regions (Table 1), at identical levels,[49] were measured in each animal for each receptor subtype. These 21 regions were selected to represent portions of the basal ganglia, cerebral cortex and subcortical areas. The areas were selected *a priori* without regard to their receptor densities. The densities of the D_1 and D_2 receptors for the four animals were averaged for each region. Because binding was determined at K_D ligand con-

Table 1. Summary of D_1 and D_2 receptor densities in the rat CNS

Region†	Receptor subtype (amount bound, fmol/mg protein*) D_1	D_2
1. Olfactory tubercle	1472 ± 36	530 ± 21
2. Med. sub. nigra reticulata	1457 ± 34	95 ± 26
3. Rostral n. accumbens	1420 ± 18	348 ± 27
4. Med. sub. nigra compacta	1354 ± 33	153 ± 33
5. Rostral striatum	1342 ± 44	562 ± 18
6. Ventral pallidum	689 ± 26	70 ± 14
7. Entopeduncular n.	600 ± 48	19 ± 14
8. Basolateral amygdaloid n.	358 ± 27	61 ± 20
9. Perirhinal cortex	288 ± 43	91 ± 53
10. Entorhinal cortex	206 ± 43	69 ± 12
11. Occipital cortex	200 ± 50	17 ± 6
12. Olfactory bulb	200 ± 50	38 ± 5
13. Globus pallidus	192 ± 33	28 ± 8
14. Sup. gray superior coll.	170 ± 27	55 ± 9
15. Lat. prefrontal cortex	92 ± 6	48 ± 4
16. Lat. sep. n. dorsal	79 ± 34	59 ± 11
17. Temporal cortex	78 ± 59	23 ± 8
18. Parietal cortex	49 ± 10	13 ± 6
19. Ventral hypothalamic n.	49 ± 24	13 ± 10
20. Central gray	46 ± 25	31 ± 10
21. Frontal cortex	41 ± 6	26 ± 15

*The values in the table are the number of receptors (fmol/mg protein) from four animals (mean ± S.E.M.). The values represent binding at a concentration equal to the K_D for each ligand as determined in the rat striatum.

†The atlas of Paxinos and Watson[49] was used to assist in determining anatomical regions. In regions of the cerebral cortex where binding is heterogeneous in different laminae, the values represent the average number of receptors in a rectangular box extending from the surface of the cortex to the underlying white matter. In regions where binding was nonlaminar, but heterogeneous (nucleus accumbens, olfactory bulb and tubercle), the entire region was averaged. Averaged values for each of the cerebral cortical regions used were used to eliminate the possibility that multiple layers would have skewed the analysis by giving excess weight to those areas.

centrations, values represent 50% of the maximal number of binding sites (B_{max}) in any region. A Pearson correlation was performed between the D_1 and D_2 receptor densities.

Rostral to caudal gradients have been reported for the D_1 and D_2 receptors in regions of the striatum, nucleus accumbens, and olfactory tubercle.[2,13,55] However, rostral to caudal gradients were not detected in these areas in the short distances examined in this study (data not shown).

Materials

[^{3}H]SCH 23390 (specific gravity 74–85 Ci/mmol) and [^{3}H]spiperone (76–95 Ci/mmol) were obtained from Amersham Corporation (Arlington Heights, IL). Mianserin was obtained from Organon (Oss, The Netherlands), dopamine from Sigma (St Louis, MO), *cis*-flupentixol from Dr John Hyttel of H. Lundbeck and Co. (Copenhagen, Denmark), SKF 38393 from Smith, Kline and French Laboratories (Philadelphia, PA), LY 171555 from Lilly Research Laboratories (Indianapolis, IN), sulpiride from Ph. Delagrange (Paris, France), SCH 23390 from Schering (Bloomfield, NJ), and guanylyl-imidodiphosphate (GmP-PnP) and guanosine-5′-*O*-(2-thiophosphate) (GDP-βS) were obtained from Boehringer–Mannheim (Indianapolis, IN).

RESULTS

Dopamine receptor distributions in the rat CNS

DA D_1 and D_2 receptors have heterogeneous distributions in the rat CNS (Table 1 and as reported by others[2,10,13,18,40]). The amount of binding varied 36-fold for the D_1 receptor and 43-fold for the D_2 receptor among the structures sampled (Table 1). Medial to lateral gradients for the D_2 receptor have been reported in the striatum,[29] but were not seen in these animals for either the D_1 or the D_2 receptors (Fig. 1).

A plot of the log transformed densities of the two receptor subtypes revealed a linear increase of one subtype with the other (Fig. 2). Pearson correlation between the two receptor subtypes was 0.80 ($P < 0.001$) for the 21 regions of Table 1.

Affinity states of dopamine receptors in striatum

The two DA receptor subtypes were found to have different affinity states in the absence of exogenous guanine nucleotides (Fig. 3; Tables 2 and 3). The D_1 receptor was primarily in the low affinity state for both the nonselective agonist DA ($R_H = 21 \pm 6\%$, $K_H = 49 \pm 24$ nM and $K_L = 2030 \pm 690$ nM) and the selective agonist SKF 38393 ($R_H = 20 \pm 14\%$, $K_H = 2 \pm 1$ nM and $K_L = 41 \pm 5$ nM). The D_2 receptor was primarily in the high affinity state for both the nonselective agonist DA ($R_H = 77 \pm 3\%$, $K_H = 43 \pm 4$ nM and $K_L = 4550 \pm 530$ nM) and the

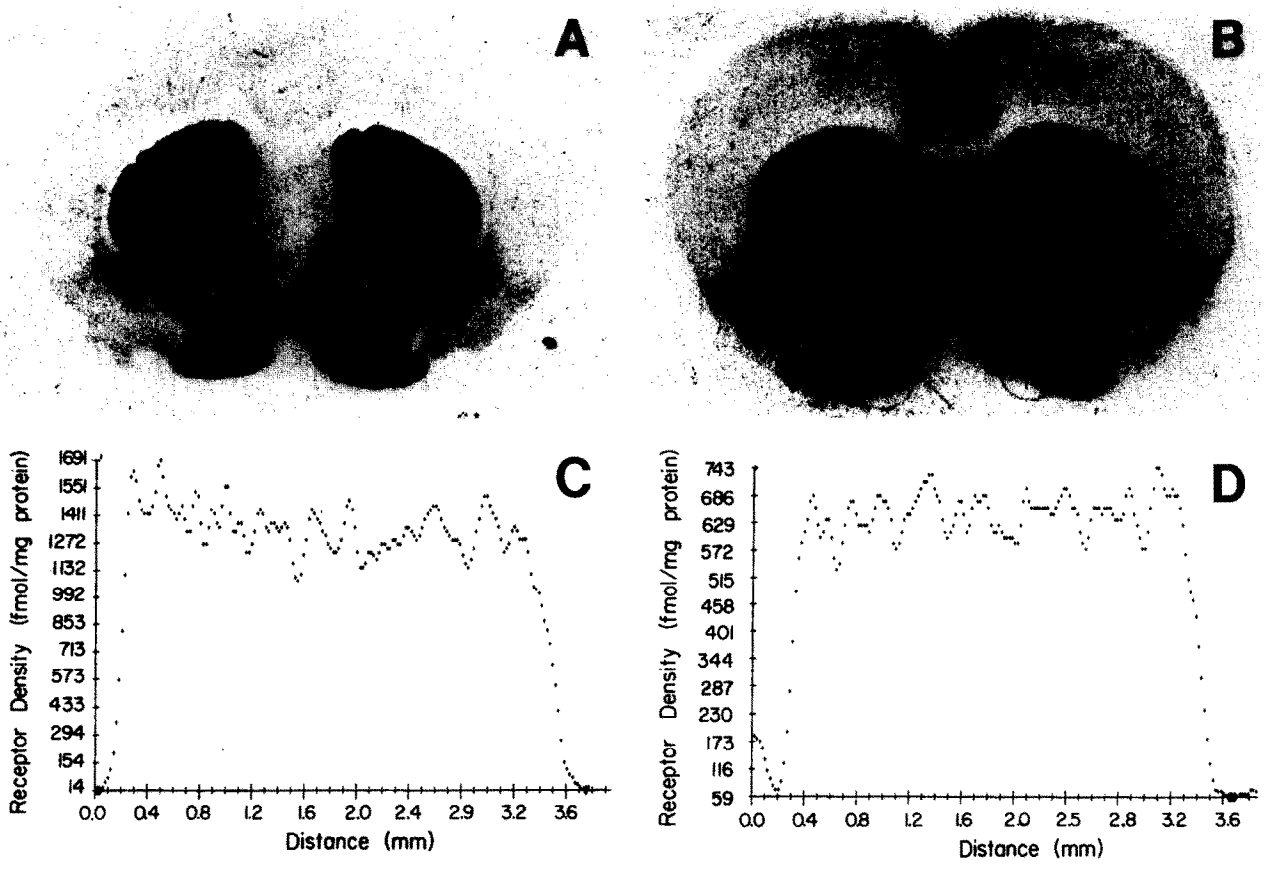

Fig. 1. Striatal dopamine receptor histograms. D_1 (A) and D_2 (B) receptor autoradiograms were used to generate receptor density versus distance histograms through the right striata for both the D_1 (C) and D_2 (D) receptors. Minor increases and decreases in receptor density can be attributed to white matter bundles coursing through the striatum and to minor random fluctuations in receptor density or ligand binding. No significant medial to lateral gradient was observed at any of the rostral levels used in this study. The LKB Ultrofilm-^{3}H was apposed to the tissue sections for different periods of time for the D_1 and D_2 receptors, so direct comparison of the amount of binding from the photographs cannot be made. Valid comparisons can be made using quantitative autoradiography and those results are presented in Table 1.

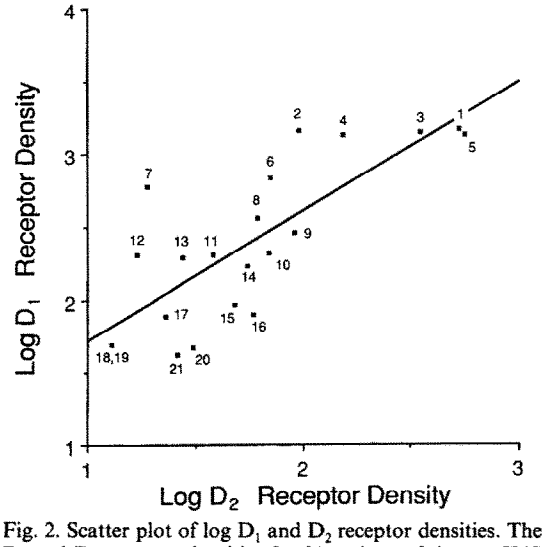

Fig. 2. Scatter plot of log D_1 and D_2 receptor densities. The D_1 and D_2 receptor densities for 21 regions of the rat CNS (Table 1) were log transformed and plotted. A Pearson correlation for these 21 regions was performed ($r = 0.80$, $P < 0.001$).

selective agonist LY 171555 ($R_H = 90 \pm 1\%$, $K_H = 63 \pm 18$ nM and $K_L = 3330 \pm 750$ nM).

In the presence of 10 μM GmP-PnP, both D_1 and D_2 receptors were entirely converted to a low affinity state ($R_L = 100\%$) for both nonselective and selective agonists (Table 2). The dissociation constant (K_L) determined in the presence of exogenous guanine nucleotide was higher than the dissociation constant (K_L) determined in the absence of guanine nucleotides from the two-site model for both receptor subtypes using both selective and nonselective agonists (Table 2). The guanine nucleotide GDP-βS (10 μM) produced an affinity state shift similar to that of 10 μM GmP-PnP for both the D_1 and D_2 receptors. In the presence of 10 μM GDP-βS, using DA as the competitor, the K_L was 8580 nM and R_L was 100% for the D_1 receptor, and the K_L was 17100 nM and the R_L was 100% for the D_2 receptor. Neither 10 μM GDP-βS nor 10 μM GmP-PnP had an effect on the amount of tritiated antagonist bound to the D_1 or D_2 receptor.

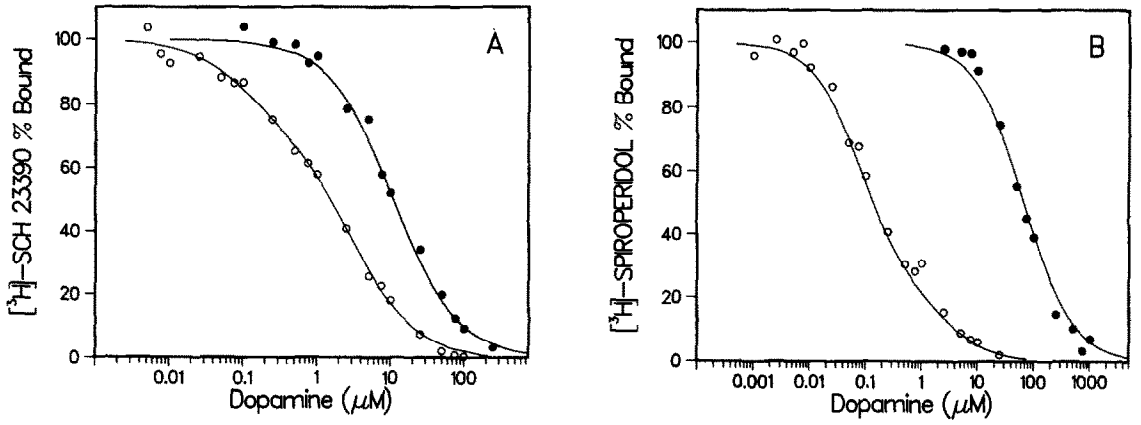

Fig. 3. Dopamine competition curves for the D_1 and D_2 receptors in striatum. Competition curves for the D_1 (A) and D_2 (B) receptors were performed in the striatum using DA as the competitor. Competitions were performed in the absence (open circles) or presence of 10 μM GmP-PnP (filled circles). The data for the two curves for each receptor subtype are from the same experiment. Values plotted are actual values, while the line plotted is from the best fit computer generated estimates for that set of data. Values for the D_1 receptor (A) in the absence of exogenous guanine nucleotide (open circles) were $K_H = 59$ nM, $K_L = 1570$ nM, $R_H = 27.7\%$, $R_L = 72.3\%$; values for the D_1 receptor in the presence of 10 μM GmP-PnP (filled circles) were $K_L = 5760$ nM, $R_L = 100\%$. Values for the D_2 receptor (B) in the absence of exogenous guanine nucleotide (open circles) were $K_H = 40$ nM, $K_L = 1350$ nM, $R_H = 79\%$, $R_L = 21\%$; values for the D_2 receptor in the presence of 10 μM GmP-PnP (filled circles) were $K_L = 25{,}500$ nM, $R_L = 100\%$.

Affinity states of dopamine receptors in other regions

The affinity states of the D_1 and D_2 receptors were also analysed in the nucleus accumbens and olfactory tubercle. Only competition curves in which the regions were present in all sections were used and only competition curves for DA were analysed. The affinity states for the D_1 receptor in both the nucleus accumbens and the olfactory tubercle were similar to those seen in the striatum (Table 3). Affinity states for the D_2 receptor in nucleus accumbens were also similar to those seen in the striatum (Table 3). The olfactory tubercle demonstrated binding that was too heterogeneous for the D_2 receptor to produce satisfactory competition curves for autoradiographic analysis.

Affinity state interactions

To determine if there was an allosteric interaction between the DA receptor subtypes that would alter binding characteristics, the affinity state of one DA receptor subtype was examined while the other DA receptor subtype was occupied by an agonist or antagonist. DA competitions for the D_1 receptor were performed in the presence of 1 μM sulpiride (to block the binding of DA to the D_2 receptor). This concentration of sulpiride did not alter the binding of

Table 2. Affinity states of dopamine D_1 and D_2 receptors in rat striatum*

	No nucleotide				10 μM GmP-PnP	
Drug	K_H (nM)†	K_L (nM)	R_H (%)‡	R_L (%)	K_L (nM)	R_L (%)
D_1 Receptor						
Dopamine	49 ± 20	2030 ± 690	21 ± 6	79 ± 6	9550 ± 5360	100
SKF 38393	2 ± 1	41 ± 5	20 ± 14	80 ± 14	89 ± 49	100
D_2 Receptor						
Dopamine	43 ± 4	4550 ± 930	77 ± 3	23 ± 3	28,500 ± 4270	100
LY 171555	63 ± 18	3330 ± 750	90 ± 1	10 ± 1	31,900 ± 310	100

*DA receptor autoradiography was performed as described in the text, using both tritiated antagonists ([³H]SCH 23390 and [³H]spiperone) at a concentration equal to their respective K_Ds. Competition curves were done in either the absence or presence of 10 μM GmP-PnP. Both antagonists displayed Hill slopes equal to one from saturation studies done previously (Richfield *et al.*[35,55]). Computer modeled parameters were constrained with the dissociation rate constants of the receptor for the tritiated antagonist fixed to the K_D of each ligand. For two-site fits, both K_H and K_L for the tritiated ligand were constrained to the same value. All curves were fit using nonlinear, least-squares computer assisted modeling as described in the text.

†Values are in nM ± S.D. for 2–3 independent experiments.

‡Values are the percentage of the total number of receptors ± S.D. for 2–3 independent experiments. In the absence of exogenous guanine nucleotide, all curves were best modeled as a two-site fit, whereas in the presence of 10 μM GmP-PnP all curves were best modeled as a one-site fit.

Table 3. Affinity states of dopamine D_1 and D_2 receptors in rat nucleus accumbens and olfactory tubercle*

Region†	K_H (nM)‡	K_L (nM)	R_H (%)§	R_L (%)
D_1 receptor				
Nucleus accumbens	22 ± 18	1570 ± 320	17 ± 2	73 ± 2
Olfactory tubercle	9 ± 4	2040 ± 700	23 ± 1	77 ± 1
D_2 receptor				
Nucleus accumbens	52 ± 31	1610 ± 1480	66 ± 19	34 ± 19

*Dopamine receptor autoradiography was performed as described in the text, using both tritiated antagonists ([^{3}H]SCH 23390 and [^{3}H]spiperone) at a concentration equal to their K_D. Competition curves were done in the absence of exogenous guanine nucleotide. Computer modeled parameters were constrained with K_H and K_L equal to the K_D for the tritiated ligand. All curves were fit using nonlinear least-squares computer assisted modeling as described in the text. All curves in the absence of exogenous guanine nucleotide were best modeled using a two-site model.

†The regions of nucleus accumbens and olfactory tubercle were selected from sections previously analysed in the striatum. Both the nucleus accumbens and olfactory tubercle had heterogeneous binding for both D_1 and D_2 receptors. The heterogeneous D_1 receptor binding in nucleus accumbens and olfactory tubercle, as well as the heterogeneous D_2 binding in the nucleus accumbens was slight, and the entire region was averaged as a single value. The heterogeneous binding produced curves with more scatter to the data, producing greater variability in the parameters estimated. The D_2 binding in olfactory tubercle was too heterogeneous to produce satisfactory curves for analysis.

‡Values are in nM ± S.D. for 2–3 independent experiments.

§Values are the percentage of the total number of receptors ± S.D. for 2–3 independent experiments.

[^{3}H]SCH 23390 to the D_1 receptor (inhibitory rate constant, $K_i > 1000\ \mu$M) and blocked most of the binding of DA to the D_2 receptor. No change in the D_1 receptor affinity state was seen. The selective D_1 agonist SKF 38393 was used as a competitor at the D_1 receptor in the presence of the selective D_2 agonist LY 171555 (10 μM). This concentration of LY 171555 did not alter binding of [^{3}H]SCH 23390 to the D_1 receptor ($K_i > 100\ \mu$M) and was adequate to block most of the D_2 receptors ($K_H = 63$ nM and $K_L = 3330$ nM for the D_2 receptor). Under these conditions, no change in the affinity state of the D_1 receptor was seen. A similar set of experiments was performed for the D_2 receptor in which DA competitions were done in the presence of 100 nM SCH 23390 ($K_i = 0.57$ nM for the D_1 receptor, with no change in the amount of D_2 receptor binding assessed using [^{3}H]-spiperone) and LY 171555 competitions were done in the presence of 10 μM SKF 38393 ($K_H = 2$ nM and $K_L = 41$ nM for the D_1 receptor and no change in the amount of D_2 receptor binding assessed using [^{3}H]spiperone). Similar to the results obtained for the D_1 receptor, no change in the affinity state of the D_2 receptor was seen in either case.

DISCUSSION

Anatomical distribution

Although the distribution of DA receptors in the rat has been reported for the D_1,[18,58] D_2,[10,30,40,45,66] or both D_1 and D_2 receptors,[13,22] no studies have specifically examined the quantitative relationship between the distributions of the two receptors. In the present study, assay conditions were similar for both receptor subtypes. The identical incubation buffer was used, both ligands were used at a concentration equal to their respective K_D, in order to label the same proportion of receptors (50% of the total number of receptors), and tritiated antagonists were used to avoid differences in affinity states recognized by agonists. Under these conditions a positive correlation was seen between the densities of the D_1 and D_2 receptors.

Attempts were made to avoid bias in the correlation performed. The same animals and adjacent tissue sections were used. Regions were selected *a priori* to include a variety of areas with previously known dopaminergic innervation, as well as areas with unverified dopaminergic innervation. The areas included had densities that spanned the range from highest densities of receptors seen in portions of the basal ganglia to areas with barely detectable levels of receptors in some regions of the cerebral cortex. Nevertheless, there are sources of potential bias that might affect the correlation seen. Some areas with heterogeneous binding were averaged to yield a single value. Although averaged values of D_1 and D_2 receptors might correlate well, smaller areas within a region might not have, thus introducing a bias that artifactually increased the correlation. In addition, some regions might not have been included that would have reduced the correlation seen. However,

no regions were eliminated from the analysis, or ignored from selection, based on density values.

The Pearson correlation seen in this study (0.80) can be compared to the correlations seen between other binding sites in the rat CNS. For example, the correlation between *N*-(1-[2-thienyl]cyclohexy)3,4-piperidine (TCP) and *N*-methyl-D-aspartate (NMDA) binding is 0.94.[38] The correlation between benzodiazepine and GABA-stimulated benzodiazepine binding is 0.88.[68a] While the correlation between the NMDA and quisqualate subtypes of glutamate receptors is similar to that seen in this study (0.76), the correlations of other glutamate subtypes are low; NMDA and kainate, 0.07, quisqualate and kainate, 0.11.[75] Furthermore, the correlation of the GABA receptor subtypes $GABA_A$ and $GABA_B$ is only 0.39 (Pearson correlation calculated from the data of Bowery *et al.*[11]). Correlations between unrelated GABA and glutamate receptor subtypes were also low (0.2, data alculated from unpublished observations of D.C.M. Chu and W. F. Maragos). The intermediate correlation seen between DA D_1 and D_2 receptors in this study suggests that a relationship may exist between the receptor subtypes, but the stoichiometry and nature of the relationship are unclear and will require other types of investigations for clarification.

Despite the regional correlation seen between D_1 and D_2 receptors, there are some regions where there is a marked discrepancy in the proportion of the two receptor subtypes, including the substantia nigra pars reticulata, endopeduncular nucleus, and the globus pallidus. The neuronal location of DA receptors in these regions differs.[43,59,71] The substantia nigra pars reticulata and entopeduncular nucleus are also unique in their metabolic responses to D_1 and D_2 agonists following 6-hydroxydopamine lesions to the substantia nigra.[67,68] These two regions had the largest deviations from the linear relationship seen between the log transformed D_1 and D_2 receptors' densities in this study. If the Pearson correlation is repeated without including these two regions, the correlation between D_1 and D_2 receptors improves to 0.90. This supports the notion that these two regions have dopaminergic characteristics that may differ from those seen in other regions of the CNS.

Autoradiographic studies using a radiolabeled agonist[16a,20a,22,58,69] are likely to underestimate the total number of DA D_1 and D_2 receptors because they bind primarily to high affinity sites at the concentration used. Guanine nucleotide insensitive D_2 agonists (CV 205-502) exist and appear to label a single homogeneous site.[16a] These noncatechol D_2 agonists label a different proportion of D_2 sites compared to other D_2 agonists (N-0437).[69] Surprisingly, the guanine nucleotide-insensitive agonist (CV-205-502) labeled only about one-quarter the number of sites that the guanine nucleotide-sensitive agonist (N-0437) labeled.[69] If the regional percentage of high and low affinity sites is similar throughout the brain, as it was for the three regions examined in this report, then the distributions described by an agonist will be approximately similar to those described with an antagonist. However, if the affinity state of a receptor changes from region to region, or under experimental or pathological conditions, then distributions described by an agonist will reflect this. This suggests that both the total number of receptors and their affinity states need to be considered in determining the regional distributions and densities of dopamine receptors.

Determination of receptor densities using agonists will also give an incorrect comparison between the densities of D_1 and D_2 receptors because the proportions of high affinity sites are quite different between them. For example, the density of D_2 receptors is reported to be greater than that of the D_1 receptors in rat striatum, when tritiated agonists are used.[22] However, when antagonists are used D_1 receptors are in considerable excess of D_2 receptors in the rat striatum.[13,55]

One question regarding the cellular location of D_1 and D_2 receptors is whether they are both located on the same neuron in a given region. Striatal neurons were hypothesized to contain both D_1 and D_2 receptors based on adenylate cyclase studies.[62] Electrophysiological evidence has also suggested that some neurons of the cat caudate nucleus may possess both functional D_1 and D_2 receptors.[46] In view of the regional correlation between DA receptor subtypes, the location of D_1 and D_2 receptors on the same neurons in one region might suggest a common mechanism of interaction in other selective regions. The reason for two receptors binding the same endogenous neurotransmitter (with nearly the same dissociation constants), but having opposing actions on adenylate cyclase remains unclear however. Recent studies continue to suggest that more than one direct biochemical effect might be ascribed to the D_1 or D_2 receptor.[12,34,42,51,61]

The recent description of behavioral[6,23,39,41,57,68] and electrophysiological[16,70,72] studies demonstrating a synergistic effect of D_1 receptor activation on D_2 receptor actions suggests that the DA receptor subtypes may have a common mode of interaction. A variety of different behaviors (including stereotypy, turning behavior, catalespy, and reserpine induced akinesia) have been found to be potentiated when DA D_1 and D_2 receptors were simultaneously stimulated.[6,23,39,41,57,70] Whether the regions mediating different behaviors contain both D_1 and D_2 receptors or receive projections from areas containing different proportions of D_1 or D_2 receptors is not known. In view of the widespread distributions of both D_1 and D_2 receptors, determining the region(s) responsible for a particular behavior may be difficult.

Affinity state comparisons

In this study, D_1 and D_2 receptors differed in the proportions of high and low affinity receptors. The D_2 receptor was primarily in the high affinity state

(R_H between 77 and 90%), whereas the D_1 receptor was primarily in the low affinity state (R_H between 17 and 23%). This difference was seen using both selective and nonselective agonists as competitors, and in different regions of the CNS including striatum and nucleus accumbens. These regions share characteristics that may not be representative of other parts of the CNS, including their high levels of DA, D_1 and D_2 receptors. However, the innervation of these regions is from both the substantia nigra pars compacta (nigrostriatal pathway) and the ventral tegmental area (mesoallocortical pathway), which together supply most of the innervation to the remaining telencephalon. Whether these affinity state proportions are true in the other regions of the CNS will require further study. In view of the regional correlation between D_1 and D_2 receptors, it would be noteworthy if this relationship was maintained throughout the CNS. It would also be interesting to determine if the characteristics of the presynaptic D_1 heteroreceptors in the entopeduncular nucleus and substantia nigra pars reticulata were similar.

The D_1 and D_2 receptors also shared a number of affinity state characteristics in this study. Agonist binding to both D_1 and D_2 receptors was clearly biphasic in the absence of guanine nucleotide, and monophasic in the presence of either GDP-βS or GmP-PnP. In the absence of exogenous guanine nucleotide, both receptors had similar high affinities for DA (K_H) that were near 40 nM (range 9–74 nM) and low affinities for DA (K_L) that were between 2 and 4 μM. The similarities in dissociation rate constants for DA at the two DA receptor subtypes suggest that neither receptor will predominate in its binding of endogenous DA. If both receptors are found in the same dendritic area of a neuron, both receptors are likely to bind released DA.

Although the affinities of the two DA receptor subtypes were found to be similar for DA the magnitude of the cellular response depends on the number of receptors, as well as their affinity states. The D_1 receptor accounts for an average of 78% of the total number of DA receptors in most regions (Table 1), but only 20% of the receptors may be in the high affinity state. Conversely, the D_2 receptor accounts for the remaining 22% of the total number of D_2 receptors, but 80–90% of the receptors may be in the high affinity state. There is evidence to suggest that a portion of the D_1 receptors in the rat striatum and substantia nigra may be spare receptors.[1] How spare DA receptors contribute to the proportion of high and low affinity states in these regions is not known. However, if both D_1 and D_2 receptors are located on the same neurons in any region, and if these affinity state proportions exist *in vivo*, these proportions will have a major influence on the cellular effects of DA.

The affinity states for the D_1 and D_2 receptors obtained from this autoradiographic study differ from those typically seen using homogenate preparations.[20,24–26,34,44,60] Many of the characteristics relating to affinity states seem to be influenced by the method used to measure them, including the dissociation constants (K_H and K_L), the proportion of high and low affinity sites (R_H and R_L), and the ability of guanine nucleotides to produce a complete shift in the affinity state.

For the D_1 receptor studied using tissue homogenates, the dissociation constants (K_H and K_L) for DA (1–197 and 740–3971) and SKF 38393 (3–8 and 114–600) were quite variable between different studies.[26,44] Likewise for the D_2 receptor, the dissociation constants determined for DA (K_H varied from 16 to 460 nM, and K_L varied from 2 to 67 μM) by different investigators were quite different.[20,25,34,60]

Of greater interest is the difference in the proportion of high (R_H) and low (R_L) affinity states determined using tissue sections compared to those determined using tissue homogenates. The proportion of high affinity state D_2 receptors in striatum was found to be much higher in tissue sections ($R_H = 77$–90%) than in homogenate preparations, where the values of R_H ranged from 28 to 56%.[20,24,71] The high percentage of R_H seen for the D_2 receptor in tissue sections was confirmed by obtaining similar values using both selective and nonselective DA agonists and in two different regions of the brain the striatum and the nucleus accumbens. This finding was originally ascribed to improved preservation of membrane properties or membrane proteins.[53] It was, therefore, unexpected that the D_1 receptor would have a proportion of high affinity sites ($R_H = 20$%) much lower than that seen for the D_2 receptor. This percentage was also confirmed by the use of both selective and nonselective agonists and by examining three different regions in the rat brain. This low proportion of high affinity state receptors for the D_1 receptor in tissue sections was also lower than that seen in homogenate studies. Values of R_H for the D_1 receptor in striatum using tissue homogenates have been reported to average 40%.[26,44] The reason for observing a low proportion of R_H for the D_2 receptor when using tissue homogenates might be due to the processes used in preparing tissues for homogenate studies. However, the reason for the observed lower proportion of R_H for the D_1 receptor in tissue sections is not clear, since identical methods and buffer were used for both the D_1 and D_2 receptors in this study. Perhaps homogenization is capable of uncoupling G-proteins from their intended receptor and allows them to interact with another receptor type, thus increasing the proportion of D_1 receptors in the high affinity agonist state determined using homogenate preparations. The role of the relative differences in affinity state proportions in terms of biochemical and behavioral functions remains a complex function.[73]

Despite the differences in R_H for the D_1 and D_2 receptors, both could be shifted entirely to a one site low affinity state with the use of exogenous guanine nucleotide. This property also differs from that seen

in homogenate studies, where a complete shift to a single low affinity state was frequently not possible for the D_1[26,44] nor for the D_2[19,24,28,74] receptors. This may indicate that some uncoupling or alteration of G-proteins occurs during homogenization.

Affinity states have been examined using homogenate preparations in different regions of the bovine brain for D_2 receptors.[19,35] Biphasic curves were seen in caudate nucleus, putamen, olfactory tubercle, and globus pallidus in the absence of exogeneous guanine nucleotide. Complete shifts to a single site were seen in the caudate nucleus in one report,[19] but not another,[35] and in the other regions examined. A maximal shift was seen only with 100 μM guanine nucleotide,[35] which is 10 times higher than that required with tissue sections in this study.

Taken together, this information suggests that the agonist binding properties of receptors in tissue sections are quite different from those in homogenate preparations. The variability in tissue preparation techniques may account for the differences between the various homogenate studies. The differences between tissue sections and homogenates may reflect differences in the preservation or coupling of integral membrane components including G-proteins. Whether the properties seen using tissue sections are a better reflection of the properties of DA receptors *in vivo* has not yet been demonstrated.

Relationship of affinity states to adenylate cyclase activity

The relationship of the affinity states for the D_1 and D_2 receptors in tissue sections is difficult to relate to the findings from studies on adenylate cyclase activity due to DA receptor binding. The D_1 receptor has been shown to produce very strong stimulation of adenylate cyclase in striatum.[62] For a long time the D_2 receptor was thought not to be linked to adenylate cyclase due to the difficulty in demonstrating DA inhibition in the striatum.[17] Subsequently, reports have demonstrated inhibition of adenylate cyclase in rat striatum due to D_2 receptor activation.[7,47,64] The magnitude of the changes in adenylate cyclase due to D_2 activation[62] and in the nucleus accumbens a lack of inhibition of adenylate cyclase has been reported,[7,33,64] despite the presence of substantial numbers of D_2 receptors, their high proportion of high affinity states, and their regulation by guanine nucleotides. Perhaps the central nervous system D_2 receptor is also linked to G_O (guanine nucleotide-binding protein not linked to adenylate cyclase), which might have an intracellular effect different from inhibition of adenylate cyclase via G_I (inhibitory guanine nucleotide-binding protein).[22,34,42,51,61,65]

While the D_1 receptor has been recently shown to have synergistic behavioral and electrophysiological actions on the D_2 receptor,[6,23,39,41,57,68] behaviors mediated solely by the D_1 receptor have been difficult to find.[52] The reason for this difficulty is not clear, but perhaps the low proportion of high affinity sites (R_H) seen in tissue sections contributes to this. The *in vivo* activity mediated by D_1 receptors may be small in comparison to that mediated by the D_2 receptor, making the separation of behaviors mediated by the D_1 receptor subtype difficult.

CONCLUSION

The present study has demonstrated a quantitative relationship between the regional distributions of DA D_1 and D_2 receptors. The significance of this correlation will depend on the cellular location and mechanism of interaction between D_1 and D_2 receptors. The proportion of high affinity sites was quite different between the two receptor subtypes, although other affinity state characteristics were similar. These results may have implications for understanding the role of the DA system in mediating different cerebral functions in normal and pathological conditions.

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