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# THE DOPAMINE RECEPTOR: DIFFERENTIAL BINDING OF d-LSD AND RELATED AGENTS TO AGONIST AND ANTAGONIST STATES

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Dopamine receptor binding in calf striatal membranes of $^{3}$H-dopamine and $^{3}$H-haloperidol appears to differentiate agonist and antagonist states of the receptor. Agonists and antagonists have selective affinities for dopamine and haloperidol sites respectively. In evaluating relative affinities for dopamine and haloperidol binding sites, we have observed that d-LSD interacts with considerable affinity at the dopamine receptor. Its similar competition for binding of the two tritiated ligands suggests that it is a mixed agonist-antagonist, which is consistent with its interactions with the dopamine-sensitive adenylate cyclase. The effects of LSD on dopamine receptor binding are stereospecific, with d-LSD being 1,000 times more potent than l-LSD. 2-Bromo-LSD has more of an antagonist profile than d-LSD for the dopamine receptor. In binding experiments methiothepin behaves like a potent and relatively pure antagonist at dopamine receptors.

Behavioral studies indicate that d-lysergic acid diethylamide (LSD) can stimulate dopamine receptors (1-3). In biochemical studies, in vitro, LSD stereospecifically enhances the activity of a dopamine-sensitive adenylate cyclase (4-6). Since LSD also blocks the dopamine stimulation of the cyclase, it behaves like a mixed agonist-antagonist in this system. While apomorphine is a potent stimulant of the dopamine-sensitive adenylate cyclase, it fails to produce the maximal augmentation elicited by dopamine so that apomorphine may not be as "pure" an agonist as dopamine (7,8). Factors accounting for the profiles of drugs as relatively pure agonists, antagonists or mixed agonist-antagonists at dopamine receptors have heretofore been unclear.

$^{3}$H-Dopamine appears to label selectively the agonist state of the dopamine receptor while $^{3}$H-haloperidol interacts selectively with the antagonist state (9). Thus dopamine and other agonists have about 30 times more affinity for $^{3}$H-dopamine than $^{3}$H-haloperidol binding sites while the reverse occurs with relatively pure dopamine antagonists such as the phenothiazines and butyrophenones. If $^{3}$H-dopamine and $^{3}$H-haloperidol do indeed label agonist and antagonist states of the dopamine receptor respectively, then drugs with similar affinities for the two sites should tend to be mixed agonist-antagonists while drugs with much higher affinity for dopamine sites would be agonists and drugs with a selectively high affinity for haloperidol sites would be relatively pure antagonists. In the present study we have evaluated the influence of LSD and related agents on specific dopamine receptor binding.

## Methods

Methods were essentially as described previously (9, 11). Caudate nuclei of fresh or frozen calf brains were homogenized in 30-40 volumes of ice cold 50 mM Tris-HCl buffer, pH 7.7 at 25° C, with a Brinkmann Polytron PT-10. The homogenate was centrifuged twice at 50,000 g for 10 min, with rehomogenization of the intermediate pellet in fresh buffer. The final pellet was homogenized in 90 volumes of cold 50 mM Tris buffer, pH 7.6 at 25° C, containing 0.1% ascorbic acid, 10 μM pargyline, and ions as follows: 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$. The suspension was warmed to 37° C for 5 min and returned to ice.

[Ethyl-1-$^3$H]dopamine, 8.4 Ci/mmol, was purchased from New England Nuclear. $^3$H-Haloperidol, 0.86 Ci/mmol, was custom tritiated by New England Nuclear and purified by thin layer chromatography. On the day of use the two radioactive drugs were diluted in 0.1% ascorbic acid to concentrations of 100 nM and 40 nM respectively.

Each incubation tube received 100 μl of diluted $^3$H-dopamine or $^3$H-haloperidol, 100 μl of various concentrations of non-radioactive drugs dissolved in 0.1% ascorbic acid, and 1.8 ml of caudate suspension. The tubes, prepared in triplicate, were incubated at 37° C for 10 min and rapidly filtered under vacuum through Whatman GF/B filters with two 5 ml rinses of cold buffer. The filters were counted in liquid scintillation counters (Packard or Nuclear Chicago) in 12 ml of Hydromix (Yorktown Research) at efficiencies of 38-45%.

Saturable or specific binding of $^3$H-dopamine was measured as the excess over blanks taken in the presence of 1 μM dopamine or 10 μM (+)-butaclamol. Blanks for $^3$H-haloperidol binding contained 100 μM dopamine or 0.1 μM (+)-butaclamol. Approximately 50% of the total $^3$H-dopamine binding was specific while about 40% of the $^3$H-haloperidol binding was specific by these criteria.

The sources of drugs were as follows: dopamine, Sigma Chem. Co.; apomorphine, Merck; (+)- and (-)-butaclamol, Ayerst Labs.; serotonin, Regis Chem. Co.; fluphenazine, E. R. Squibb and Sons; haloperidol, McNeil Labs; d- and l-LSD and psilocin, the NIMH-FDA Committee on Scheduled Substances; Br-LSD, Sandoz; cyproheptadine, Smith, Klein and French; methiothepin, Roche.

## Results and Discussion

As we previously reported (9), dopamine is about 30 times more potent in competing for $^3$H-dopamine binding sites ($K_i$ 20 nM) than for $^3$H-haloperidol binding sites ($K_i$ = 600 nM)(Table 1). In dramatic contrast haloperidol is about 400 times more potent in displacing $^3$H-haloperidol than $^3$H-dopamine binding with respective $K_i$ values of 2.0 and 800 nM. Because the regional variations and relative drug specificities of both the binding of $^3$H-dopamine and $^3$H-haloperidol are very similar (9), we feel that both dopamine and haloperidol bind to the dopamine receptor. The most likely explanation for the marked difference in agonist and antagonist potencies for the two binding sites is that $^3$H-dopamine labels an agonist state of the dopamine receptor while $^3$H-haloperidol labels an antagonist state of the receptor. These conclusions are consistent with similar data obtained with binding to other neurotransmitter receptors in the brain (13). Thus glycine and strychnine may bind to agonist and antagonist states respectively of the glycine receptor (14, 15). An extensive body of evidence supports differential labeling of agonist and antagonist states of the opiate receptor (16-18). The muscarinic cholinergic receptor in the brain can be differentially labeled by agonists and antagonists (19, 20). $^3$H-Serotonin appears to label selectively the agonist state of the postsynaptic serotonin receptor while $^3$H-LSD labels both agonist and antagonist states of the serotonin receptor (21-23).

TABLE 1

Inhibition of $^3$H-Dopamine and $^3$H-Haloperidol Binding by LSD and Related Agents

| Drug | $K_i$, nM $^3$H-Dopamine | $^3$H-Haloperidol | "Relative Agonist Index" |
|---|---|---|---|
| Dopamine | 20 | 600 | 300 |
| Apomorphine | 8 | 60 | 75 |
| d-LSD | 29 | 20 | 6.9 |
| l-LSD | 50,000 | 20,000 | - |
| 2-Br-LSD | 53 | 4 | 0.76 |
| Serotonin | 14,000 | 30,000 | 21.4 |
| Psilocin | 17,000 | 13,000 | 7.6 |
| Methiothepin | 180 | 1.4 | 0.08 |
| Methysergide | 320 | 45 | 1.4 |
| Cyproheptadine | 980 | 65 | 0.67 |
| Fluphenazine | 160 | 1.3 | 0.08 |
| Haloperidol | 800 | 2 | 0.025 |

For each drug listed competition for binding of both ligands was measured at 3 or more concentrations of drug and the concentration which inhibited binding by 50%, the $IC_{50}$, was derived by log-probit analysis. Binding of $^3$H-dopamine was independently determined (by Scatchard plots) to have a $K_D$ of about 20 nM while that of $^3$H-haloperidol was about 2 nM. These values were used to convert $IC_{50}$ values to apparent $K_i$'s (inhibition constants) according to the equation $K_i = IC_{50}/(1 + C/K_D)$, where C is the concentration of radioactive ligand (5 nM for $^3$H-dopamine and 2 nM for $^3$H-haloperidol). Each value listed is the mean of 3 or more independent determinations which varied by less than 30%. The "relative agonist index" was calculated as the following ratio of ratios: the $K_i$ of the drug on haloperidol binding over the $K_i$ of haloperidol on haloperidol binding (2 nM) divided by the $K_i$ of the drug on dopamine binding over the $K_i$ of dopamine on dopamine binding (20 nM).

In studies of the dopamine-sensitive adenylate cyclase, apomorphine does not elicit as great a maximal augmentation of cyclase activity as does dopamine (7,8) and thus does not seem as "pure" an agonist as dopamine. Interestingly, while dopamine has 30 times greater affinity for $^3$H-dopamine than $^3$H-haloperidol sites, apomorphine displays only about a 7 fold greater affinity for dopamine than haloperidol binding (Table 1). As an index of the relative agonist and antagonist propensities of drugs in their interaction with the dopamine receptor, we computed the following ratio: the relative affinity of a drug compared to dopamine for the agonist sites divided by the relative affinity of the drug compared to haloperidol for the antagonist sites. Higher ratios of the "relative agonist" index are thus associated with "purer" agonists and lower numbers with "purer" antagonists. Mixed agonist-antagonists should have intermediate values. Dopamine, presumably the most pure agonist, has an index of 300 while the pure antagonist haloperidol has an index of 0.025. Apomorphine has the profile of an agonist but one less "pure" than dopamine, with an index of 75.

Previously we observed that phenothiazines have a relatively greater affinity than butyrophenones for dopamine binding sites than haloperidol binding sites (9). This has been confirmed in the present study for fluphenazine, a potent phenothiazine, which has a relative agonist index of 0.08, according to which it might not be as "pure" an antagonist as haloperidol.

Does the "relative agonist" index have any predictive value biochemically and behaviorally? d-LSD has similar affinities for $^3$H-dopamine and $^3$H-haloperidol binding sites and a relative agonist index of 6.9 suggesting that it should behave as a mixed agonist-antagonist. This is consistent with results from studies of the dopamine-sensitive adenylate cyclase, in which d-LSD can both enhance cyclase activity and antagonize the cyclase stimulation caused by dopamine (4,5). The interactions of LSD with the dopamine receptor are highly stereospecific with the d-isomer displaying about 1,000 times greater affinity than the l-isomer for both $^3$H-dopamine and $^3$H-haloperidol binding sites. This is consistent with the stereospecificity of the actions of LSD on the dopamine-sensitive adenylate cyclase.

2-Bromo-LSD has about 5 times greater affinity than d-LSD for $^3$H-haloperidol sites but slightly over half the affinity of d-LSD for $^3$H-dopamine sites resulting in a "relative agonist" index of 0.76. Thus, we would predict it to behave more like an antagonist than d-LSD. In confirmation of this prediction Von Hungen and co-workers observed that while LSD both stimulates and blocks the dopamine sensitive adenylate cyclase, 2-bromo-LSD can antagonize the actions of dopamine but cannot itself stimulate the cyclase (4,5). Behavioral studies show d-LSD to be a directly acting dopaminergic agonist. In unilaterally nigro-striatal lesioned rats d-LSD causes contralateral rotation (2,3) whereas 2-bromo-LSD does not (2).

The actions of LSD on dopamine receptor binding do not appear to correlate with the psychedelic actions of this drug. Thus 2-bromo-LSD, which is very weak or inactive in its psychedelic effects, is still about as potent as LSD at the dopamine receptor, although the differences discussed above may prove to be a relevant determinant. The relative activity of these two drugs at presumed postsynaptic serotonin sites is also similar to that for dopamine sites (21). However the potent psychedelic agent psilocin has negligible affinity for dopamine receptor binding.

Methysergide, which differs from LSD in the substitution of a methyl group at C-1, is only about half as potent as d-LSD in competing for $^3$H-haloperidol binding and 1/10 as potent as d-LSD in competing for $^3$H-dopamine binding resulting in a "relative agonist" index of 1.4. We would predict it to be a mixed agonist-antagonist but substantially weaker in interacting with dopamine receptors than d-LSD. In behavioral studies methysergide has been reported to potentiate apomorphine-induced stereotyped behaviors (24, 25).

Cyproheptadine, a classical serotonin antagonist, can block the dopamine-sensitive adenylate cyclase at high doses (4, 5). It has only 1/3 and 1/30 the affinity of d-LSD for $^3$H-haloperidol and $^3$H-dopamine binding sites respectively with a "relative agonist" index of .67 consistent with a dopamine antagonist activity in these high doses. Its potency on $^3$H-haloperidol binding is about the same as that of the very weak neuroleptic agent promazine (9) and it has not been found to be behaviorally active as a dopamine agonist (26). By contrast to the relatively weak activity of cyproheptadine, our data support pharmacologic evidence that methiothepin is a potent dopamine antagonist (26). Its affinity for $^3$H-haloperidol sites ($K_i$ = 1.4 nM) is similar to that of haloperidol itself and it is about 130 times more potent in competing for $^3$H-haloperidol than $^3$H-dopamine binding sites resulting in a "relative agonist" index of 0.08.

In summary, the specific binding of $^3$H-dopamine and $^3$H-haloperidol to calf striatal membranes provides a simple, sensitive and specific assay for the dopamine receptor. Predictions that differential affinities of drugs for dopamine and haloperidol binding sites will characterize pure agonists, pure antagonists, and mixed agonists-antagonists have been confirmed in evaluations of LSD and related agents.

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REFERENCES

1. A.K. DIXON, Experientia 24, 743-747 (1968).
2. L. PIERI, M. PIERI, and W. HAEFELY, Nature 252, 586-588 (1974).
3. K. FUXE, L.F. AGNATI, H. CORRODI, B.J. EVERITT, T. HOKFELT, A. LOFSTROM, and U. UNGERSTEDT, Advances in Neurology, Volume 9, edited by D.B. Calne, T.N. Chase and A. Barbeau, pp. 223-242, Raven Press, New York (1975).
4. K. VON HUNGEN, S. ROBERTS, and D.F. HILL, Nature 252, 588-589 (1974).
5. K. VON HUNGEN, S. ROBERTS, and D.F. HILL, Brain Res. 94, 57-66 (1975).
6. M. DA PRADA, A. SANER, W.P. BURKARD, G. BARTOLINI, and A. PLETSCHER, Brain Res. 94, 67-73 (1975).
7. J.W. KEBABIAN, G.L. PETZOLD, and P. GREENGARD, Proc. Natl. Acad. Sci., USA, 69, 2145-2149 (1972).
8. R. MILLER, A. HORN, L. IVERSEN, and R. PINDER, Nature 250, 238-241 (1974).
9. I. CREESE, D.R. BURT, and S.H. SNYDER, Life Sci. 17, 993-1002 (1975).
10. D.R. BURT, S.J. ENNA, I. CREESE, and S.H. SNYDER, Neurosciences Abstracts 1, 404 (1975).
11. D.R. BURT, S.J. ENNA, I. CREESE, and S.H. SNYDER, Proc. Natl. Acad. Sci., USA, in press (1975).
12. P. SEEMAN, M. WONG, and J. TEDESCO, Neurosciences Abstracts 1, 405 (1975).
13. S.H. SNYDER, Biochem. Pharmac. 24, 1371-1374 (1975).
14. A.B. YOUNG, and S.H. SNYDER, Proc. Natl. Acad. Sci., USA 71, 4002-4005 (1974).
15. A.B. YOUNG, and S.H. SNYDER, Mol. Pharmacol. 10, 790-809 (1974).
16. C.B. PERT, and S.H. SNYDER, Mol. Pharmacol. 10, 868-879 (1974).
17. G.W. PASTERNAK, and S.H. SNYDER, Nature 253, 563-565 (1975).
18. S.H. SNYDER, Nature 257, 185-189 (1975).
19. N.J. BIRDSALL, A.S.V. BURGEN, and E.C. HULME, J. Supramol Struct., in press (1975).
20. S.H. SNYDER, K.J. CHANG, M.J. KUHAR, and H.I. YAMAMURA, Fed. Proc. 34, 1915-1921 (1975).
21. J.P. BENNETT, and S.H. SNYDER, Brain Res. 94, 523-544 (1975).
22. S.H. SNYDER, and J.P. BENNETT, Pre- and Postsynaptic Receptors, edited by E. Usdin and W.E. Bunney, Jr., pp. 191-206, Marcel Dekker, New York (1975).
23. J.L. BENNETT, and G.K. AGHAJANIAN, Life Sci. 15, 1935-1944 (1974).
24. W.J. WEINER, C. GOETZ, and H.L. KLAWANS, Acta Pharmacol et Toxicol. 36, 155-160 (1975).
25. J. ROTROSEN, B.M. ANGRIST, M.B. WALLACH, and S. GERSHON, Eur. J. Pharmacol. 20, 133-135.
26. B. COSTALL, and R.J. NAYLOR, Eur. J. Pharmacol. 29, 206-222, (1974).