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# PRELIMINARY COMMUNICATION

## CONVERSION OF DOPAMINE $D_1$ RECEPTORS FROM HIGH TO LOW AFFINITY FOR DOPAMINE

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This study indicates that dopamine $D_1$ receptors convert from a state having high affinity for dopamine to one having low affinity for dopamine. The dopamine $D_1$ receptor stimulates adenylate cyclase [1] and both of these sites are 50% occupied or stimulated by 1 to 10 micromolar dopamine [see Refs. in 2]. Since sodium ions and guanine nucleotides help convert other receptors from their high-affinity state for agonists to their low-affinity state [3-7], we tested whether these factors could also have a similar converting effect on $D_1$ dopamine receptors.

The $D_1$ receptor is sensitive to nanomolar concentrations of cis-flupenthixol [8-10] and of the benzazepine SCH 23390 [or R-(+)-8-chloro-3-methyl-5-phenyl-7-ol-benzazepine; Refs. 11,12]. Although [$^3$H]-cis-flupenthixol labels $D_1$ and $D_2$ dopamine receptors [8-10], the $D_1$ receptor can be selectively labeled by [$^3$H]-cis-flupenthixol in the presence of 10 nM spiperone which occludes the $D_2$ receptors [13]. The $D_1$ receptor can also be selectively labeled by [$^3$H]-SCH 23390, since SCH 23390 is much more selective at $D_1$ receptors than at $D_2$ receptors [11,12].

Homogenates of rat brain striatum (fresh) or calf caudate nucleus (frozen at -70°) were used (see Refs. 14,15 for methods). The buffer contained 50 mM Tris-HCl (pH 7.4 at 20°), 5 mM KCl, 4 mM $MgCl_2$, 1.5 mM $CaCl_2$, 1 mM EDTA acid, 12 μM nialamide and 0.1% ascorbic acid; when added, NaCl was 120 mM. The final concentrations of [$^3$H]-cis-flupenthixol (10.8 Ci/mmole; New England Nuclear, Boston, MA) and [$^3$H]-N-propylnorapomorphine (61.5 Ci/mmole; New England Nuclear) were between 0.25 and 0.54 nM, while that for [$^3$H]-SCH 23390 (3.7 Ci/mmole; Nuclear Research Center, Beer-Sheva) was 1.4 nM. The final concentration of tissue was 0.8 mg original tissue per ml of incubation medium; for [$^3$H]-SCH 23390, however, it was 2.5 mg original tissue/ml. The final total volume was 1.5 ml. The suspensions were incubated for 2 h at 20° (at which time equilibrium had occurred) and then filtered using a Titertek cell harvester [15]. Three experiments (in triplicate) were done for each variable (see Ref. 15 for further details).

Figure 1 illustrates that SCH 23390 inhibited the binding of [$^3$H]-cis-flupenthixol at $D_1$ and $D_2$ dopamine receptors. In the presence of 10 nM spiperone, which served to occlude the $D_2$ receptors, SCH 23390 inhibited the binding of [$^3$H]-cis-flupenthixol from a single population of binding sites, the $D_1$ dopamine receptors.

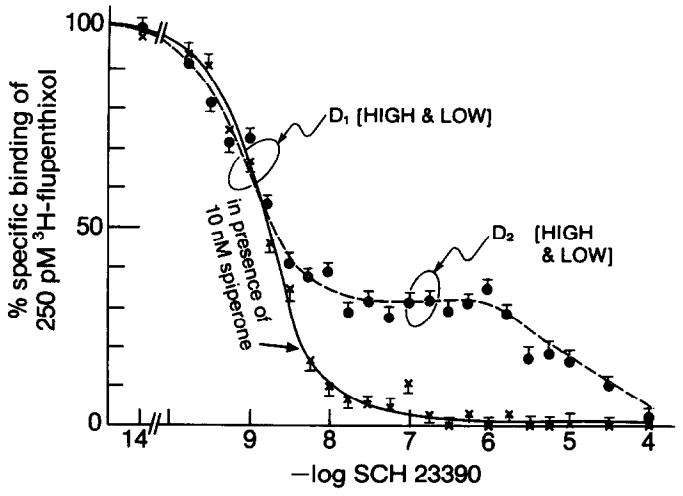


Fig. 1. Inhibition of [$^3$H]-cis-flupenthixol binding by SCH 23390 at $D_1$ and $D_2$ receptors (rat striatum) in absence and presence of 10 nM spiperone (to occlude $D_2$ receptors). NaCl absent. Specific binding was defined as that inhibited by $10^{-6}$M (+)-butaclamol. Total binding was 1200 bound cpm per tube.

Figure 2 illustrates that dopamine recognized the binding of [$^3$H]-cis-flupenthixol to $D_1$ receptors having two subpopulations of $D_1$ receptors, one of which having high affinity ($D_1^{high}$) for dopamine, the other having low affinity ($D_1^{low}$) for dopamine. These experiments were done in the presence of 10 nM spiperone to preclude the attachment of [$^3$H]-cis-flupenthixol to $D_2$ dopamine receptors. The $D_1^{high}$ and $D_1^{low}$ components appeared clearly separate only in the absence of NaCl. In the presence of 120 mM NaCl the high-affinity phase ($D_1^{high}$) was obliterated (Figure 2), all the [$^3$H]-cis-flupenthixol binding now taking place at $D_1^{low}$, indicating that all the $D_1$ receptors had converted into the low-affinity state for dopamine.

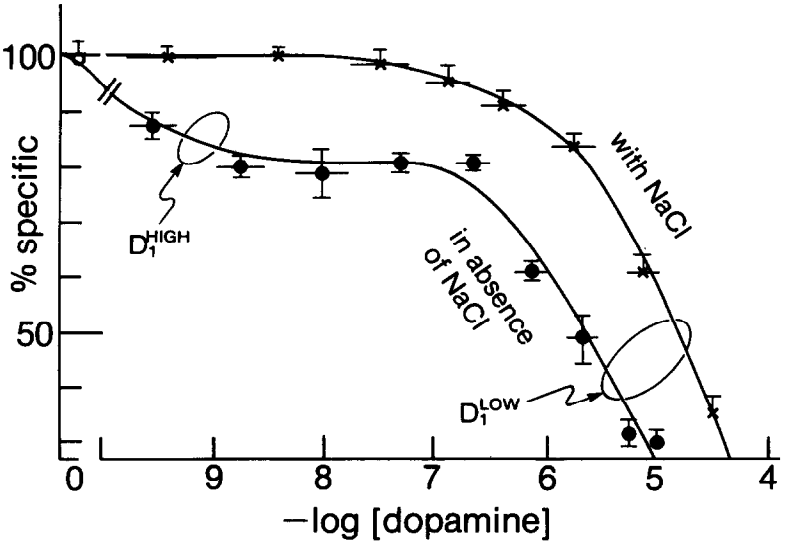


Fig. 2. Competition between [$^3$H]-cis-flupenthixol and dopamine at $D_1$ receptors (rat striatum) in the presence of 10 nM spiperone. Total binding of 0.3 nM [$^3$H]-cis-flupenthixol was 1800 cpm/tube in absence of NaCl, and 2000 cpm/tube in presence of 120 mM NaCl. Specific binding defined by 1 μM (+)-butaclamol.

The conversion of $D_1^{high}$ into $D_1^{low}$ was also detected using [$^3$H]-SCH 23390, as shown in Figure 3. Dopamine inhibited the binding of [$^3$H]-SCH 23390 at $D_1^{high}$ with a dissociation constant ($K_D$) for dopamine of 1.2 nM, and inhibited at $D_1^{low}$ with a dopamine $K_D$ of 740 nM, using computer-assisted analysis [7]. [$^3$H]-SCH 23390 was more selective than [$^3$H]-cis-flupenthixol in labeling $D_1$ receptors, since [$^3$H]-SCH 23390 had an extremely low affinity for $D_2$ receptors (to be published). Figure 3 illustrates that the combination of 120 mM NaCl and 100 μM guanine nucleotide (guanylimidodiphosphate; Gpp[NH]p; Sigma Chemical Co., St. Louis, MO) almost completely converted the $D_1^{high}$ sites into $D_1^{low}$ sites at 20°. Since the conversion of $D_1^{high}$ into $D_1^{low}$ (Figs. 2 and 3) is almost identical in principle to that which occurs for $D_2$ receptors in the brain [7] and in the anterior pituitary gland [16], it is likely that complete conversion would occur at 37°. NaCl (120 mM) alone converted about 15% of the $D_1^{high}$ sites into $D_1^{low}$ sites (data not shown). The density ($B_{max}$) of [$^3$H]-SCH 23390 sites (obtained by Scatchard analysis) was identical in the absence and presence of NaCl.

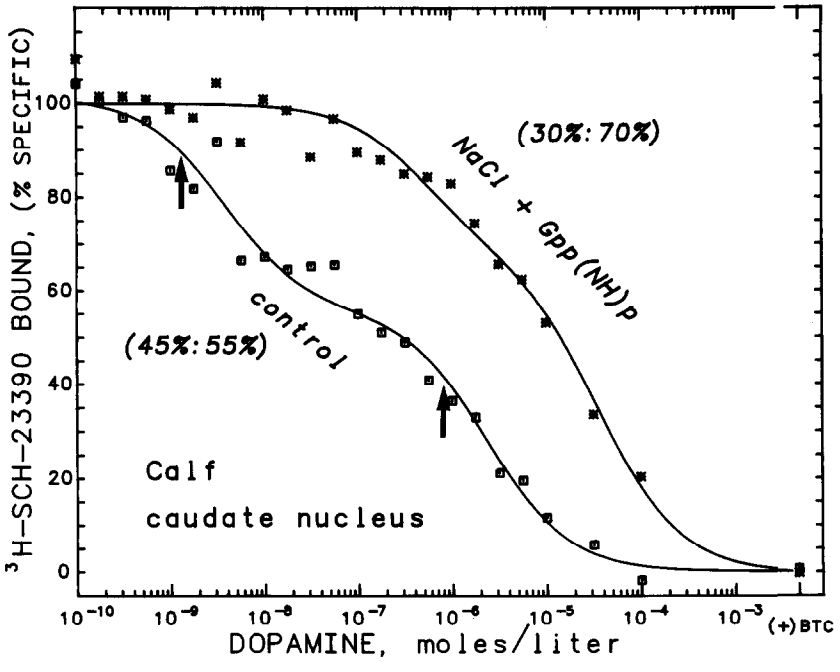


Fig. 3. Competition between [$^3$H]-SCH 23390 and dopamine at $D_1$ receptors (calf caudate nucleus) in absence and presence of 120 mM NaCl and 100 μM Gpp[NH]p. Total binding was 650 dpm/tube at 1.4 nM [$^3$H]-SCH 23390. 20°. Specific binding defined by 1 μM (+)-butaclamol (BTC). Arrows indicate dopamine $K_D$ values, using [$^3$H]-SCH 23390 $K_D$ values of 650 pM (without NaCl) and 250 pM (with NaCl) which had been independently obtained from a saturation isotherm (Scatchard). Numbers in brackets are (% $D_1^{high}$; % $D_1^{low}$).

It was also possible to demonstrate the disappearance of $D_1^{high}$ by using [$^3$H]-N-propylnorapomorphine to label the high-affinity sites of both $D_1$ and $D_2$. As shown in Figure 4, approximately 20% of the total binding of 0.54 nM [$^3$H]-N-propylnorapomorphine was to $D_1^{high}$, as recognized and displaced by SCH 23390 in the absence of NaCl. In the presence of 120 mM NaCl, however, these $D_1^{high}$ sites were completely absent, only $D_2^{high}$ sites remaining.

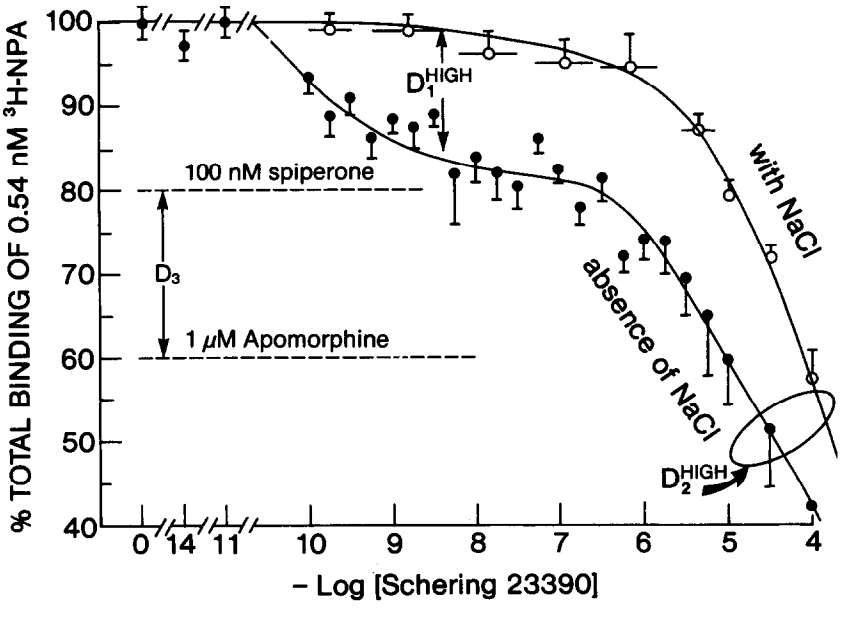


Fig. 4. Competition between [$^3$H]-N-propylnorapomorphine and SCH-23390 at the high-affinity states of $D_1$ and $D_2$ receptors ($D_1^{high}$ and $D_2^{high}$) in the absence and presence of 120 mM NaCl (rat striatum). The total binding of 0.54 nM [$^3$H]-NPA was 3500 dpm/tube (no NaCl) and 1750 dpm/tube (with 120 mM NaCl). The magnitude of the binding to the "$D_3$" site [13,17] is given by the difference in binding in the presence of excess spiperone and excess apomorphine, as shown by the dashed lines.

The value of 20% for the $D_1^{high}$ sites in Fig. 4 is identical to the magnitude of the so-called "$D_3$" site, originally defined [13,17] as a dopaminergic site with nanomolar agonist affinity and micromolar antagonist affinity. These data, therefore, support the proposal

[10] that the $D_3$ site is the same entity as $D_1^{high}$. Thus, recognizing that $D_3$ is $D_1^{high}$, and that "$D_4$" is $D_2^{high}$ [7], there are now only two types of dopamine receptors in the nervous system, $D_1$ and $D_2$, each able to convert its high-affinity state into a low-affinity state for dopamine. This is illustrated in Fig. 5. As discussed previously [18], it is possible that $D_1$ is like the vascular $DA_1$ dopamine receptor [19], and that $D_2$ is similar to the vascular $DA_2$ dopamine receptor [19], but this has not yet been experimentally confirmed.

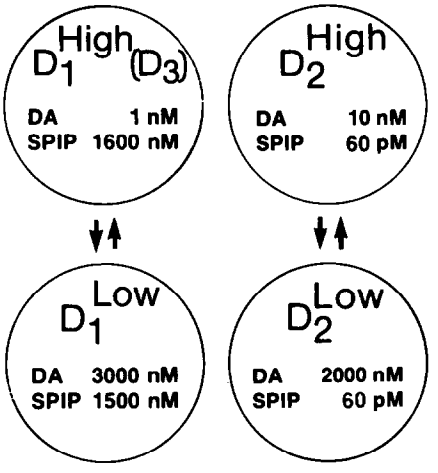


Fig. 5. Summary of dissociation constants and nomenclature for the brain $D_1$ and $D_2$ dopamine receptors. $D_1^{high}$ was formerly "$D_3$", and $D_2^{high}$ was formerly "$D_4$" [Ref. 7]. The $K_D$ values are for dopamine (DA) and spiperone (SPIP). This scheme is in agreement with that of Leff and Creese [10].

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## References

1. J.W. Kebabian and D.B. Calne, Nature, Lond. 277, 93 (1979).
2. P. Seeman, Pharmacol. Rev. 32, 229 (1980).
3. M. Rodbell, Nature, Lond. 284, 17 (1980).
4. T. Michel, B.B. Hoffman and R.J. Lefkowitz, Nature, Lond. 288, 709 (1980).
5. L.E. Limbird, J.L. Speck and S.K. Smith, Molec. Pharmac. 21, 609 (1982).
6. M. Hamblin and I. Creese, Life Sci. 30, 1587 (1982).
7. D. Grigoriadis and P. Seeman, Can. J. neurol. Sci. 11, 108 (1984).
8. J. Hyttel, Prog. Neuropsychopharmac. 2, 329 (1978).
9. A.J. Cross and F. Owen, Eur. J. Pharmac. 65, 341 (1980).
10. S.E. Leff and I. Creese, Trends pharmac. Sci. 4, 463 (1983).
11. L.C. Iorio, A. Barnett, F.H. Leitz, V.P. Houser and C.A. Korduba, J. Pharmac. exp. Ther. 226, 462 (1983).
12. J. Hyttel, Eur. J. Pharmac. 91, 153 (1983).
13. S.J. List, K.A. Wreggett and P. Seeman, J. Neurosci. 2, 895 (1982).
14. P. Seeman, C. Ulpian, K.A. Wreggett and J.W. Wells, J. Neurochem. 43, 221 (1984).
15. P. Seeman and C. Ulpian, Eur. J. Pharmac. 94, 145 (1983).
16. S. George, M. Watanabe and P. Seeman, in Dopamine Receptors (Eds. C. Kaiser and J. Kebabian), p.93. American Chemical Society, Washington, DC (1983).
17. S. List, M. Titeler and P. Seeman, Biochem. Pharmac. 29, 1621 (1980).
18. P. Seeman, Biochem. Pharmac. 31, 2563 (1982).
19. L.I. Goldberg and J.D. Kohli, Commun. Psychopharmac. 3, 447 (1979).