Antiparkinsonian drug doses and neuroleptic receptors

M. Titeler and P. Seeman

Department of Pharmacology, University of Toronto, Toronto (Canada M5S 1A8), 13 February 1978

Summary. The clinical potency of 3 drugs, apomorphine, N-propylnorapomorphine, and bromocryptine, have been found to be closely correlated to their potencies in competing for ^{3}H-haloperidol and ^{3}H-spiroperidol both of which label the dopamine receptor. This correlation indicates that the direct binding assay may be used to predict clinical potencies of anti-parkinsonian drugs, and indicates that agonists as well as antagonists compete potently for ^{3}H-neuroleptic binding.

There are now 3 radioactive ligands which meet the criteria for identifying brain dopamine receptors. These are ^{3}H-haloperidol[2-5], ^{3}H-spiroperidol[6-8] and ^{3}H-dihydroergocryptine[9-10], the latter employed in the presence of excess phentolamine. The ^{3}H-dopamine[3,11,12] and ^{3}H-apomorphine[13] radioligands themselves, however, do not seem to meet all the criteria. For example, although ^{3}H-dopamine and ^{3}H-apomorphine reveal saturable sites which have high affinity[13,14] and which are found in dopamine-rich regions of the brain, their binding is not inhibited by dopaminergic agonists or antagonists at concentrations which correlate with the pharmacological potencies on a dopaminergic response. In order to explain these observations, therefore, it has been suggested that ^{3}H-haloperidol might label the antagonist conformation state of the dopamine receptor while ^{3}H-dopamine labels the agonist conformation, a so-called 'two-state' hypothesis.

This communication considers a simple hypothesis which is based on the dopaminergic antiparkinsonian drug doses; namely, that the 2 high affinity sites for ^{3}H-dopamine and ^{3}H-neuroleptic are separate and noninterconvertible entities, and that the high affinity ^{3}H-neuroleptic site is probably the classical post-synaptic receptor with relatively low affinity for dopamine.

Materials and methods. Binding assays were done on calf caudate homogenates. The fresh caudate tissues were homogenized in 10 vol. of ice-cold TEAN buffer (15 mM Tris-HCl, pH 7.4, 5 mM Na_2EDTA, 1.1 mM ascorbate and 12.5 µM nialamide), using a Teflon-glass homogenizer (0.16 mm clearance; 500 rpm; 15 up-down strokes). This homogenate was incubated at 37 °C for 1 h, subdivided into 200 mg wet tissue per vial, and stored frozen at −20 °C for periods of up to 3 months. Immediately before the assay, the 200-mg sample was thawed and centrifuged (15 min at 44,000 × g) at 4 °C. The supernatant was discarded and the pellet was resuspended in 5 ml of TEAN buffer, using a ground-glass homogenizer (5 strokes by hand). The homogenate was then homogenized further using a Brinkmann

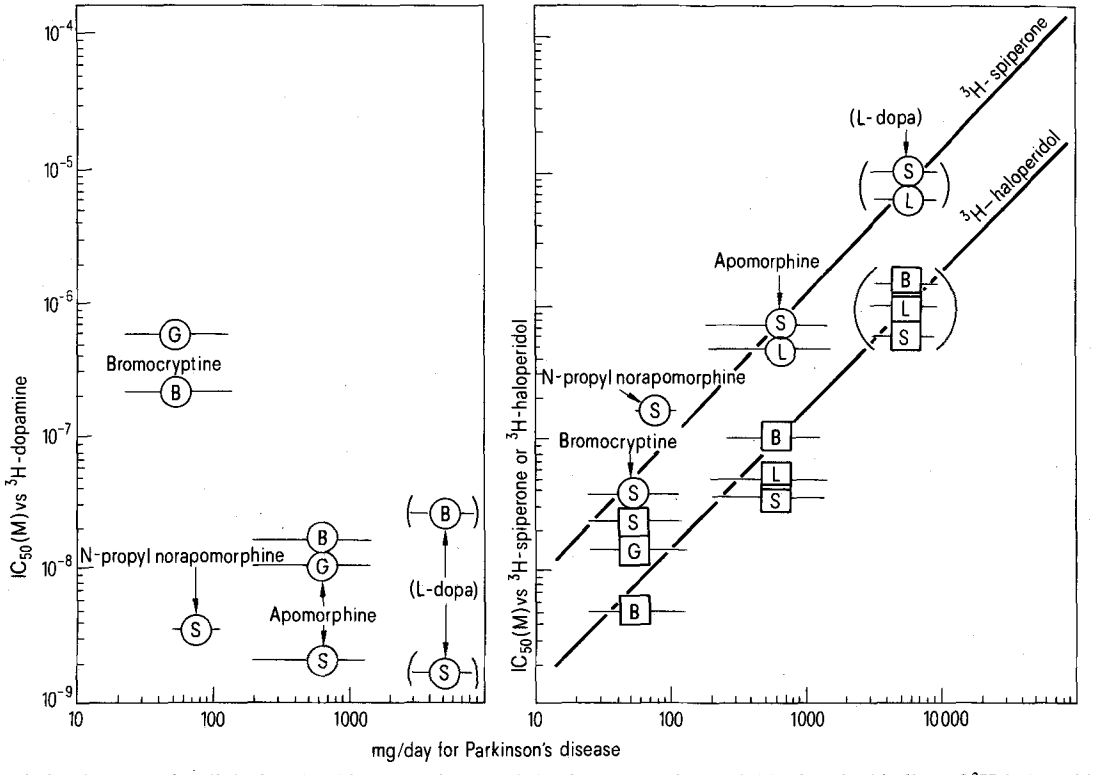

Fig. 1. Correlation between the clinical antiparkinsonian doses and the drug potencies for inhibiting the binding of ^{3}H-haloperidol or ^{3}H-spiperone, but not ^{3}H-dopamine.

The IC_{50}-values are the in vitro concentrations which inhibit binding by 50% in crude homogenates of rat or calf brain striatum. The IC_{50}-values labelled G are from Lew et al.[23] (calf for ^{3}H-dopamine; rat for ^{3}H-haloperidol), B are from Burt et al.[14] (calf), L are from Leysen et al.[6] (rat), while those labelled S are from this laboratory (calf; unpublished or from Seeman et al.[15]). The antiparkinsonian range of dosages are: apomorphine: 200–1400 mg/day, with a mean of 600 mg/day[24-26]; N-propylnorapomorphine: 60–90 mg/day[25]; bromocryptine: 42 mg/day[27], 79 mg/day[28], 70 mg/day[29], 120 mg/day[30], 26 mg/day[31], 26 mg/day[32]. Lergotrile has not been included because it is not considered clinically effective[33]. The standard clinical doses for L-DOPA are generally 3–9 g/day (without a DOPA-decarboxylase inhibitor). The IC_{50}-values are for dopamine in this case, not for L-DOPA. Rigourously, the data for L-DOPA should not be mixed with the data for dopamine since they are 2 different chemicals; however, dopamine is the active metabolite of L-DOPA and the main point is that this drug is weak both in vivo and in vitro.

Polytron (PT-10) at a setting of 7.0 (full scale = 10) for 20 sec. The final protein concentration of the suspension was 1.1 mg ml^{-1}. For the binding assay, samples were added to a glass test tube (12 × 75 mm) as follows: 100 µl of 600 nM (+)-butaclamol or (−)-butaclamol (the final butaclamol concentration was 100 nM in the ^{3}H-haloperidol but 1 µM in the ^{3}H-dopamine assay[8]): 100 µl of buffer containing different concentrations of antipsychotic drugs: 200 µl of ^{3}H-haloperidol (6.4 nM, 9.66 Ci $mmole^{-1}$) or ^{3}H-dopamine (3.5 nM, 8.4 Ci $mmole^{-1}$) or ^{3}H-spiroperidol (10.5 nM, 26.4 Ci $mmole^{-1}$); and 200 µl of membrane suspension. After incubation at room temperature (20–21 °C) for 30 min, 0.5 ml of the mixture was filtered (Whatman GF/B glass fibre filter, 2.4 cm), using a 600-mmHg vacuum, followed by washing with 5 ml of ice-cold TEAN buffer. The filters were monitored for ^{3}H by liquid scintillation. The stereospecific component of binding was defined as that amount of ^{3}H-haloperidol or ^{3}H-dopamine bound in the presence of (−)-butaclamol (inactive neuroleptic) minus that bound in the presence of (+)-butaclamol (active neuroleptic). Each experiment was done in sextuplicate, and each antipsychotic drug was tested at least 3 times,

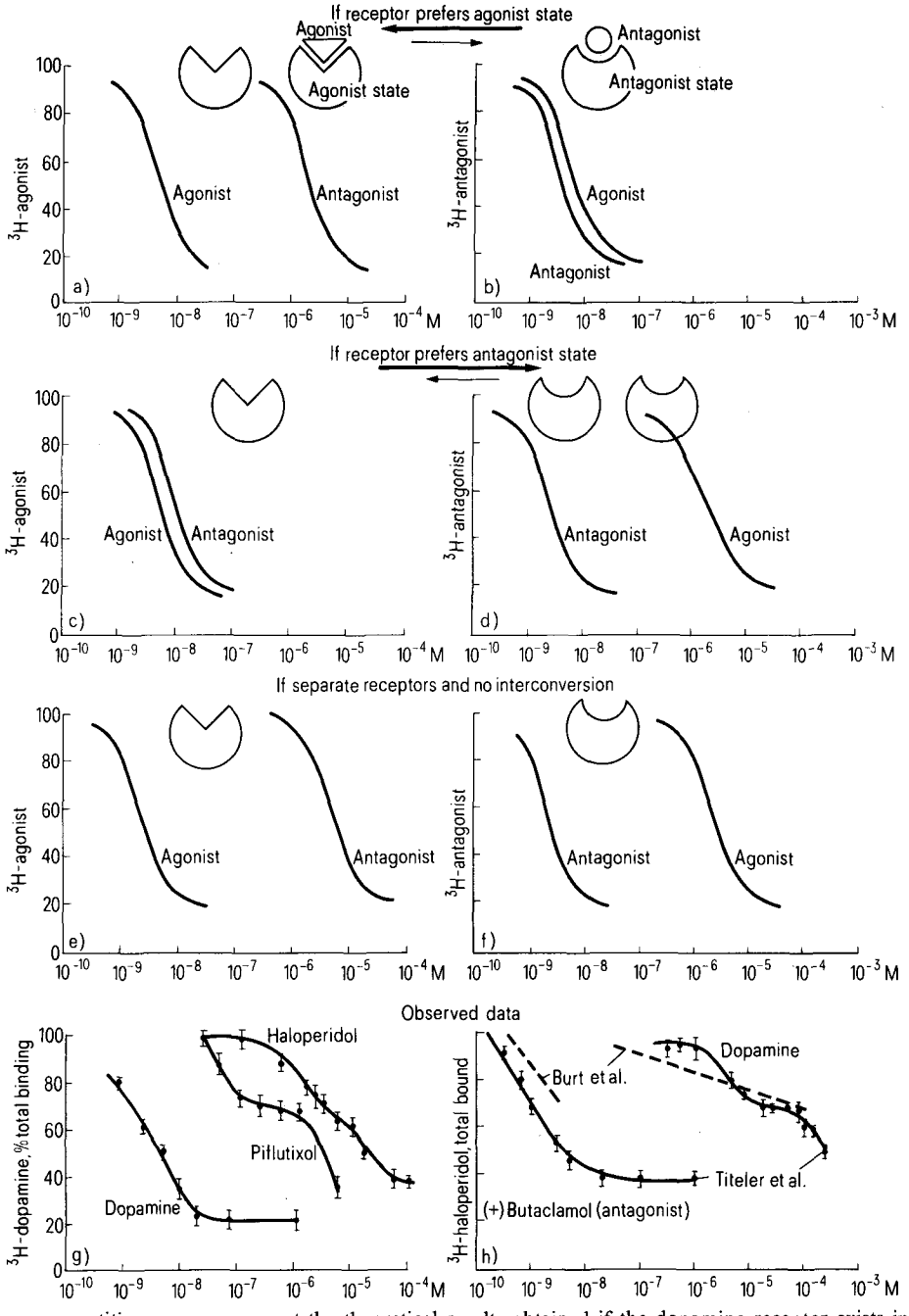

Fig. 2. *a* and *b* These competition curves represent the theoretical results obtained if the dopamine receptor exists in 2 interconvertible conformations, with the agonist state thermodynamically more stable. *c* and *d* The next competition curves (second from the top) represent the results that would be obtained if the receptor interconverts and prefers the antagonist state. Neither a, b or c, d resemble the experimentally observed competition data (section g, h at bottom). *e* and *f* (second from bottom): Here there is no interconversion of dopamine receptor conformations, but rather 2 separate, noninterconvertible sites. The expected data here do fit the experimentally observed competition curves (section g, h, bottom).

with most tested 9 times. The ^{3}H-spiroperidol assay was performed identically to the ^{3}H-haloperidol binding assay except that 0.5 nM was the final concentration of tritiated ligand.

Results and discussion. The concentrations of apomorphine and bromocryptine which inhibit the specific binding of ^{3}H-dopamine or ^{3}H-apomorphine by 50% (i.e., the IC_{50}-values) should at least correlate with their clinical dopaminergic antiparkinsonian potencies, according to the two-state hypothesis. This is not so, however, as shown in figure 1 (left). On the other hand, the IC_{50}-values for apomorphine, bromocryptine and N-propylnorapomorphine against ^{3}H-neuroleptic binding do exhibit a crude correlation to their antiparkinsonian potencies (figure 1). Although it would be preferable to relate the IC_{50}-values to the unbound concentrations (in plasma) of these antiparkinsonian drugs, such data are not available.

Qualitative considerations are shown in figure 2. Figure 2, a and b indicate the competition results expected if the agonist conformation is the preferred state in the two-state model. Figure 2, a shows that the agonist would readily (i.e. in the nM region) compete with ^{3}H-agonist while the antagonist would less readily compete (i.e. in the µM region); this is because the agonist has no difficulty fitting the ^{3}H-agonist site while the antagonist poorly 'fits' the ^{3}H-agonist conformation. Figure 2, b shows that the antagonist has no difficulty competing with ^{3}H-antagonist, and that the agonist also has no difficulty displacing the ^{3}H-antagonist because the receptor 'prefers' the agonist conformation. Figure 2, c and d shows results expected if the antagonist shape is preferred by the receptor.

The main point in figure 2, a–d is that, regardless which state is preferred by the receptor, one should not reasonably expect a low potency competition curve in both a and b or in both c and d. Apparently only a model wherein the receptors are separate and do not interconvert would be expected to reveal such a condition. This is shown in figure 2, e and f. Figure 2, e illustrates that the agonist readily competes with ^{3}H-agonist while the antagonist does not; figure 2, f shows that the antagonist easily displaces the ^{3}H-antagonist but the agonist does not. The results observed experimentally[8,14,15], summarized in figure 2, g and h do match figure 2, e and f but not figure 2, a and b or figure 2, c and d.

Further evidence difficult to explain in the two-state model is the fact that (+)-butaclamol, a potent dopamine antagonist, and bromocryptine, a potent dopamine agonist, have identical binding properties[8,10] when competing for ^{3}H-haloperidol and ^{3}H-dopamine sites. The results are readily accounted for by a simple model of separate receptors. It seems reasonable to postulate, therefore, that ^{3}H-dopamine or ^{3}H-apomorphine are tagging a site different from that labelled by the ^{3}H-neuroleptics.

This high affinity site for ^{3}H-dopamine, having a K_D of about 1 nM, does not appear to have properties expected of a traditional post-synaptic receptor according to the following considerations. a) In vitro physiological results on peripheral tissues generally reveal K_D-values in the µM concentration region for acetylcholine[16,17] and noradrenaline[13,18], but not in the nM region. b) Dopamine receptors in *Aplysia* neurones have threshold actions in the µM range[20] rather than in the nM range. c) This apparent low affinity of dopamine and other neurotransmitters for their receptors (i.e. K_D-values in the µM region) may be reflected in the massive doses of transmitter precursors needed clinically to replete dopaminergic or serotoninergic function with L-DOPA[21] or tryptophan[22]. The synapse anatomy guarantees that, despite the high K_D, sufficient transmitter will reach the receptors; exogenous drugs must have, however, rather high affinities (with K_D values as low as 1 nM) in order to attach specifically to the receptor.

In summary, it is hypothesized that the ^{3}H-neuroleptic ligand (and not ^{3}H-dopamine) identifies the traditional type of post-synaptic dopamine receptor, that the value of about 1 µM for the K_D of the dopamine receptor (as measured using ^{3}H-neuroleptic) may be physiologically plausible, and that the dopaminergic antiparkinsonian drug doses may thus correlate with their potencies against ^{3}H-neuroleptic binding. If the hypothesis is valid, it would indicate that the high affinity site (K_D of about 1 nM) for ^{3}H-dopamine would identify some other receptor site in the neuroleptic, possibly the 'autoreceptor' postulated by Carlsson[34].

1 This work was supported by the Ontario Mental Health Foundation, the W. Garfield Weston Foundation and the Medical Research Council of Canada.

2 P. Seeman, Biochem. Pharmac. *26*, 1741 (1977).
3 P. Seeman, M. Chau-Wong, J. Tedesco and K. Wong, Proc. nat. Acad. Sci. USA *72*, 4376 (1975).
4 I. Creese, D.R. Burt and S.H. Snyder, Life Sci. *17*, 993 (1975).
5 I. Creese, D.R. Burt and S.H. Snyder, Life Sci. *17*, 1715 (1975).
6 J.E. Leysen, W. Gommeren and P.M. Laduron, Biochem. Pharmac. *27*, 307 (1977).
7 J.Z. Fields, T.D. Reisine and H.I. Yamamura, Brain Res. *136*, 578 (1977).
8 M. Titeler, P. Weinreich, D. Sinclair and P. Seeman, Proc. nat. Acad. Sci. USA, *75*, 1153 (1978).
9 M. Titeler, P. Weinreich and P. Seeman, Proc. nat. Acad. Sci. USA *74*, 3750 (1977).
10 M.G. Caron, M. Beaulieu, V. Raymond, B. Gagné, J. Drovin, R.J. Lefkowitz and F. Labrie, J. biol. Chem. *253*, 2244 (1978).
11 P. Seeman, M. Wong and T. Lee, Fedn Proc. *33*, 246 (1974).
12 D.R. Burt, S.H. Enna, I. Creese and S.H. Snyder, Proc. nat. Acad. Sci. USA *72*, 4655 (1975).
13 P. Seeman, T. Lee, M. Chau-Wong, J. Tedesco and K. Wong, Proc. nat. Acad. Sci. USA *72*, 4354 (1976).
14 D.R. Burt, I. Creese and S.H. Snyder, Molec. Pharmac. *12*, 800 (1976).
15 P. Seeman, T. Lee, M. Chau-Wong and K. Wong, Nature *261*, 717 (1976).
16 A.J. Ariens, Ann. N.Y. Acad. Sci. *139*, 606 (1967).
17 J.O. Dolly and E.A. Barnard, FEBS Lett. *46*, 145 (1974).
18 R. Andersson, S. Holmberg, N. Svedmyr and G. Äberg, Acta med. scand. *191*, 241 (1972).
19 L.T. Williams and R.J. Lefkowitz, Science *192*, 791 (1976).
20 J.W. Swann, C.N. Siback and D.O. Carpenter, Soc. Neurosci. *7*, 189 (1977).
21 M.D. Yahr (ed.), Adv. Neurol. vol. 2, Treatment of Parkinsonism – The role of dopa-decarboxylase inhibitors, Raven Press, New York (1973).
22 E. Hartman, Am. J. Psychiat. *134*, 366 (1977).
23 J.Y. Lew, F. Hata, T. Ohashi and M. Goldstein, J. neural Transm. *41*, 109 (1977).
24 G.C. Cotzias, P.S. Papavasiliou and J.Z. Ginos, in: The Basal Ganglia, p. 305. Ed. M.D. Yahr. Raven Press, New York 1976.
25 G.C. Cotzias, P.S. Papavasiliou, E.S. Tolosa, J.S. Mendez and M. Bell-Midura, N. Engl. J. Med. *294*, 567 (1976).
26 S.E. Duby, G.C. Cotzias, P.S. Papavasiliou and W.H. Lawrence, Archs Neurol. *27*, 474 (1972).
27 D.B. Calne, P.D. Teychenne, P.N. Leigh, A.N. Bamji and J.K. Greenacre, Lancet *2*, 1255 (1974).
28 R. Kartzinel, M. Perlow, P. Teychenne, A.C. Gielen, M.M. Gillespie, D.A. Sadowsky and D.B. Calne, Lancet *1*, 272 (1976).
29 A. Leiberman, M. Kupersmith, E. Estey and M. Goldstein, N. Engl. J. Med. *295*, 1400 (1976).
30 J. Gerlach, Acta neurol. scand. *53*, 189 (1976).
31 A. Lieberman, M. Zolfaghari, D. Boal, H. Hassouri, B. Vogel, A. Battista, K. Fuxe and M. Goldstein, Neurology, Minneap. *26*, 405 (1976).
32 J.D. Parkes, C.D. Marsden, I. Donaldson, A. Galae-Debono, J. Walters, G. Kennedy and P. Asselman, J. Neurol. Neurosurg. Psychiat. *29*, 184 (1976).
33 A. Lieberman, T. Miyamoto, A.F. Battista and M. Goldstein, Neurology, Minneap. *25*, 459 (1975).
34 A. Carlsson, in: Pre- and Post-synaptic receptors, p. 49. Ed. E. Usdin and W.E. Bunney. Marcel Dekker Inc., New York 1975.