ADVANCES IN PHARMACOLOGY AND THERAPEUTICS

Proceedings of the 7th International Congress of Pharmacology

General Editors: J. R. BOISSIER, P. LECHAT & J. FICHELLE

Volume 5

NEUROPSYCHOPHARMACOLOGY

Editor: C. DUMONT

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Editor

C. DUMONT
Romainville

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Introduction

The scientific contributions at the 7th International Congress of Pharmacology were of considerable merit. Apart from the sessions organised in advance, more than 2,200 papers were presented, either verbally or in the form of posters, and the abundance of the latter in the congress hall is a good indication that this particular medium of communication is becoming increasingly attractive to research workers, and offers scope for discussions which combine an elaborate, thorough approach with a certain informality.

It would have been preferable to have published the entire congress proceedings within the framework of the reports. That was, however, physically impossible, and the organisers had to adopt a realistic solution by publishing only the main lectures, symposia and methodological seminars. The amount of material presented necessitated the printing of ten volumes, each volume containing congress topics regrouped according to their relevant content and subject areas. This system of division may give rise to criticism on account of its artificiality, and we readily admit that certain texts could have been placed in more than one volume. We are asking the reader to excuse this arbitrariness, which is due to the editors' personal points of view.

I draw attention to the fact that most of the symposia finish with a commentary which the chairmen had the option of including, presenting their personal opinions on one or several points. We think that such an addition will facilitate reflection, discussion, indeed even controversy.

The launching of the scientific programme for this congress began in September 1975 on returning from the last meeting in Helsinki. Long and delicate discussions took place in the Scientific Programme Committee and with the International Advisory Board. Should it be a pioneer, 'avant-garde' congress? Or one laid out like a balance-sheet? Should we restrict the congress to the traditional bounds of pharmacology, or extend the range of papers to cover the finest discipline? The choice was difficult, and the result has been a blend of the two, which each participant will have appreciated in terms of his training, his tastes, and his own research.

A certain number of options, however, were taken deliberately: wide scope was given to toxicology, from different points of view, and to clinical pharmacology, a subject much discussed yet so badly practised; the founding of two symposia devoted

to chemotherapy of parasitic diseases which are still plagues and scourges in certain parts of the world; a modest but firm overture in the field of immunopharmacology, which up until now was something of a poor relation reserved only for clinical physicians; the extension of methodological seminars, in view of the fact that new techniques are indispensable to the development of a discipline.

We have been aware since the beginning that, out of over 4,000 participants who made the journey to Paris, not one could assimilate such a huge body of knowledge. Our wish is that the reading of these reports will allow all of them to become aware of the fantastic evolution of pharmacology in the course of these latter years. If one considers pharmacology as the study of the interactions between a "substance" and a living organism, then there is no other interpretation. Nevertheless, one must admit that there exists a period for describing and analysing a pharmacological effect, and that it is only afterwards that the working mechanism can be specified; a mechanism which will permit these "substances" to be used for the dismantling and breaking down of physiological mechanisms, a process which justifies Claude BERNARD'S term, "chemical scalpel".

The reader will be able to profit equally from more down-to-earth contributions, more applied to therapeutics, and less "noble", perhaps, for the research worker. He will realise then that his work, his research and his creative genius are first and foremost in the service of Man, and will remember this statement from Louis PASTEUR:

> "Let us not share the opinion of these narrow minds who scorn everything in science which does not have an immediate application, but let us not neglect the practical consequences of discovery."

I would like to renew my thanks to my colleagues in the Scientific Programme Committee and also to the members of the International Advisory Board, whose advice has been invaluable. I owe a particular thought to J J BURNS, now the past-president of IUPHAR, who granted me a support which is never discussed, and a staunch, sincere friendship. The Chairmen have effected an admirable achievement in the organisation of their proceedings, and in making a difficult choice from the most qualified speakers. The latter equally deserve our gratitude for having presented papers of such high quality, and for having submitted their manuscripts in good time.

The publisher, Robert MAXWELL, has, as always, put his kindness and efficiency at our service in order to carry out the publication of these reports. But none of it would have been possible without the work and competence of Miss IVIMY, whom I would like to thank personally.

My thanks again to the editors of the volumes who, in the middle of the holiday period, did not hesitate to work on the manuscripts in order to keep to the completion date.

Finally, a big thank you to all my collaborators, research workers, technicians and secretaries who have put their whole hearts into the service of pharmacology. They have contributed to the realisation of our hopes for this 7th International Congress, the great festival of Pharmacology. Make an appointment for the next one, in 1981, in Tokyo.

Jacques R BOISSIER
Chairman
Scientific Programme Committee

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Invited Lectures

Anti-schizophrenic Drugs: Membrane Receptor Sites of Action

Philip Seeman

(In collaboration with J. Tedesco, M. Titeler & E. J. Hartley)
Pharmacology Department, University of Toronto, Toronto, Canada M5S 1A8

Neuroleptic drugs, introduced in 1952, are effective in controlling many of the symptoms of schizophrenia. Since the neuroleptics are particularly effective in preventing the delusions and hallucinations of paranoid schizophrenia, it is possible that the sites of neuroleptic action in the brain may yield some clue as to the chemical derangement in schizophrenia.

Of the many sites of neuroleptic action which have been proposed, only those which are affected by nanomolar concentrations (1-100 nM) ought to be further considered as being specific and clinically significant. This is because the therapeutic concentrations of neuroleptics in plasma water are between 0.1 and 50 nM (Ref. 53).

Since the neuroleptics are very fat-soluble, surface-active and membrane-soluble (Refs. 52,53,58), it is essential to distinguish the specific sites of action (which are stereoselective and require three-point attachment of the drug) from the non-specific sites (which are hydrophobic and non-selectively accept highly fat-soluble drugs). The stereoselective actions of (+)-butaclamol are essential in making this distinction (Fig. 1). The in vitro neuroleptic sites which fulfill these criteria include both pre-synaptic and post-synaptic sites of action:

Post-Synaptic Sites for Neuroleptics

A. Receptors with high affinity (nM) for ^{3}H-neuroleptics.
B. Receptors with low affinity (μM) for dopamine.
C. Dopamine-sensitive adenylate cyclase with low affinity (μM) for dopamine (Refs. 26,31,64).
D. Dopamine receptors for ^{3}H-dihydroergocryptine and ^{3}H-LSD (Refs. 3,12,68-72).

Pre-Synaptic Sites for Neuroleptics

E. Dopamine autoreceptors with high affinity (nM) for dopamine.
F. Receptors (with low?) affinity for ^{3}H-neuroleptics.
G. Sites which release ^{3}H-dopamine upon stimulation; blocked by neuroleptics (Ref. 55).
H. Tyrosine hydroxylase, wherein neuroleptics reverse the apomorphine-induced inhibition of this enzyme (Ref. 50).

^{3}H-Neuroleptic Receptors

Of the many sites studied in vitro for neuroleptics, the site with the highest affinity for neuroleptics is that which specifically binds the ^{3}H-neuroleptics themselves. ^{3}H-Haloperidol, the first radio-neuroleptic to be studied in detail, was prepared for this laboratory by I.R.E. Belgique in 1974 with a specific activity of 9.6 Ci/mmole, permitting one to test for specific binding in the nM region (Refs. 62,54). Fig. 1 indicates the approach for measuring stereospecific or specific binding of a ^{3}H-neuroleptic to neural tissue homogenates.

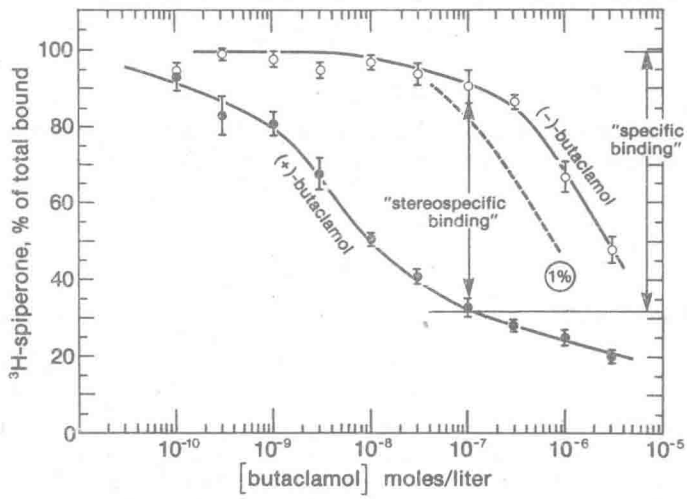

Fig. 1. The specific or stereospecific binding of a ^{3}H-neuroleptic to calf caudate homogenate. The concentration of ^{3}H-spiperone was 0.8 nM. "1%" indicates data expected if (-)-butaclamol contained 1% (+)-butaclamol.

Since (-)-butaclamol has little effect at 100 nM, specific binding of the ^{3}H-neuroleptic is generally equal to stereospecific binding. Hitherto, the ^{3}H-neuroleptics used have been ^{3}H-haloperidol (Refs. 2-5,14-18,20,54,56,57,61),

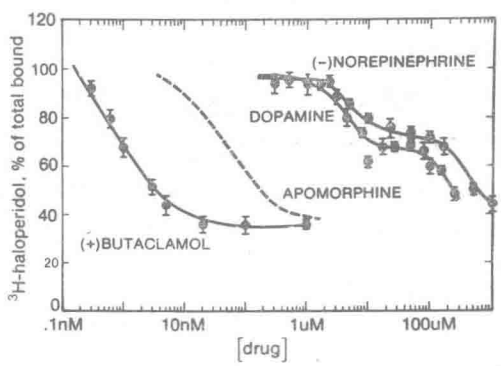

Fig. 2. Dopamine is the most effective transmitter in competing for the binding of ^{3}H-haloperidol to calf caudate homogenate (adapted from Ref. 70).

^{3}H-spiperone (Refs. 30,29,35,36,22,19,24,25), and ^{3}H-pimozide (Ref. 1). Specific binding of ^{35}S-chlorpromazine (Ref. 52) and ^{14}C-fluphenazine were not detected.

Of all the neurotransmitters, dopamine is the most effective in competing against the binding of ^{3}H-neuroleptics, suggesting that the ^{3}H-neuroleptic labels dopamine receptors (Fig. 2). In the rat frontal cortex, however, Leysen et al. (36) found that serotonin displaced ^{3}H-spiperone at 3200 nM compared to 250,000 nM for dopamine. This may indicate that ^{3}H-spiperone might also bind to serotonin sites (cf. Whitaker & Seeman, Refs. 71,72). Recent work in this lab (Hartley et al., in preparation) indicates that serotonin and dopamine are equally effective (3000 nM) in displacing ^{3}H-spiperone in the calf frontal cortex. In the calf caudate there is only one set of high affinity sites for ^{3}H-neuroleptics (Fig. 3), and data for ^{3}H-spiperone and ^{3}H-haloperidol give a similar pattern (Table 1).

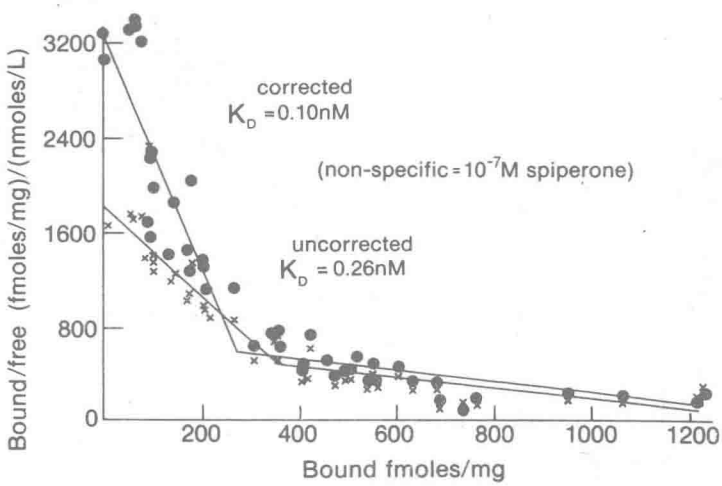

Fig. 3. The specific binding of ^{3}H-spiperone to calf caudate has a very low dissociation constant of 0.1 nM. The total spiperone concentrations were corrected or converted to the free concentrations (cf. Ref. 13), since 15-50% (cf. Ref. 28) of the isotope was bound to the tissue. Adapted from Hartley & Seeman (Ref. 24).

TABLE 1: IC_{50} VALUES (nM) FOR CALF CAUDATE OR RAT STRIATUM

Radioligand	^{3}H-haloperidol	^{3}H-spiperone	^{3}H-dopamine		^{3}H-apomorphine	
Tissue	calf	calf	calf	rat	calf	
nM ligand	2.1	0.1	1.2		1.5	3
free nM ligand	0.97	0.05[24]	1.2		0.86	1.5
non-specific binding	100 nM (±)-butaclamol	spiperone or butaclamol	1000 nM (±)-but.		1000 nM (±)-but.	200 nM apo.
AGONISTS						
Apomorphine	40[57]	750	2[57]	45[57]	1.3[57]	3.8[61]
Dopamine	600[57]	17500[70]	9[57]	1[53A]	3.6[56]	2.0[61]
(-)-Noradrenaline	100000[70]	100000[70]	46[56]		15[56]	30[61]
Adrenaline	5200[4]		68[56]		20[56]	
Isoproterenol	>10000[4]		>10000[4]		1400[56]	
Serotonin	31600[35]	3200[35]	100000[56]		5000[56]	
ANTAGONISTS						
Chlorpromazine	29[57]	170[35]	2000[57]	420[57]		900[70]
Haloperidol	1.2[57]	5.6	300[57]	35[57]		900[70]
Spiperone	0.07[57]	0.5[57]	4000[56]			2200

The competition of various drugs against the specific binding of ^{3}H-haloperidol is much more effective than against ^{3}H-spiperone (Table 1 and Refs. 35,36), but the use of ^{3}H-haloperidol requires about 3 times more tissue.

The IC_{50} values for various neuroleptics in competing against the specific binding of ^{3}H-haloperidol (calf caudate) correlate very well with their clinical antipsychotic potencies (Fig. 4). This simple radioreceptor system can thus be used as a test for screening new neuroleptics, active neuroleptic metabolites or new anti-Parkinsonian drugs. Equally important is the astonishing fact that these $IC_{50\%}$ values are virtually identical to the therapeutic neuroleptic concentrations in the water phase in plasma from patients being treated with these drugs (53).

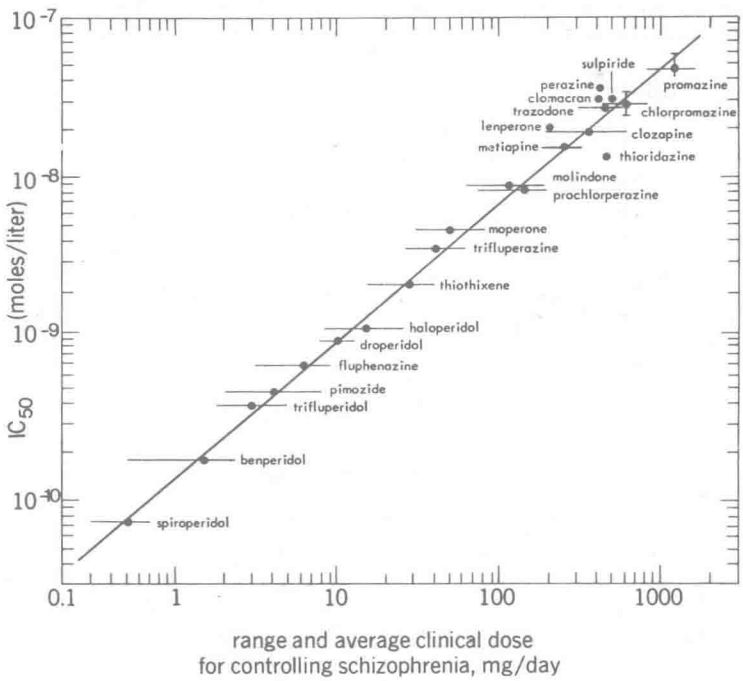

Fig. 4. Correlation between the clinical antipsychotic potencies of neuroleptics and the neuroleptic concentrations which inhibit (by 50%) the specific binding of 2.1 nM ^{3}H-haloperidol to calf caudate homogenate ($IC_{50\%}$) values. Adapted from Refs. 53,57 (cf. 16).

Receptors for ^{3}H-Dopamine, ^{3}H-Apomorphine and ^{3}H-ADTN

Receptors with high affinity for ^{3}H-dopamine, ^{3}H-apomorphine or ^{3}H-ADTN are generally found in the same tissues harbouring high affinity sites for the ^{3}H-neuroleptics. As shown in Fig. 5 and Table 1, the K_D values for these ligands are between 1 and 10 nM (cf. Refs. 2,7). In collaboration with Dr. G. Woodruff and Dr. J. Poat, who provided ^{3}H-ADTN (8 Ci/mmole; also see Ref. 49), we found that the binding of ^{3}H-ADTN to calf caudate was inhibited by 50% at 1 nM N-propyl-norapomorphine, 3 nM dopamine, 3 nM epinine, 5 nM ADTN, 10 nM apomorphine, 100 nM (-)-noradrenaline, 500 nM bromocryptine and 50000 nM phentolamine (using 500 nM ADTN as a baseline for non-specific binding). In general, the concentration of any drug which inhibits the binding of ^{3}H-dopamine is virtually the same as that which inhibits the binding of ^{3}H-apomorphine (Ref. 61). The data in Fig. 2 indicate that dopamine displaces 2 nM ^{3}H-haloperidol in two phases, suggesting that 2 nM ^{3}H-haloperidol bound to two high affinity sites for ^{3}H-haloperidol. Furthermore, the neuroleptics also displaced 3 nM ^{3}H-apomorphine in two phases, possibly

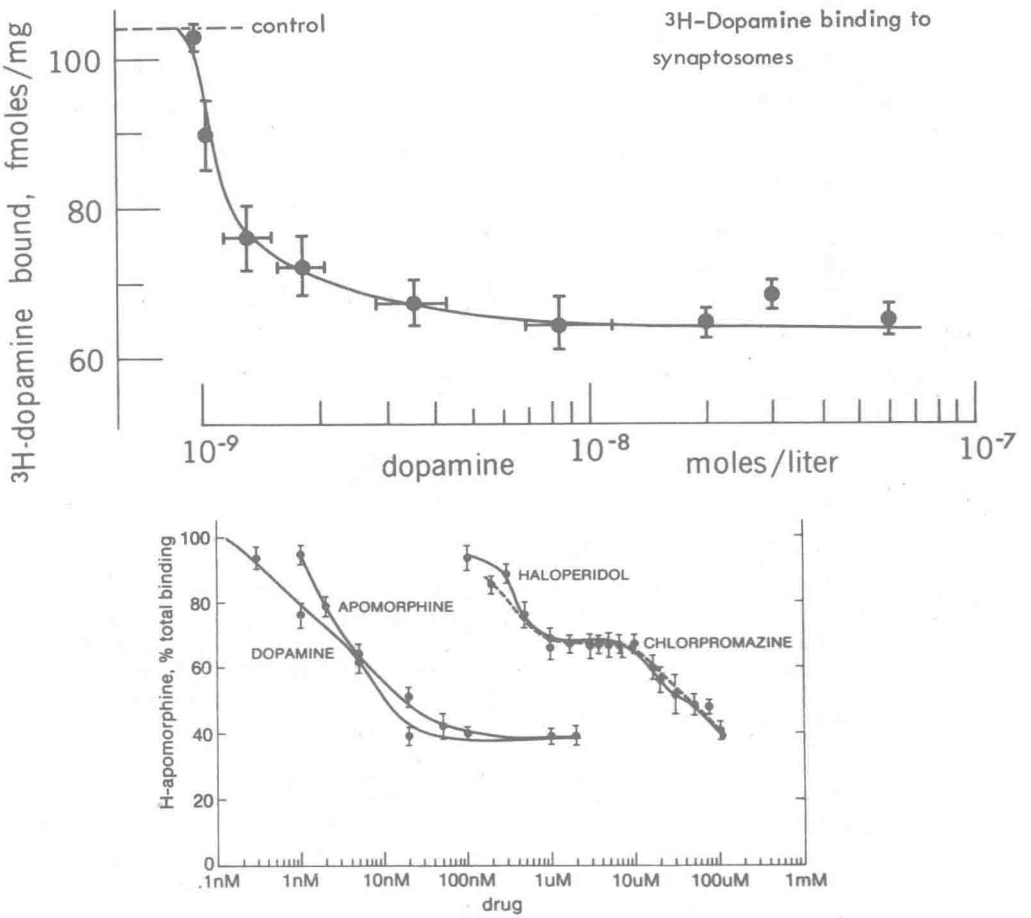

Fig. 5. The high affinity binding of ^{3}H-dopamine (top) and ^{3}H-apomorphine (bottom) to rat and calf caudate homogenates, respectively. The K_D for ^{3}H-dopamine was 0.6 nM (adapted from Ref. 53A; centrifugation method). The neuroleptics displayed 3 nM ^{3}H-apomorphine in two phases suggesting the possible existence of two high affinity sites for apomorphine/dopamine (bottom adapted from Ref. 70).

indicating two high affinity sites for ^{3}H-apomorphine. An alternate explanation for these biphasic patterns, however, may be that during the prolonged exposure for 30 min (in the incubation period of the radioreceptor assay), the high molarities (1000-10,000 nM) of dopamine may actually unmask or expose new sites for ^{3}H-apomorphine (Fig. 6).

Is the High Affinity ^{3}H-Neuroleptic Site the Same as that for ^{3}H-Dopamine?

As noted (Table 1, Fig. 2 and Fig. 5), it requires μM concentrations of neuroleptics to displace nM concentrations of ^{3}H-dopamine or ^{3}H-apomorphine; it also requires μM concentrations of dopamine to displace nM concentrations of ^{3}H-neuroleptics. To explain this apparent paradox, it has been suggested that the dopamine receptors may exist in "two states", an agonist-state and an antagonist state, and that the states interconvert (Refs. 4,14).

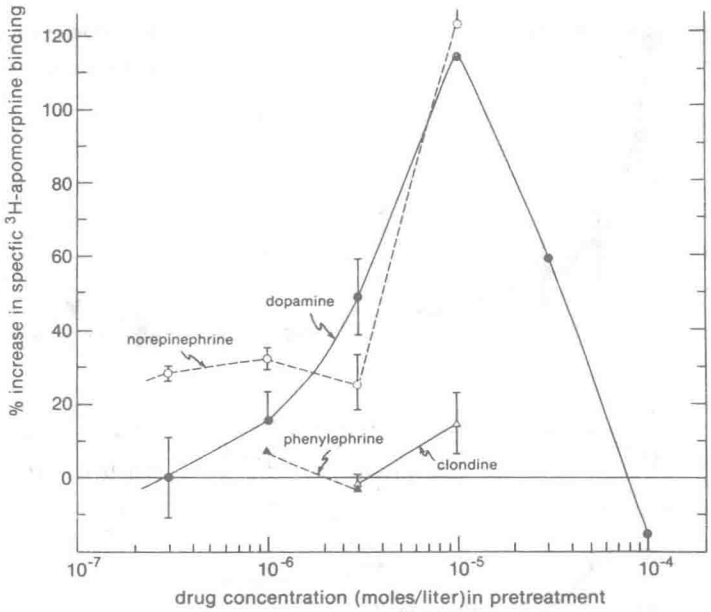

Fig. 6. After pretreating the calf caudate homogenate to μM concentrations of dopamine or noradrenaline, and then extensively washing the homogenates, the % of specifically bound ^{3}H-apomorphine increased, possibly indicating an unmasking of sites. Adapted from McManus, Hartley & Seeman (Ref. 42).

Current evidence, however, tends to favour the idea that the high affinity ^{3}H-neuroleptic receptor and the high affinity ^{3}H-dopamine (^{3}H-apomorphine) receptor are separate and non-interconverting sites, as based on the following evidence:

1) Lesions of the nigral dopamine neurones (using 6-OH-dopamine injected into the medial forebrain bundle in rats) lowered the amount of ^{3}H-apomorphine bound by the striatum (Fig. 7), but elevated the amount of ^{3}H-neuroleptic bound (Ref. 47; cf. 18).

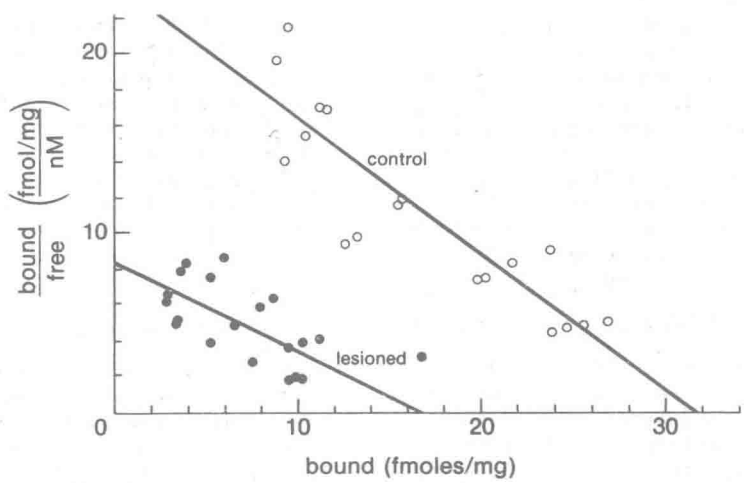

Fig. 7. 6-OH dopamine lesions of nigral cells reduced the binding of ^{3}H-apomorphine by the striatum (Ref. 47).

2) The latter finding suggests that much of the high affinity sites for ^{3}H-apomorphine may be pre-synaptic, while those for ^{3}H-neuroleptics may be post-synaptic. Compatible with this is our recent finding that intrastriatal kainic acid reduced the binding of ^{3}H-spiperone, but not that of ^{3}H-apomorphine (Weinreich et al., to be published).

3) Post-mortem analysis of untreated Parkinson's diseased human brains reveals less binding of ^{3}H-apomorphine by the striatum, compatible with the loss of nigral neurones (Fig. 8). At the same time, however, we detected more binding of ^{3}H-haloperidol by the putamen, compatible with more post-synaptic receptors associated with denervation of the nigral cells (Fig. 8, bottom).

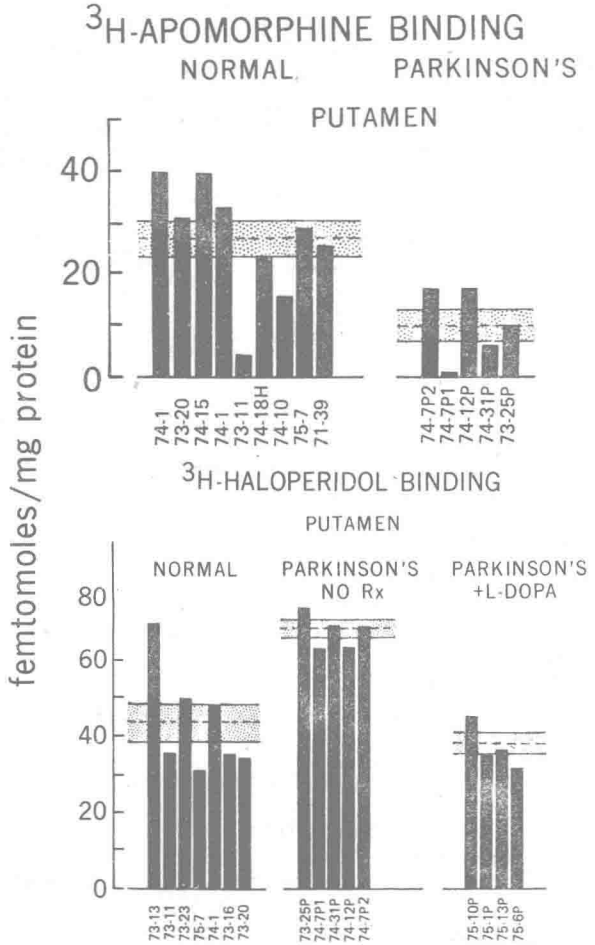

Fig. 8. The binding of ^{3}H-apomorphine by Parkinsonian putamens was low, while that for ^{3}H-haloperidol was high (untreated patients), suggesting the former ligand labels pre-synaptic sites, while the latter ligand labels post-synaptic sites (from Ref. 33).

4) The analysis shown in Fig. 9 indicates that the observed data (bottom of Fig.) do not fit any possible interconversion two-state hypothesis. The data of Burt et al. in that Fig. refer to Ref. 4 while those of Titeler et al., refer to Ref. 70.