The Dopamine/Neuroleptic Receptor

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ABSTRACT: The neuroleptic/dopamine receptor, with its picomolar affinity for potent neuroleptics, is the functional dopamine receptor of the brain. This receptor has been termed the D_2 dopamine receptor, and it inhibits or interferes with dopamine-stimulated adenylate cyclase. This D_2 receptor has two states, each having different affinity for dopamine. The high-affinity state, termed D_2^{high}, has a 10 nM affinity for dopamine and is the functional correlate for dopamine autoreceptors and for the dopamine receptor in the pituitary gland. The low-affinity state, termed D_2^{low}, has a 2000 nM affinity for dopamine, and may possibly represent the desensitized state of the dopamine receptor or the functional post-synaptic receptor.

RÉSUMÉ: Le récepteur neuroleptique/dopamine, avec son affinité picomolaire pour les neuroleptiques puissants, est le récepteur dopaminergique fonctionnel du cerveau. Ce récepteur fut appelé le récepteur dopaminergique D_2; il inhibe ou interfère avec l'adénylate cyclase stimulée par la dopamine. Ce récepteur D_2^{high}, a une affinité à 10 nM pour la dopamine et correspond à l'état fonctionnel des autorécepteurs dopaminergiques et des récepteurs dopaminergiques de la glande pituitaire. L'état de basse affinité, D_2^{low}, a une affinité à 2000 nM pour la dopamine, et représente probablement l'état désensibilisé du récepteur dopaminergique.

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Dopamine receptors occur in high density in the putamen and caudate nucleus with densities of about 11 pmoles of receptors per gram of tissue. Progressively lower densities are found in the globus pallidus, the substantia nigra, the median eminence and anterior pituitary gland, the area postrema, the ventral tegmental region, the retina, and the paraolfactory cortex (see List and Seeman, 1981 for further Refs.).

Although dopamine receptors were first detected about 10 years ago (Seeman et al., 1974, 1975a, b), the conditions for measuring their precise absolute concentrations in different brain regions are still being developed (Seeman et al., 1982).

Definition of a dopamine receptor

A neurotransmitter receptor is a membrane-located protein which when stimulated by the transmitter results in an electrical or chemical effect. A receptor should be affected by drug doses or concentrations which correlate with the drug doses or concentrations causing a particular brain response subserved by that receptor.

A dopamine receptor is defined as that receptor which is more sensitive to dopamine than to any other neurotransmitter. Thus, the primary criterion of a dopamine receptor is that it be most sensitive to dopamine, less sensitive to noradrenaline and even less sensitive to serotonin. If one includes exogenous drugs, such as bromocriptine, apomorphine and ADTN (6, 7-dihydroxy-2-aminotetralin), a dopamine receptor is defined as one which has the following rank order of dopaminergic agonist potencies:

Bromocriptine > apomorphine = ADTN > dopamine > noradrenaline > serotonin

A dopamine receptor and a "dopaminergic site" generally have the same rank order of sensitivities to dopamine agonists; a dopamine receptor, however, has a functional correlate, while the functional correlate of a "dopaminergic site" is one which remains to be established.

Classification of central and peripheral dopaminergic sites and receptors

Subclassification of the dopaminergic sites (and/or states) depends on the absolute molarities of agonists and antagonists to which the sites are sensitive. Thus, the nomenclature used in this laboratory is based solely on the absolute sensitivities of the site to three drugs: dopamine, spiperone and sulpiride. This is summarized in Table 1 and Fig. 1.

The sites and/or states (in Table 1) are defined according to two criteria: A) by the order of magnitude of the absolute molarities (uM or nM) of dopamine and spiperone that were 50% effective in vitro; and B) by whether or not the site was sensitive to R-sulpiride of S-sulpiride.

Dopamine-stimulated adenylate cyclase, or the D_1 site:

Dopamine-stimulated adenylate cyclase, first detected in 1972 by Kebabian et al., has been termed the D_1 site (Kebabian and Calne, 1979). The D_1 site is sensitive to *micromolar* concentrations of dopamine as well as to *micromolar* concentrations of spiperone,

Table 1: Definitions of Dopaminergic Sites and States

	Central nervous system				Peripheral tissues	
	D_1	D_2^{low}	D_2^{high}	D_3	DA_1	$DA_2 = D_2^{high}$
Dopamine C_{50}	μM	μM	nM	μM	μM	nM
Spiperone IC_{50}	μM	nM	pM	μM	nM	nM
Sulpiride-sensitive?	No	S-sulpiride		No	R-sulpiride	S-sulpiride

The C_{50} or IC_{50} values are the concentrations which either stimulate or inhibit the site by 50%.

As explained in the test, we had previously used the term "D_4" instead of D_2^{high} because we were not sure that all the D_2^{high} sites could be converted to D_2^{low} sites (Wreggett and Seeman, 1983a, b). Since we have now established that all the D_2^{high} sites can indeed be converted to their low-affinity state (see later Figs.), it is no longer necessary to use the term "D_4".

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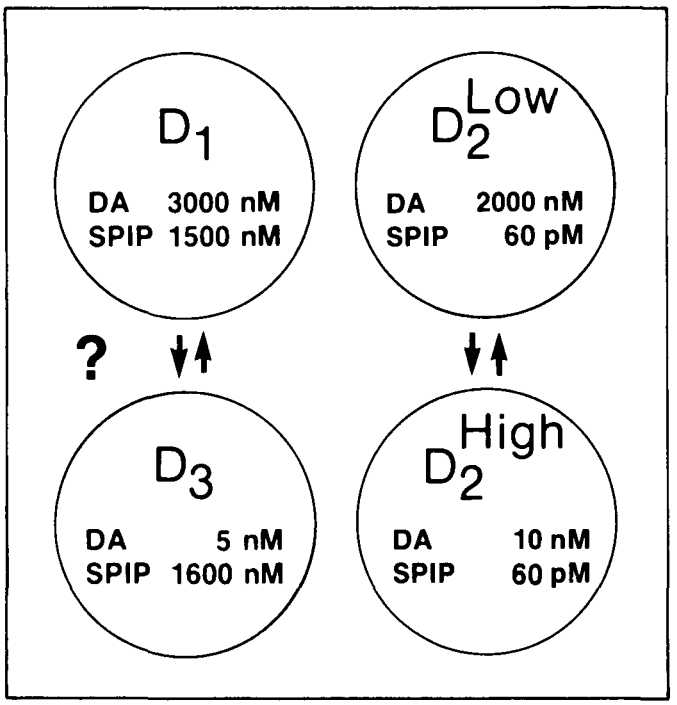

Figure 1 — Dopamine-stimulated adenylate cyclase is the D_1 site which has low affinity (μM) for neuroleptics and for dopamine (μM). The dopamine/neuroleptic receptor is termed the D_2 dopamine receptor. This D_2 receptor has a very high affinity for neuroleptics (60 picomolar dissociation constant for spiperone), and has two states of affinity for dopamine agonists: the high-affinity state has a 10 nanomolar dissociation constant for dopamine (D_2^{high}), while the low-affinity state has a 2000 nanomolar dissociation constant for dopamine. The D_3 site is one which has a high affinity for dopamine (5 nM), but a very low affinity for neuroleptics. It has been suggested that some of the D_1 and D_3 sites inter-convert, but there is no direct evidence for this yet. The D_2 receptor is the only dopaminergic site that presently has functional correlates in the nervous system. The D_2^{high} site is the functional site for the anterior pituitary gland.

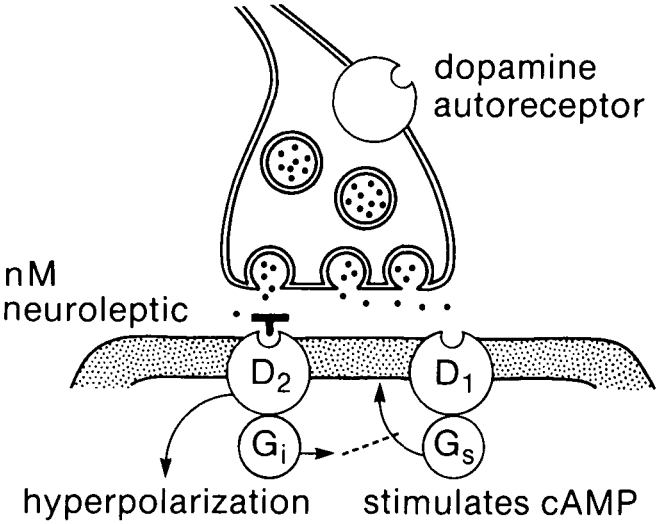

Figure 2 — A possible anatomical arrangement for the D_1 site, the D_2 receptor, and the dopamine autoreceptor. The D_1 site results in dopamine-stimulated adenylate cyclase via a G_s protein. The D_2 receptor in the brain striatum may interfere with the release (and production) of cyclic AMP caused by D_1 (Stoof and Kebabian, 1981). The D_2 interference may be via a G_i protein, as is the case in the pituitary gland (Meunier et al., 1980; Cote et al., 1981). Dopamine autoreceptors are sensitive to very low concentrations of dopamine (nanomolar) and thereby inhibit the release of dopamine from the nerve terminals.

but is extremely *insensitive* to the substituted benzamides, such as sulpiride or metoclopramide (Refs. in Seeman, 1980). These properties thus define the D_1 site for any tissue response or for the competition for the binding of any ^{3}H-ligand. The neural or behavioural role of the D_1 site is not yet known.

The neuroleptic/dopamine receptor, or the D_2 dopamine receptor:

The neuroleptic/dopamine receptor, having much higher affinity and selectivity than the D_1 site for all neuroleptics, was first detected in 1975 by Seeman et al. (1975a, b). This receptor has been termed the D_2 dopamine receptor, as suggested by Spano (see Kebabian and Calne, 1979). The D_2 dopamine receptor inhibits adenylate cyclase in the anterior pituitary gland (De Camilli et al., 1979) and in the intermediate lobe of the pituitary (Meunier et al., 1980; Cote et al., 1981). There is good (but indirect) evidence for a similar type of inhibition in the brain striatum (Stoof and Kebabian, 1981). Fig. 2 depicts the fact that both the D_1 site and the D_2 receptor are located on the same post-synaptic membrane.

At present, the D_2 site is the only dopaminergic site (labelled by a ^{3}H-ligand) which warrants being called a "receptor". This is because the IC_{50} values of agonists and antagonists at this site correlate very well with their doses which elicit various dopaminergic behaviours (rotation, locomotion, anti-Parkinson action, psychotomimetic action, emesis and stereotypy), as exemplified in Fig. 3 (Seeman, 1980).

The D_2 dopamine receptor is experimentally characterized by its picomolar affinity for spiperone (an antagonist), and by having both nanomolar and micromolar affinity states for dopamine itself. Since spiperone recognizes both the D_2^{high} and the D_2^{low} states with equal affinity (60 pM), radioactive ^{3}H-spiperone (between 10 and 1000 pM) is used experimentally to measure the density of brain D_2 dopamine receptors (see Fig. 4).

Fig. 5 illustrates the two states of dopamine sensitivity of the D_2 dopamine receptor. The D_2^{high} state is sensitive to about 10 nM dopamine, while the D_2^{low} state is sensitive to approximately 2000 nM dopamine, these values being most readily apparent in the absence of NaCl.

The proportion of D_2 receptors in the high and low affinity states can be regulated by sodium ions (see Fig. 5), and by guanine nucleotides (Zahniser and Molinoff, 1978; De Lean et al., 1982; Sibley et al., 1982).

Up until now it has not been possible to convert *all* the *brain* D_2^{high} sites into D_2^{low} sites by means of high concentrations of guanine nucleotides (see Huff and Molinoff, 1982; Wreggett et al., 1982; Wreggett and Seeman, 1983a, b). A complete conversion has been found for D_2 receptors in anterior pituitary tissue (De Lean et al., 1982; Sibley et al., 1982), but not until now for brain tissue.

Figs. 5 and 6 illustrate for the first time a complete conversion of D_2^{high} into D_2^{low} in brain tissue. This conversion occurred at 37° in the presence of NaCl and a guanine nucleotide, and could only be demonstrated if allowance was made for the fact that ^{3}H-spiperone also labelled serotonin S_2 receptors in the rat brain striatum (List and Seeman, 1981; Wreggett and Seeman, 1983a, b).

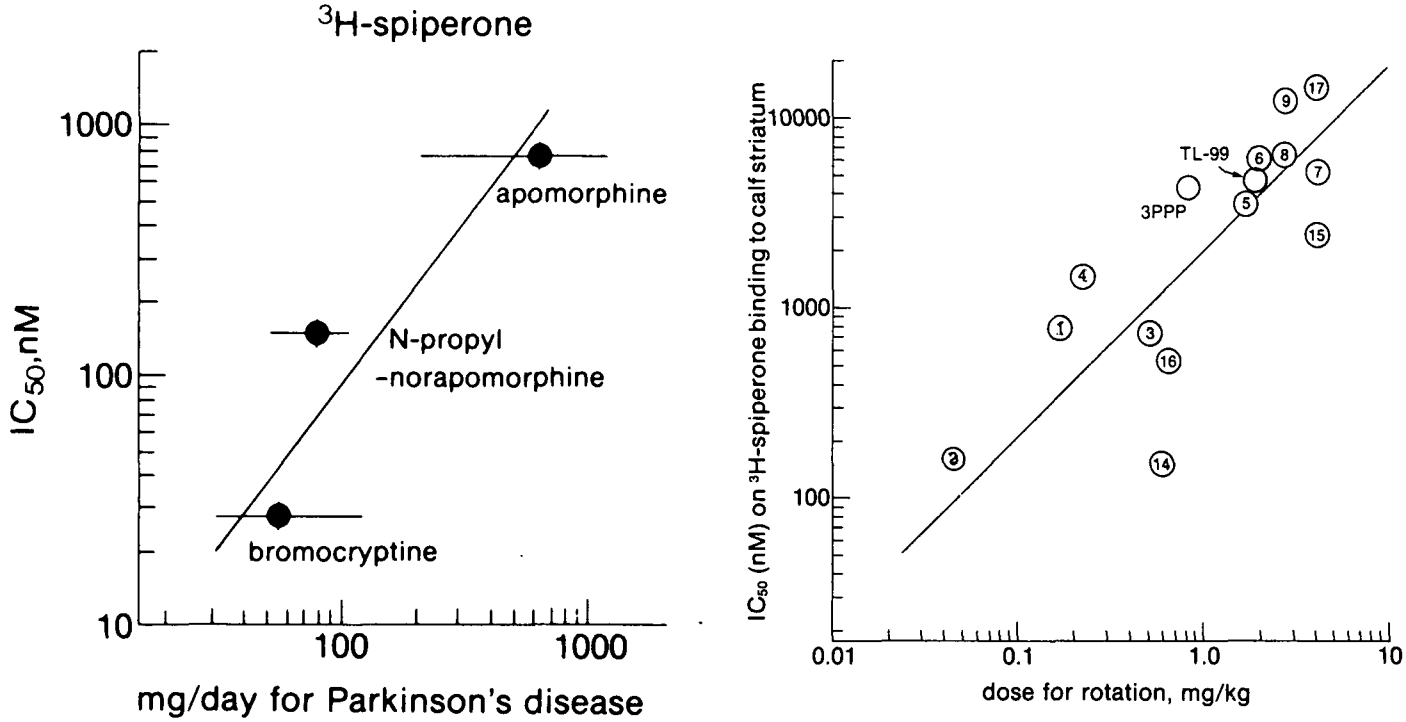

Figure 3 — The clinical doses for anti-Parkinson action correlate with the IC_{50} values for ^{3}H-spiperone at D_2 dopamine receptors. The doses of dopamine agonists which elicit contralateral turning (in 6-hydroxy-dopamine-lesioned rats unilaterally lesioned in the substantia nigra) correlate with the IC_{50} values for these dopamine agonists on ^{3}H-spiperone binding to D_2 dopamine receptors. Further details and references in Seeman (1980).

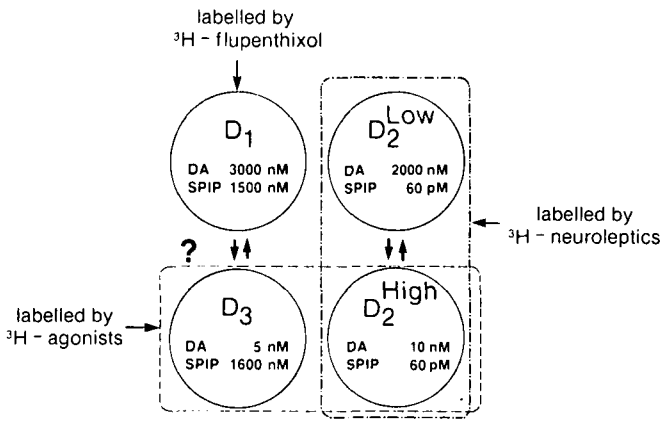

Figure 4 — Illustrating how the dopaminergic sites and receptor states are measured experimentally. For technical reasons, it is only possible to label sites which have an affinity for a ^{3}H-ligand which is less than 20 nM. The D_1 site has a 4 nM affinity for ^{3}H-flupenthixol. The D_2 dopamine receptor has a 60 pM affinity for ^{3}H-spiperone. Low concentrations of ^{3}H-dopamine readily label the D_3 site, as well as the D_2^{high} state, because of their high affinity for dopamine.

Fig. 5, for example, shows that (in the absence of NaCl) dopamine inhibited the binding of ^{3}H-spiperone (to rat brain striatum) in three phases. Dopamine's high-affinity phase of inhibition (D_2^{high}) had a dissociation constant of about 10 nM dopamine. The low-affinity phase of inhibition (D_2^{low}) had a dissociation constant of about 3000 nM dopamine. This experiment (Fig. 5) was done in the presence of 50 nM ketanserin which served to occlude as much as possible the serotonin sites from becoming occupied by ^{3}H-spiperone (see also List and Seeman, 1981; Wreggett and Seeman, 1983a, b).

The third phase, affected by 10^{-5} to 10^{-4}M dopamine, represents the displacement of ^{3}H-spiperone by dopamine at a serotonin receptor or site. This was shown by separate experiments where under conditions when all the D_2 receptors were selectively blocked (by 10 μM S-sulpiride), it was found that serotonin much more effectively inhibited (at 10^{-5}M) the binding of ^{3}H-spiperone than did dopamine (10^{-4}M). Thus, in the range between 10^{-5}M and 10^{-4}M, dopamine inhibits the binding of ^{3}H-spiperone to serotonin receptors. In other words, 50 nM ketanserin (in Fig. 5) was insufficient to occlude the serotonin sites from being occupied by ^{3}H-spiperone.

As Fig. 5 illustrates, dopamine inhibited the binding of ^{3}H-spiperone in three phases in the absence of NaCl, two of these phases being associated with the dopamine receptor, as mentioned above. In the presence of both NaCl and guanine nucleotide, however, dopamine exhibited two phases for the inhibition of ^{3}H-spiperone, one phase representing a single population of dopamine receptors completely in the D_2^{low} state, and the other phase representing a single population of serotonin receptors.

Fig. 6 also illustrates conversion in the presence of both NaCl and a guanine nucleotide. The example shown is for ADTN which inhibited the binding of ^{3}H-spiperone in three phases, the D_2^{high} phase having a K_D of 3.4 nM and the D_2^{low} phase having a K_D of 155 nM, and where the proportions of dopamine receptors in the high- and low-affinity phases were about equal (in the absence of NaCl). Although 50 nM ketanserin was used to occlude the serotonin sites, ADTN did displace ^{3}H-spiperone from a third site, the serotonin sites. ADTN recognized these sites at μM concentrations.

In the presence of NaCl (Fig. 6), many of the D_2^{high} sites were converted into the D_2^{low} state, since ADTN recognized

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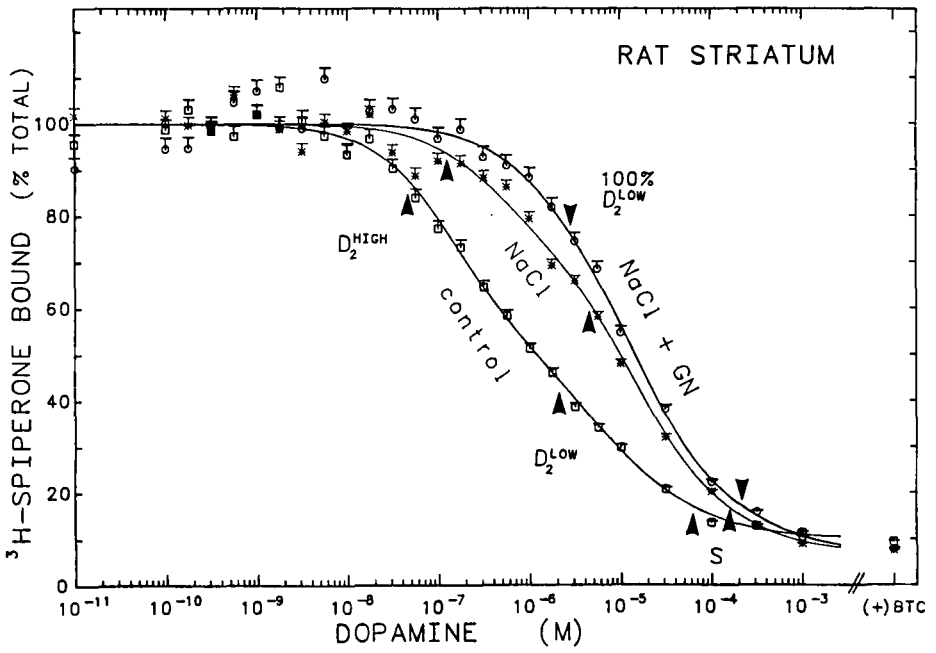

Figure 5 — Dopamine inhibits the binding of ^{3}H-spiperone to D_2 dopamine receptors in three phases (rat striatum). The high-affinity phase (D_2^{high}) occurs at about 10 nM (dissociation constant), while the low-affinity phase occurs at about 2000 nM (dissociation constant). Although 50 nM ketanserin was present to occlude as many serotonin sites as possible from being occupied by ^{3}H-spiperone, dopamine also inhibited binding of ^{3}H-spiperone at serotonin sites between 10^{-5} and 10^{-4}M dopamine. In the presence of NaCl and guanine nucleotide, however, dopamine inhibited the ^{3}H-spiperone fron binding at a single population of D_2^{low} receptors (K_D = 3000 nM dopamine) and at a single population of serotonin receptors (K_D = 200 μM dopamine). Thus, all the D_2^{high} sites had converted completely into the D_2^{low} state.

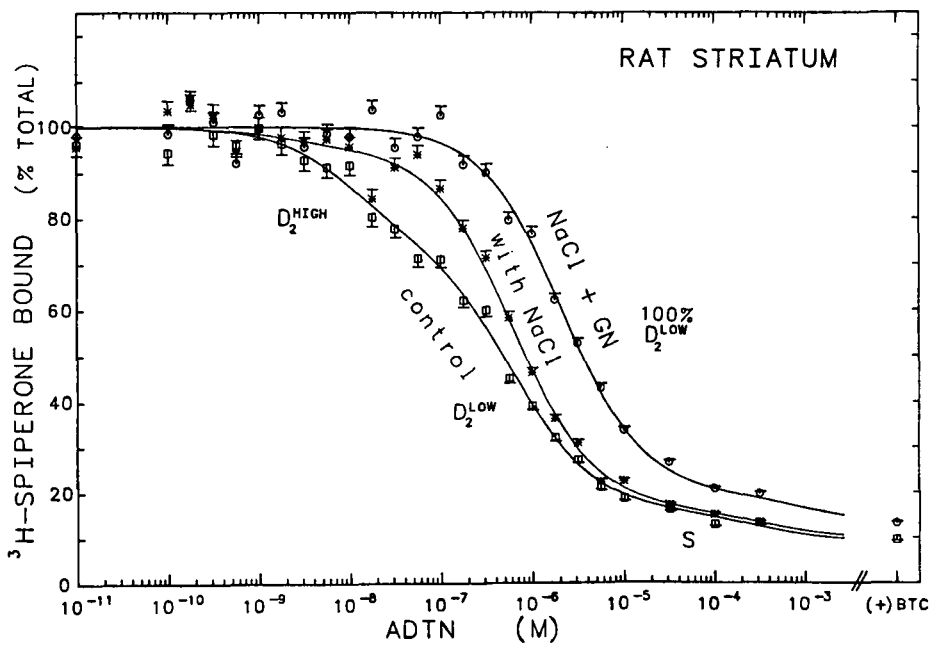

Figure 6 — Illustrating conversion of the D_2^{high} sites into the D_2^{low} state in rat striatum. In the control experiment (without NaCl; data on left side), the dopamine agonist (ADTN) revealed that 47% of ^{3}H-spiperone bound to dopamine receptors in the high state, and 45% to receptors in the low state with 8% to serotonin sites. The addition of NaCl resulted in 20% of the ^{3}H-spiperone binding to dopamine receptors in the high state, 75% to receptors in the low state, and 5% to serotonin receptors (see text). The addition of NaCl and guamine nucleotide (GN) resulted in more ^{3}H-spiperone binding (75%) to dopamine receptors completely (100%) in the low-affinity state with 5% binding to serotonin receptors. Although 50 nM ketanserin was present throughout to block serotonin receptors, this was insufficient to block all the serotonin sites.

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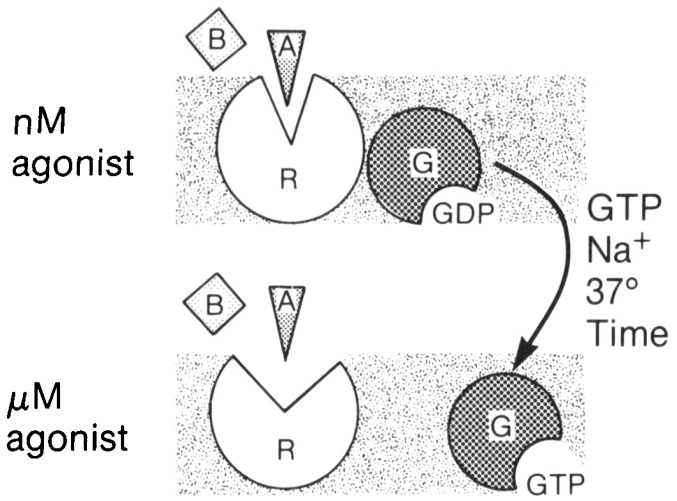

Figure 7 — Diagram of a ternary complex model for the two states of the D_2 dopamine receptor. A = nM dopamine agonist; B = neuroleptic; G = nucleotide-binding protein; R = dopamine receptor.

20% of the ^{3}H-spiperone at D_2^{high} sites (K_D = 3.7 nM ADTN), 75% at D_2^{low} sites (K_D = 187 nM ADTN) and 5% at S_2 sites (K_D = 6.7 μM ADTN).

Fig. 7 is a diagram illustrating the foregoing interpretation. This Figure merely depicts a ternary complex model for the dopamine receptor (De Lean et al., 1982).

We have previously used the term "D_4" to denote D_2^{high} (Seeman, 1980), because it was then not possible to know whether all the D_2^{high} sites could be converted into the D_2^{low} state. Thus, D_4 was a term that could be applied to conversion-resistant D_2 sites. Fig. 5, however, indicates that complete conversion does occur in brain tissue, and, thus, there is no longer any need to use the D_4 nomenclature.

The D_3 dopaminergic site:

The D_3 dopaminergic site is defined by its high affinity (nM) for dopamine and its low affinity (uM) for neuroleptics (List et al., 1979, 1980; Sokoloff et al., 1980). The rank order of potencies of the dopaminergic congeners at the D_3 site generally follows that for the D_2 sites, with one important exception: bromocriptine is particularly weak at the D_3 site.

There is as yet no known functional correlate of the D_3 site. Creese (1981) has suggested that it may be a different state of the D_1 site. Certainly this would be consistent with the fact that bromocriptine is weak at both the D_1 and D_3 sites.

Earlier work had suggested that the D_3 site was located on the nigrostriatal dopamine neurones since the density of these D_3 sites, as detected by ^{3}H-dopamine binding, were reduced in the putamen and caudate nucleus in Parkinson's disease (Lee et al., 1981). More recently, however, it has been found by Creese and colleagues that the amount of ^{3}H-dopamine binding appears to depend on the amount of endogenous dopamine in the tissue. Thus, if the dopamine content is low, as in Parkinson's disease striatum, then the amount of ^{3}H-dopamine binding would also be low.

Presynaptic receptors and autoreceptors for dopamine:

There is a considerable literature on presynaptic dopamine receptors (see Fig. 1; Seeman, 1982, for references). Certain adrenergic nerve terminals in the peripheral nervous system contain dopamine receptors which inhibit the release of noradrelanine. These dopamine receptors, termed DA_2 receptors, have sensitivities to dopamine agonists and antagonists which are virtually identical to those for the D_2^{high} dopaminergic sites in the central nervous system. This similarity suggests that the central D_2^{high} sites and the peripheral DA_2 sites may be identical.

The presynaptic dopamine receptors (autoreceptors) are sensitive to nanomolar concentrations of dopamine as well as to nanomolar concentrations of neuroleptics. Thus, the autoreceptors may be synonymous with the D_2^{high} site. Thus, if autoreceptors are indeed similar to the D_2^{high} site, one ought to detect a correlation between drug action on autoreceptors with drug action on the binding of ^{3}H-spiperone. In fact, such a correlation does exist (Fig. 8).

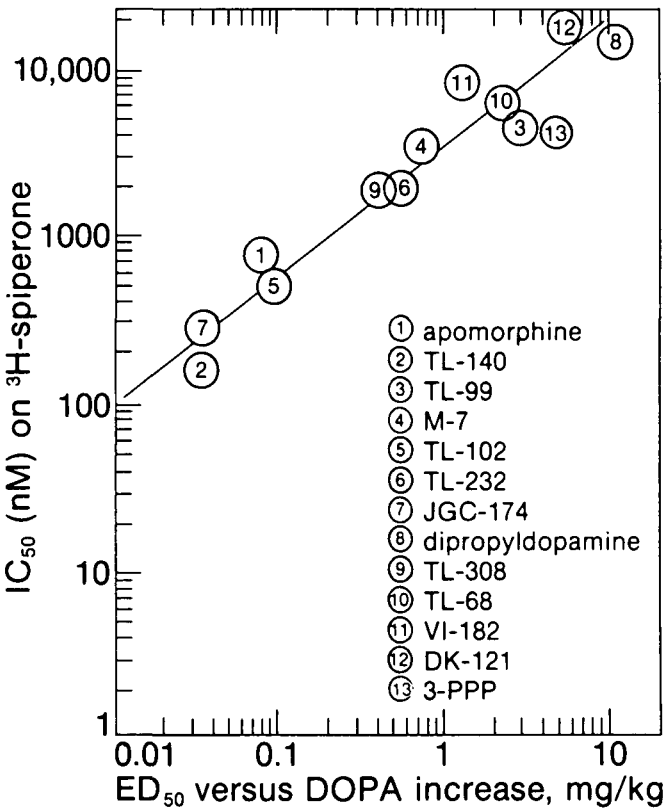

Figure 8 — The in vivo *potencies of various dopamine agonists on dopamine autoreceptors correlate very well with the potencies of these agonists to inhibit the binding of ^{3}H-spiperone to D_2 dopamine receptors. The ED_{50} values were the doses that reversed the gammabutyrolactone-induced elevation of DOPA by 50% (see Rusterholz et al., Goodale et al., and R.P. Long References in Seeman, 1980). The IC_{50} values are the concentrations which 50% inhibited the specific binding to calf brain striatum (Seeman, 1980). Adapted from Seeman (1980), which contains further details and chemical structures of the agonists.*

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