A COMPARATIVE STUDY OF [^{3}H]HALOPERIDOL AND [^{3}H]SPIROPERIDOL BINDING TO RECEPTORS ON RAT CEREBRAL MEMBRANES

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1. Introduction

The development of receptor binding techniques has greatly assisted in the identification, localisation and characterisation of neurotransmitter receptors in the central nervous system. This approach has been of considerable interest in the case of the dopamine receptor since there is evidence that malfunction of this neurotransmitter system may relate to certain neurological [1] and psychiatric disorders [2].

A number of laboratories have reported that [^{3}H]-haloperidol binds with high affinity in a saturable manner to membranes prepared from cerebral areas rich in dopamine, and the potency of a number of neuroleptic drugs to displace the high affinity binding component of this ligand closely matches their clinical efficacy [3–5]. However, the specific binding of [^{3}H]haloperidol is small (30–40% total binding) and in our hands preliminary data suggested that this neuroleptic binds to more than one population of 'specific' membrane sites. It was felt therefore, that this ligand might not be suitable for the accurate assessment of binding sites particularly in areas with low numbers of dopamine receptors.

Spiroperidol is more potent than haloperidol in its ability to block dopamine receptors, assessed behaviourally [6] and biochemically [4] and a recent abstract has reported that this neuroleptic binds with high affinity to membranes prepared from rat and human corpus striatum [7]. In this communication we have compared the binding characteristics of this new ligand, with those of [^{3}H]haloperidol, to membranes prepared from various rat cerebral areas.

2. Materials and methods

[^{3}H]Spiroperidol (26 Ci/mmol) and [^{3}H]haloperidol (13 Ci/mmol) were obtained from New England Nuclear Co. All other drugs were obtained from commercial sources.

Male Wistar rats (120–150 g) were used in all experiments. The animals were decapitated, the brain removed and dissected on ice as follows. Using the point of divergence of the optic nerves as a guide, a coronal cut was made 1 mm forward of this, thus excising the rostral part of the brain. A further cut was made 2 mm caudal to this resulting in a transverse slice of tissue. From this slice both the corpora striata were removed and, with cuts through both rhinal sulci at a tangent to the original position of the striata, an area of cortex was separated from the limbic forebrain. The head of the corpus striatum was dissected from the rostral piece of brain tissue and included with that from the slice.

Tissue was homogenised in 40 vol. ice cold 0.32 M sucrose. A crude purification was achieved by an initial centrifugation at 1000 × *g* for 15 min at 4°C, the pellet being discarded and the supernatant centrifuged at 50 000 × *g* for 45 min. This pellet was rehomogenised in approx. 10 vol. buffer containing 15 mM Tris–HCl, pH 7.4, 5 mM Na_2EDTA, 1.1 mM ascorbate and 12.5 μM nialamide. The homogenate was incubated at 37°C for 15 min and then stored at −20°C for future use.

In the binding assays, membrane suspensions (100–600 μg protein) were incubated at 37°C with [^{3}H]spiroperidol (0.05–2.0 nM) or [^{3}H]haloperidol

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(0.5–16 nM) and appropriate concentration of drug under test, in 50 mM Tris–HCl buffer, pH 7.8, in final vol. 1 ml for [^{3}H]spiroperidol and 0.5 ml for [^{3}H]haloperidol. After 15 min the samples were rapidly diluted with 2 ml ice cold buffer and filtered under reduced pressure through Whatman glass fibre discs (GF/B) and the filters washed with 3 X 4 ml buffer. The filters were then shaken with Triton X 100–toluene scintillator, and the radioactivity determined by liquid scintillation counting. In every experiment non-specific binding was determined by measuring the radioactivity obtained when incubations were carried out in the presence of 300 μM dopamine, and specific binding was defined as the difference between total and non-specific binding.

3. Results and discussion

3.1. *Specific binding of [^{3}H]haloperidol and [^{3}H]-spiroperidol to membranes from various regions of rat brain*

The specific binding of both neuroleptic drugs to cerebral membranes was saturable and of high affinity while the non-specific component increased linearly over the range of ligands used. Specific [^{3}H]spiroperidol binding constituted 80–90% total binding at half saturation in striatal membranes (fig.1). Scatchard analysis of the specific binding isotherm yields an apparent dissociation constant (K_d) of 0.14 nM and receptor density 0.54 pmol/mg protein. The specific binding of [^{3}H]haloperidol, on the other hand, was of considerably lower affinity (K_d 2.3 nM) and only constituted 30–40% total binding (fig.1). However, an almost identical number of binding sites were labelled by both dopaminergic ligands in rat striatal membranes.

At concentrations greater than 1.2 nM [^{3}H]spiroperidol and 10–12 nM [^{3}H]haloperidol, Scatchard analysis revealed the presence of lower affinity binding sites (fig.2). This low affinity component was present to varying degrees in different brain areas and we are at present attempting to evaluate the significance of these sites.

The affinity of [^{3}H]spiroperidol and [^{3}H]haloperidol for membranes prepared from the limbic forebrain, cortex and cerebellum were remarkably similar to the respective values for the ligands in the striatum (table 1). Moreover, the large regional variation in receptor density was reflected by both [^{3}H]neuroleptics suggesting that both ligands bind to the same receptor sites. The only disparity in this comparison was observed in the limbic forebrain where dopamine displacable [^{3}H]spiroperidol binding was greater than [^{3}H]haloperidol binding. However, recent experiments

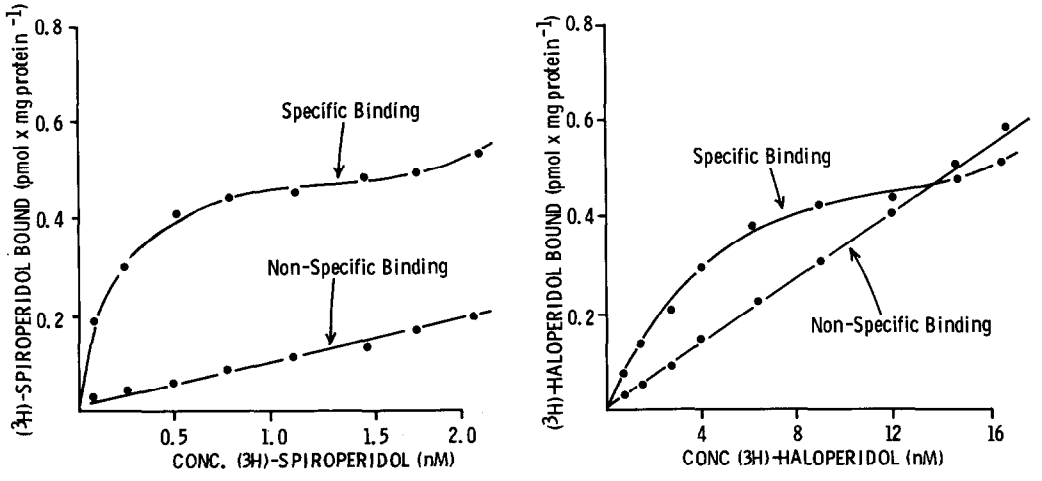

Fig.1. Binding of [^{3}H]spiroperidol and [^{3}H]haloperidol to rat corpus striatum membranes. Tissue was incubated with increasing concentrations of ^{3}H-labelled ligand. Non-specific binding is that observed in the presence of 300 μM dopamine. Binding in the absence of 300 μM dopamine less the non-specific binding represents specific binding.

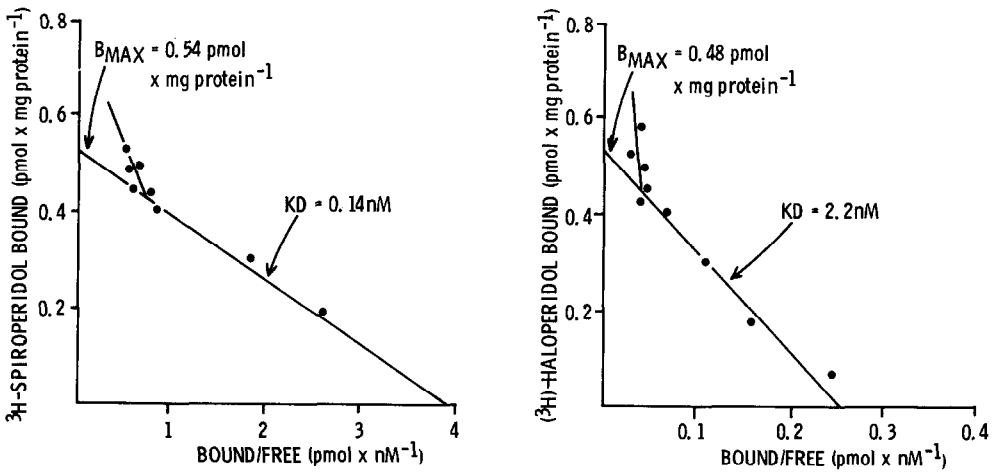

Fig.2. Scatchard analysis of [^{3}H]spiroperidol and [^{3}H]haloperidol binding to rat corpus striatum membranes. Points have been plotted from the data in fig.1.

(in preparation) suggest that whereas in cortex and striatum dopamine maximally displaces [^{3}H]spiroperidol to the same degree as competing cold neuroleptics, this catecholamine inhibits binding to a greater extent than neuroleptics in the limbic forebrain. The nature of this discrepancy remains to be established.

3.2. *Pharmacological characterisation of [^{3}H]neuroleptic binding sites*

Specific binding of [^{3}H]spiroperidol and [^{3}H]haloperidol to striatal membranes was displaced by low concentrations of neuroleptic drugs. This displacement was stereospecific for flupenthixol and butaclamol, α-(*cis*)-flupenthixol and (+)-butaclamol being considerably more potent than β-(*trans*)-flupenthixol and (−)-butaclamol, respectively (fig.3). Spiroperidol was the most potent drug in its ability to displace specific [^{3}H]spiroperidol binding and the affinity constants (K_i) for this drug and haloperidol agree well with their dissociation constants (K_d) obtained by direct binding. The relative potencies of the other neuroleptics to compete for [^{3}H]spiroperidol binding sites in rat striatum (table 2) are more or less identical with those obtained against [^{3}H]haloperidol by ourselves (not shown) and by other workers [3,4] and bear a good correlation with the clinical potencies of the neuroleptics [3]. α and β Adrenoceptor antagonists were considerably weaker than the neuroleptics and of the agonists apomorphine and dopamine were more potent than noradrenaline and isoprenaline (table 2).

Table 1
Affinity and regional distribution of [^{3}H]spiroperidol and [^{3}H]haloperidol binding to rat cerebral membranes

	[^{3}H]Spiroperidol binding		[^{3}H]Haloperidol binding	
	K_d (nM)	B_{max} (pmol · mg protein^{-1})	K_d (nM)	B_{max} (pmol · mg protein^{-1})
Corpus striatum	0.14	0.54	2.2	0.48
Limbic forebrain	0.12	0.29	2.3	0.16
Cortex	0.15	0.23	2.3	0.24
Cerebellum	0.27	0.06	2:3	0.04

Data was calculated by Scatchard analysis from triplicate determinations

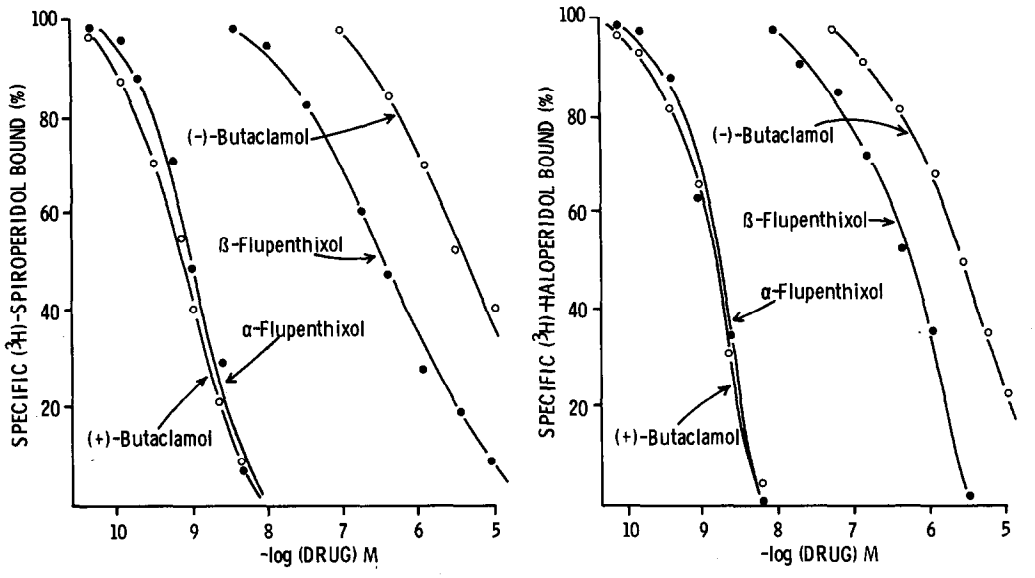

Fig.3. Competition for [^{3}H]spiroperidol and [^{3}H]haloperidol binding sites on rat striatal membranes. Increasing concentrations of each non-radioactive drug were added to tubes containing approx. 0.5 nM [^{3}H]spiroperidol or 4 nM [^{3}H]haloperidol. Specific binding is that which was displaced by 300 μM dopamine. Each point is the mean of duplicate determinations.

Table 2
Drug inhibition of [^{3}H]spiroperidol and [^{3}H]haloperidol binding to rat striatal membranes

Drug	K_i (nM)	
Spiroperidol	0.29 ± 0.03	
(+)-Butaclamol	0.80 ± 0.09	
α-Flupenthixol	0.90 ± 0.09	
Fluphenazine	2.0 ± 0.3	
Haloperidol	2.2 ± 0.2	
Trifluperazine	4.4 ± 0.5	
Thioridazine	9.8 ± 1.4	
β-Flupenthixol	45 ± 7	
Cyproheptadine	60 ± 8	(2)
Apomorphine	140 ± 18	
(−)-Butaclamol	500 ± 90	
Dopamine	1200 ± 160	
Phentolamine	1900 ± 230	
(+) or (−) Propranolol	5000 ± 650	
(−)-Noradrenaline	10 000 ± 1600	
Isoprenaline	> 100 000 ±	(2)
Clonidine	> 100 000 ±	(1)
Metiamide	> 100 000 ±	(1)

Membranes were incubated with at least 5 concentrations of drug and the K_i value calculated from the equation $K_i = IC_{50}/(1 + s/K_d)$, where IC_{50} is the concentration of drug required to inhibit specific binding by 50%, s is the concentration of radioactive ligand (approx. 0.5 mM [^{3}H]spiroperidol) and K_d is the dissociation constant. Results are means ± standard errors for 3 experiments, except where indicated

4. Conclusions

It appears from the binding properties, regional distribution and pharmacological characterisation, that [^{3}H]spiroperidol and [^{3}H]haloperidol bind to identical sites in rat brain. Moreover, as suggested previously, the distribution of the binding sites and potencies of dopaminergic agonists and antagonists indicate that the specifically labelled sites are closely associated with the 'dopamine receptor'. Thus the sites show a crude correlation with the degree of dopaminergic innervation in the different cerebral regions with the striatum showing the greatest enrichment of binding sites and the cerebellum the least. The appreciable number of receptors found in the area of cortex used in this study possibly relates to the supragenual and suprarhinal dopaminergic systems described [8].

Spiroperidol is one of the most potent neuroleptics assessed by pharmacological and clinical studies [6]. The present findings indicate that its high affinity binding and consequent high specific/non-specific binding ratio make it a much better ligand than [^{3}H]-haloperidol for 'dopamine receptor' binding studies.

Acknowledgements

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