[^{3}H]SPIROPERIDOL BINDING TO BRAIN NEUROTRANSMITTER RECEPTORS

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1. Introduction

[^{3}H]Spiroperidol (spiperone) has been widely used to study dopamine receptors in brain using ligand-binding assays [1–4]. This ligand will also bind to serotonin receptors [5], so the interpretation of [^{3}H]spiroperidol binding data may be complex where a tissue contains both dopaminergic and serotoninergic sites. In the striatum it has been assumed that [^{3}H]spiroperidol binding is largely to dopaminergic sites [5,6]. However, several reports have appeared suggesting heterogeneous populations of striatal binding sites [7–10] although the nature of the sites has not been determined. Here we present the results of some experiments designed to provide a quantitative picture of [^{3}H]spiroperidol binding in bovine caudate nucleus (a major component of the striatum); we show that [^{3}H]spiroperidol may be used to label either dopaminergic or serotoninergic sites in caudate nucleus and that this tissue contains comparable numbers of these 2 types of site.

2. Materials and methods

[^{3}H]Spiroperidol (21 Ci/mmol) was obtained from The Radiochemical Centre, Amersham. We acknowledge generous gifts of (+)-butaclamol (Ayerst Labs., Farnborough), mianserin hydrochloride (Beecham Pharmaceut. Ltd., Brentford) and spiroperidol (Janssen Pharmaceut., Marlow). All other chemicals were of the highest purity available and were obtained from commercial sources.

[^{3}H]Spiroperidol binding to a microsomal preparation of bovine caudate nucleus, obtained as in [11], was assayed by a rapid filtration method. [^{3}H]Spiroperidol (0.01–5 nM) was incubated with bovine caudate microsomal preparation (0.1 mg protein/ml) at 25°C for 20 min with the addition of competing ligands where appropriate in 1 ml final vol. of a Hepes–phosphate–saline buffer (pH 7.4) [12] containing 0.1 mM dithiothreitol and 10 μM pargyline. At the end of the incubation 5 ml ice-cold buffer was added and the mixture was filtered rapidly under vacuum through a presoaked Whatman GF/F glass fibre filter. The filter was washed quickly with 2 portions (5 ml) of ice-cold buffer and transferred to 10 ml scintillant (as in [12] or Fisofluor 1 (Fisons Ltd., Loughborough)) containing 1 ml water, for determination of bound radioactivity. Free [^{3}H]-spiroperidol concentrations were determined for each assay by subtraction according to [12]. Total specific [^{3}H]spiroperidol binding was defined as that binding displaceable by 1 μM non-radioactive spiroperidol and constituted ~90% of the total binding (av. free [^{3}H]spiroperidol 0.23 nM). The total specific binding could be divided into a dopaminergic component (displaced by 0.1 mM dopamine) and a serotoninergic component (displaced by 0.3 μM mianserin) as discussed below.

3. Results and discussion

In initial experiments, the characteristics of the total specific binding of [^{3}H]spiroperidol to a microsomal preparation of bovine caudate nucleus were assessed in displacement experiments. The neuroleptic drug (+)-butaclamol displaced the specific binding potently [fig.1; IC_{50} ([drug] giving 50% inhibition of binding) 6 nM] in agreement with [1–4] but there was little indication from the data that the drug could distinguish multiple classes of binding site. When the selective serotonin receptor antagonist mianserin [13] was used, however, the displacement data (fig.1) were clearly biphasic suggesting 2 separate classes of

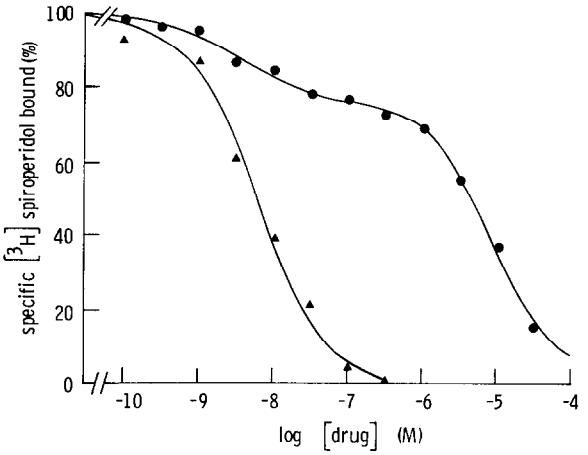

Fig.1. Displacement of [^{3}H]spiroperidol binding by mianserin (•) and (+)-butaclamol (▲). Increasing concentrations of either drug were included in the standard assay and points were determined in duplicate (av. free [^{3}H]spiroperidol 0.23 nM (•), 0.21 nM (▲)). Theoretical curves are shown for binding of (+)-butaclamol to a single class of sites and for binding of mianserin to 2 classes of site as described.

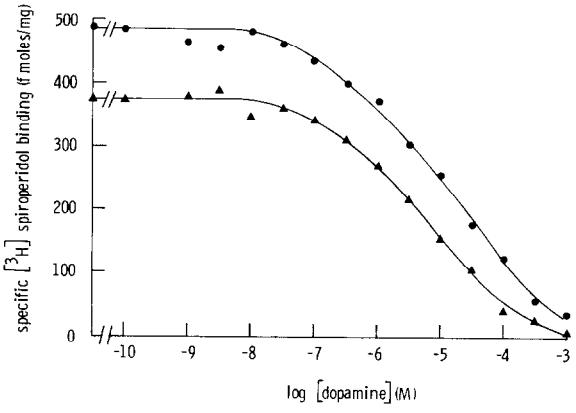

Fig.2. Displacement of [^{3}H]spiroperidol binding by dopamine in the presence (▲) and absence (•) of 0.1 μM mianserin. Increasing concentrations of dopamine were included in the standard assay using an av. free [^{3}H]spiroperidol 0.23 nM. Each point was determined in quadruplicate and a parallel experiment was performed including 0.1 μM mianserin throughout.

binding site with different affinities for mianserin. The data were resolved into displacement at 2 classes of site:

(i) Sites constituting 23% of the total specific binding and giving an IC_{50} for mianserin of 3 nM which is likely to be serotoninergic;

(ii) Sites constituting 77% of the total specific binding giving an IC_{50} for mianserin of 9 μM which is likely to be dopaminergic. High concentrations of (+)-butaclamol or mianserin displaced all the specifically bound [^{3}H]spiroperidol showing that each ligand was binding to the same total number of binding sites. Consequently, specific [^{3}H]spiroperidol binding defined by displacement with (+)-butaclamol (0.1–1 μM) will contain dopaminergic and serotoninergic contributions.

Specific [^{3}H]spiroperidol binding was also displaced by agonist ligands including dopamine. Displacement by dopamine occurred over a wide range of concentrations (fig.2) and the binding data did not conform to that predicted for combination at a single class of sites (Hill slope 0.45). Dopamine displaced all the specific binding at high concentrations indicating that it binds to the serotoninergic sites as well as the dopaminergic sites. When 0.1 μM mianserin was included throughout (in order to eliminate [^{3}H]-spiroperidol binding to the serotoninergic sites without affecting binding at the dopaminergic site, see fig.1), the shape of the dopamine displacement curve (fig.2) was largely unaltered (Hill slope 0.5) indicating that the binding of dopamine to the dopaminergic sites alone showed complex characteristics. There was, however, a slight shift in the IC_{50} for dopamine displacement (from 13 μM without mianserin to 5 μM with mianserin) indicating that binding of dopamine at the serotoninergic sites occurred only at high dopamine concentrations (>0.1 mM).

These results suggested that the dopaminergic and serotoninergic binding sites could be examined separately using [^{3}H]spiroperidol binding with an appropriate choice of condition. The dopamine displacement data suggested that specific [^{3}H]spiroperidol binding to the dopaminergic sites may be defined as that binding displaceable by 0.1 mM dopamine whereas the mianserin displacement data suggested that specific [^{3}H]spiroperidol binding to the serotoninergic sites may be defined as that binding displaceable by 0.3 μM mianserin. Accordingly, saturation binding experiments were performed with increasing concentrations of [^{3}H]spiroperidol:

(i) Using 0.1 mM dopamine to define specific dopaminergic binding:

(ii) Using 0.3 μM mianserin to define specific serotoninergic binding.

The results were analysed using Scatchard plots (fig.3)

and indicated a single class of dopaminergic binding sites for [^{3}H]spiroperidol (fig.3A) (K_d 80 pM; 610 fmol/mg protein) and a single class of serotoninergic binding sites for [^{3}H]spiroperidol (fig.3B) (K_d 1.0 nM; 718 fmol/mg protein). The use of 0.1 mM dopamine therefore offers an alternative to the use of 2-amino-6,7-dihydroxytetralin suggested in [14] for defining the dopaminergic sites.

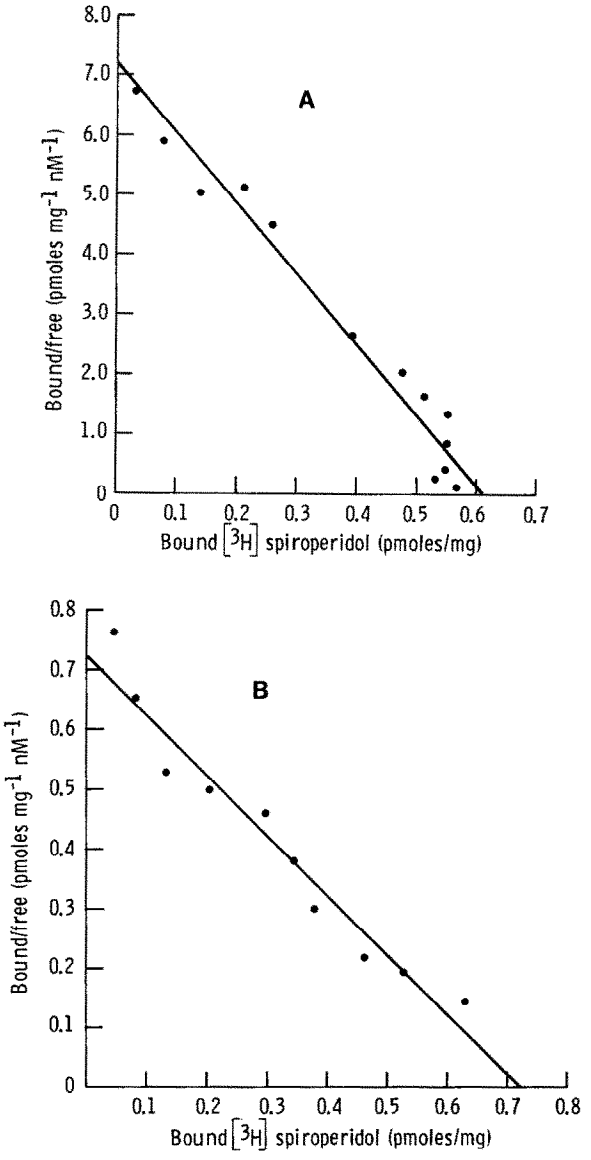

Fig.3. Scatchard analysis of [^{3}H]spiroperidol binding to dopaminergic receptors (A, defined using 0.1 mM dopamine) and serotoninergic receptors (B, defined using 0.3 μM mianserin).

In conclusion, these results show that there are comparable numbers of dopaminergic and serotoninergic binding sites for [^{3}H]spiroperidol in bovine caudate nucleus. As the 2 classes of site have different affinities for the radioligand, the relative proportions of each site labelled will depend upon the radioligand concentration, so that care must be exercised in the interpretation of ligand-binding assays using [^{3}H]-spiroperidol in this tissue, especially if (+)-butaclamol is used to define the specific binding. It is clear, however, that using appropriate conditions, it is possible to use a single radioligand, [^{3}H]spiroperidol, for studying dopaminergic or serotoninergic receptors.

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