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D_2 DOPAMINE RECEPTORS IN RAT STRIATUM ARE HOMOGENEOUS AS REVEALED BY LIGAND-BINDING STUDIES

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Abstract—D_2 dopamine receptors in rat striatum have been analysed using the binding of [^{3}H]domperidone. Competition experiments were performed with classical dopamine antagonists and antagonists reported by other workers to discriminate D_2 dopamine receptor sub-classes. In all cases competition data conformed to a single binding site interaction so that there is no evidence for heterogeneity of the D_2 dopamine receptor.

D_2 dopamine receptors have been studied extensively using the radioligand-binding technique with a variety of radioligands [1]. Although generally D_2 dopamine receptors appear homogeneous with respect to antagonist binding [2, 3], Sokoloff and colleagues have reported data consistent with subtypes of the D_2 dopamine receptor which they have termed D_2 and D_4. Whilst both subtypes display equal affinities for binding classical D_2 dopamine receptor antagonists, the D_4 subtype displays a selectivity for binding certain substituted benzamide drugs [4, 5]. In addition agonist binding to D_2 sites is more sensitive to guanine nucleotides than agonist binding to D_4 sites.

Using [^{3}H]spiperone as radioligand we have failed to find any evidence for such receptor subtypes in bovine brain [6]. Sokoloff and colleagues, however, used rat brain and [^{3}H]domperidone as radioligand [4, 5, 7] in their studies reporting receptor subtypes. Accordingly we have investigated these possible receptor subtypes in rat brain using [^{3}H]domperidone binding and can find no evidence for such subtypes.

EXPERIMENTAL SECTION

Materials

[^{3}H]Domperidone (Benzene ring ^{3}H, 56.4 Ci/mmole) was purchased from New England Nuclear, bovine serum albumin was from Sigma (London). All other chemicals were obtained from the suppliers given in Refs 2 and 3.

Methods

Preparation of rat striatal homogenate. Twenty random-bred male albino rats (Glaxo AHA strain), about 200 g were killed by cervical dislocation, the striata were rapidly dissected out and pooled into 20 vol. of HEPES (20 mM) buffered sucrose (0.32 M) (pH 7.4 at 4°) and homogenised in a glass–Teflon homogeniser (0.18 mm radial clearance) at 1000 rpm for 10 strokes. The homogenate was centrifuged (50,000 *g*, 20 min), the supernatant was discarded and the pellet was resuspended in 15 vol. of HEPES buffered sucrose, pH 7.4 at 4° and recentrifuged under the same conditions. The wash was again repeated and the final pellet was resuspended in 4 vol. of HEPES buffered physiological medium [8] at a protein concentration of about 2 mg/ml. The final homogenate was divided into 2 ml aliquots and stored at −70° for up to 2 weeks. Protein concentration was determined by a modification of the method of Lowry *et al.* [9] using bovine serum albumin as a standard and employing a precipitation step with trichloroethanoic acid (10%).

[^{3}H]*Domperidone binding assay.* [^{3}H]Domperidone binding to rat brain dopamine D_2 receptors was determined essentially as described in [10]. Briefly, brain homogenate (0.15 mg protein/ml) was incubated at 30° for 30 min in triplicate in a final volume of 1 ml of HEPES buffered physiological medium [8] with [^{3}H]domperidone (0.25 nM for displacement studies, 25 pM–5 nM for saturation analyses) and other drugs at the concentrations indicated.

In order to terminate the assays the contents of each assay tube were filtered through Whatman GF/B glass fibre filter sheets on a Brandel cell harvester and the filter was washed with 15 ml of ice-cold sodium chloride (150 mM), sodium phosphate (10 mM), bovine serum albumin (0.01% w/v) pH 7.4. Filters were dried by a stream of air and the radioactivity was determined by liquid scintillation spectroscopy.

Data analysis. Saturation binding and displacement data were analysed by non-linear, least squares computer curve fitting as described in [2]. Where necessary, data from saturation analysis were corrected for the amount of total radioligand bound to membranes as described in [8]. The amount of total radioligand bound was generally less than 10% and always less than 15%.

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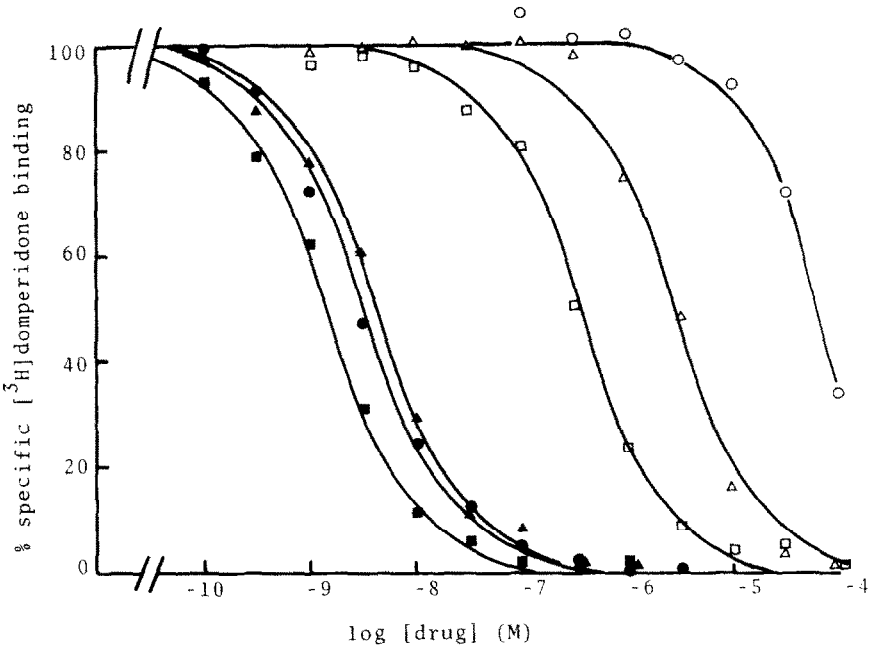

Fig. 1. Displacement of [^{3}H]domperidone binding to rat striatal D_2 dopamine receptors by (+)-butaclamol (●), (−)-butaclamol (○), haloperidol (■), α-flupenthixol (▲), ketanserin (□) and mianserin (△). Displacement experiments were performed as described. Data points are the means of triplicate determinations from representative experiments replicated as in Table 1. Errors were generally less than 10%. The curves are the best fit curves to a one binding site model (equation 1 of [2] with n = 1) (IC_{50} values (nM) (+)-butaclamol 3, haloperidol 1.4, α-flupenthixol 4, ketanserin 330, mianserin 2700, the curve for (−)-butaclamol was fitted by eye).

RESULTS

The binding of [^{3}H]domperidone to rat striatal homogenates could be displaced by a series of dopamine antagonists (α-flupenthixol, (+)-butaclamol, haloperidol) and the different antagonists each displaced to the same plateau level of binding when used at concentrations of 0.1–1 μM (Fig. 1). Analysis of the displacement curves indicated that binding was to a single class of sites in each case as the data fitted well to one binding site models. This displaceable binding has been taken as specific [^{3}H]domperidone binding and has been defined in subsequent experiments as that displaceable by 1 μM (+)-butaclamol. Specific binding comprised typically 55–60% of the total binding but non-specific binding could be reduced by the inclusion of 0.01% bovine serum albumin in the assay wash buffer (Fig. 2) when specific binding amounted to 70% of the total. As this level of albumin had no effect on the absolute level of specific binding (data not shown) it was included in all assays hereafter. Specific [^{3}H]domperidone binding was saturable and of high affinity (K_d 0.47 ∓ 0.22 nM, B_{max} 429 ∓ 25 fmol/mg, mean ∓ SD, 3 experiments) as defined by saturation analysis and a similar value was derived from association and dissociation rate experiments (k_{+1} 0.32 ∓ 0.01 nM^{-1} min^{-1}; k_{-1} 0.12 ∓ 0.01 min^{-1}, mean ∓ SD, 3 experiments, K_d 0.37 nM). Saturation analysis indicated that binding of [^{3}H]domperidone was to a single class of sites (Hill coefficient of one), these are D_2 dopamine receptors as defined by the displacement data cited earlier. D_1 receptor binding is absent as all the [^{3}H]domperidone binding can be displaced by sulpiride with high affinity and a pseudo Hill coefficient close to one. Similarly $5HT_2$ serotonin receptor binding and α_1-adrenergic receptor binding are absent as displacements by mianserin, ketanserin and prazosin are of low affinity (Fig. 1, Table 1).

Displacement of [^{3}H]domperidone was then attempted with compounds reported to discriminate between putative D_2 receptor subtypes in *in vitro* and *in vivo* tests [4, 5, 7] (substituted benzamides DO710 and sulpiride) and *in vivo* tests [11] (dibenzazepines clozapine and loxapine). Each of these compounds displaced all the specifically bound [^{3}H]domperidone (Fig. 3) and when the displacement data were analysed, pseudo Hill coefficients close to one were obtained (Table 1). Displacement data were in fact fitted very well by one binding site models (Fig. 3) so there is no evidence for heterogeneity of D_2 dopamine receptors in these experiments.

DISCUSSION

In this report we have examined the ligand-binding properties of D_2 dopamine receptors in rat striatum using the binding of [^{3}H]domperidone. This radioligand provides a selective tool for labelling D_2 receptors as shown here. Displacement of

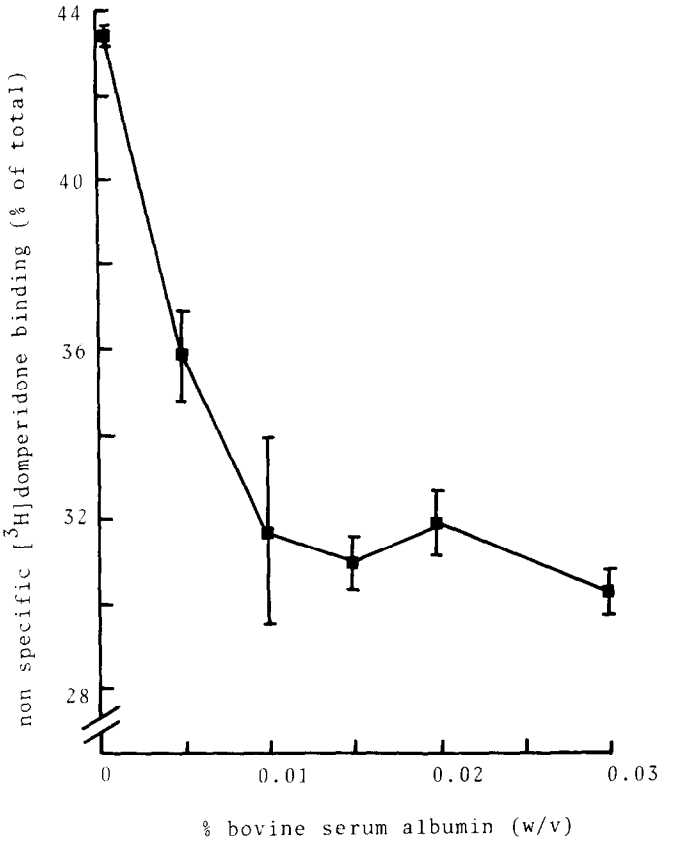

Fig. 2. The effect of bovine serum albumin in assay wash buffer on non-specific [^{3}H]domperidone binding to rat striatal membranes. Non-specific [^{3}H]domperidone binding was defined as the binding in the presence of 1 μM (+)-butaclamol. Different concentrations of bovine serum albumin were included in the assay wash buffer. Data points are mean ∓ range of duplicate determinations.

Table 1. Displacement of [^{3}H]domperidone binding from rat striatal D_2 dopamine receptors

Displacing substance	K_i (nM)	Number of experiments
(+)-Butaclamol	1.5 ∓ 0.4	3
(−)-Butaclamol	>10,000	2
Haloperidol	0.5 ∓ 0.1	3
α-Flupenthixol	2.4 ∓ 0.7	3
Ketanserin	105 ∓ 11	3
Mianserin	980 ∓ 50	3
Prazosin	>10,000	1
Substituted benzamides		
DO710	0.7 ∓ 0.1	3
Sulpiride	5.3 ∓ 0.4	3
Dibenzazepines		
Clozapine	81.2 ∓ 5.8	3
Loxapine	7.5 ∓ 0.7	3

Displacement of [^{3}H]domperidone binding was performed as in Figs 1 and 3 and data were analysed by non-linear least squares computer curve fitting as in Ref. 2. The data were fitted best by a single binding site model in all cases and are given as mean ∓ SD. Analysis using a form of the Hill equation [2] gave pseudo Hill coefficients: DO710 (0.97 ∓ 0.09), sulpiride (0.91 ∓ 0.04), clozapine (1.05 ∓ 0.03), loxapine (1.06 ∓ 0.02).

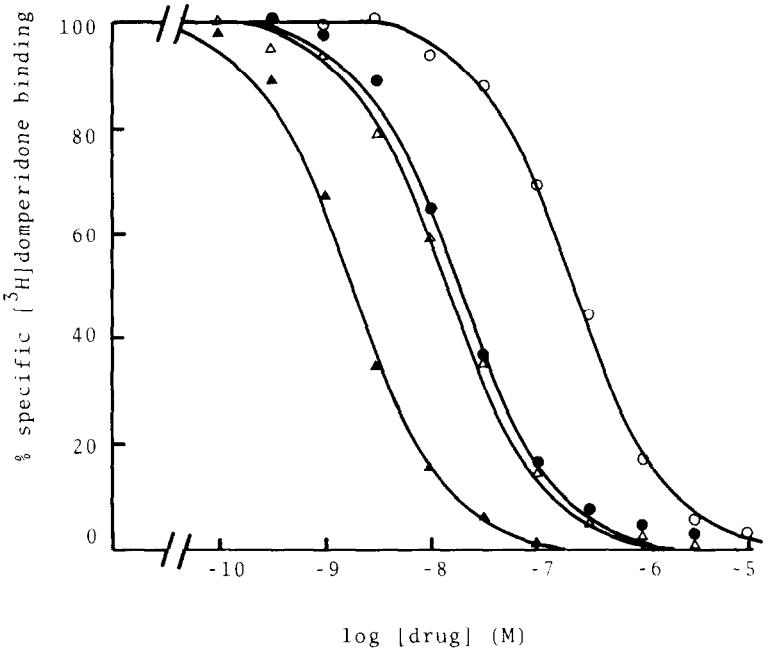

Fig. 3. Displacement of [^{3}H]domperidone binding to rat striatal D_2 dopamine receptors by DO710 (▲), sulpiride (△), clozapine (○) and loxapine (●). See legend to Fig. 1 and Table 1 for details. The curves are the best fit curves to a one binding site model (equation 1 of [2] with n = 1) (IC_{50} values (nM) DO710, 1.9, sulpiride, 15, clozapine, 230, loxapine, 19).

[^{3}H]domperidone binding from rat striatal D_2 receptors by classical dopamine antagonists as well as antagonists of the substituted benzamide and dibenzazepine classes showed that striatal D_2 receptors were a uniform class of sites. No evidence was obtained for binding site heterogeneity.

In other more limited studies sulpiride binding to rat striatal D_2 receptors has been studied by displacement and no evidence of heterogeneity found [12, 13] in support of the present data. These results do, however, conflict with those reported by Sokoloff and colleagues [4, 5, 7] of a substituted benzamide selective D_2 receptor subtype (D_4 receptor). In [5, 7] substituted benzamide drugs were reported to show up to tenfold differences in potency for binding to the D_2 and D_4 sites. In the present study such behaviour would have been evident from pseudo Hill coefficients less than one in displacement experiments. Pseudo Hill coefficients were in fact uniformly close to one in the present study. The origin of these differences is unclear but seems unlikely to be due to rat strain differences (AHA in the present study, Sprague–Dawley in [4, 5, 7]) or slight differences in assay conditions.

The number of D_2 dopamine receptors in rat striatum labelled by [^{3}H]domperidone was similar to the number in bovine caudate nucleus labelled by [^{3}H]spiperone [2, 6]. Similarly, in a range of bovine tissues antagonist binding to D_2 dopamine receptors was homogeneous indicating no heterogeneity within a tissue [2, 3, 6]. It is notable, however, that the affinities of ligands are generally higher in rat striatum compared with those in bovine striatum and this must reflect a species difference. The ratio of affinities in rat striatum compared to those in bovine striatum is generally about ten-fold but for the substituted benzamides it is higher (ratio: 94, DO710; 24 sulpiride). Thus although these compounds fail to discriminate sub-types of receptor within a tissue these data suggest that the substituted benzamide drugs have an ability to discriminate species differences between D_2 receptors.

In summary, therefore, from [^{3}H]domperidone binding studies in rat striatum and displacement with a series of antagonists we find no evidence for D_2 dopamine receptor heterogeneity. In all the tissues we have studied so far, bovine brain [2, 6], bovine anterior pituitary [3] and rat striatum, D_2 receptors appear to be a homogeneous population of sites within a tissue as defined by antagonist binding.

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