Heterogeneity of D₂ dopamine receptors in different brain regions

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The binding of [³H]spiperone has been examined in membranes derived from different regions of bovine brain. In caudate nucleus, nucleus accumbens, olfactory tubercle and putamen binding is to D₂ dopamine and 5HT₂ serotonin receptors, whereas in cingulate cortex only serotonin 5HT₂ receptor binding can be detected. D₂ dopamine receptors were examined in detail in caudate nucleus, olfactory tubercle and putamen using [³H]spiperone binding in the presence of 0.3 μM-mianserin (to block 5HT₂ serotonin receptors). No evidence for heterogeneity among D₂ dopamine receptors either between brain regions or within a brain region was found from the displacements of [³H]spiperone binding by a range of antagonists, including dibenzazepines and substituted benzamides. Regulation of agonist binding by guanine nucleotides did, however, differ between regions. In caudate nucleus a population of agonist binding sites appeared resistant to guanine nucleotide regulation, whereas this was not the case in olfactory tubercle and putamen.

INTRODUCTION

D₂ dopamine receptors have been studied extensively using the ligand-binding technique with a variety of radioligands, e.g. [³H]domperidone (Baudry *et al.*, 1979; Lazareno & Nahorski, 1982), [³H]haloperidol (Burt *et al.*, 1976; Seeman *et al.*, 1975), [³H]spiperone (Leysen *et al.*, 1978) and [³H]YM09151-2 (Niznik *et al.*, 1985). The general pattern that emerges from these studies is of a homogeneous class of antagonist binding sites. Agonist binding is, however, heterogeneous and can be described in terms of two classes of site with different agonist affinity (Withy *et al.*, 1981; De Lean *et al.*, 1982; Hamblin *et al.*, 1984; Wreggett & De Lean, 1984; Simmonds *et al.*, 1986). Agonist affinities are reduced in the presence of guanine nucleotides and generally there is a conversion of higher affinity agonist sites into lower affinity agonist sites (Sibley *et al.*, 1982; Hamblin *et al.*, 1984; Wreggett & Seeman, 1984).

Sokoloff and his colleagues have, however, provided evidence in rat brain and pituitary for heterogeneity of D₂ receptors on the basis of ligand-binding studies (Sokoloff *et al.*, 1984, 1985) and behavioural tests (Martres *et al.*, 1984). These workers have delineated a D₂ receptor located in anterior pituitary and a D₄ receptor enriched in olfactory bulb; both subtypes are found in striatum. Whereas classical dopamine antagonists bind to both subtypes with equal affinity, certain substituted benzamide drugs show a higher affinity for the D₄ subtype. Agonist binding to the D₂ subtype is modulated by guanine nucleotides, whereas this is not seen at D₄ receptors.

There is also some evidence for heterogeneity of D₂ receptors from other behavioural studies (Costall & Naylor, 1975; Gower & Marriott, 1982), from electrophysiological studies (White & Wang, 1983), from binding studies *in vivo* (Ogren *et al.*, 1984), from the effects of guanine nucleotides on agonist binding (De Keyser *et al.*, 1985) and from mechanistic studies (Stoof & Verheijden, 1986). In many of these studies differences

were implicated between receptors in limbic and striatal brain regions and either substituted benzamide or dibenzazepine drugs were described as showing some selectivity towards limbic receptors versus striatal receptors.

In the present paper we have investigated at the biochemical level possible heterogeneity of D₂ receptors labelled with [³H]spiperone in terms of the binding of substituted benzamide drugs and dibenzazepine drugs and guanine nucleotide effects on agonist binding. We have studied in detail receptors from striatal areas (caudate nucleus and putamen) and a limbic area (olfactory tubercle) of bovine brain and have obtained less extensive data on nucleus accumbens and cingulate cortex.

EXPERIMENTAL

Materials

[³H]Spiperone (16 Ci/mmol) was obtained from Amersham International. We acknowledge generous gifts of the following substances: butaclamol (Ayerst Laboratories, Farnborough, Hants., U.K.), clebopride (Laboratories Amirall, Barcelona, Spain), clozapine (Sandoz), domperidone and haloperidol (Janssen Pharmaceutica, Beerse, Belgium), DO710 (Professor C. G. Wermuth, CNRS, Strasbourg, France), flupenthixol (Lundbeck, Denmark), loxapine (Lederle), mianserin (Organon), prazosin (Pfizer Central Research, Sandwich, Kent), sulpiride (Ravizza Laboratories), remoxipride (Astra, Lakemedel, Sweden). All other chemicals were of the highest purity available and were obtained from commercial sources.

Preparation of bovine brain membranes

Caudate nucleus, olfactory tubercle, nucleus accumbens, putamen and cingulate cortex were dissected from bovine brains (obtained from Nottingham City abattoir, kept at 4 °C and used within 2 h after death) and

Abbreviation used: Gpp[NH]p, guanosine 5'-[βγ-imido]triphosphate.

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homogenized separately in 0.32 M-sucrose solution containing 20 mM-Hepes, pH 7.4 (9 ml/g wet weight of tissue) with a Teflon/steel homogenizer (0.18 mm radial clearance, 10 strokes, 800 rev./min). The homogenate was centrifuged (1500 g, 10 min), and the supernatant was kept at 4 °C while the pellet was rehomogenized in 4 vol. of ice-cold sucrose/Hepes and centrifuged (1500 g, 10 min). The final pellet was discarded and the supernatants were pooled and centrifuged (126 000 g, 60 min). The supernatant was discarded and the pellet was resuspended (2 ml/g of tissue) in an ice-cold Hepes/phosphate/saline buffer, pH 7.4 (Golds *et al.*, 1980). The resuspended mitochondrial/microsomal preparation was stored at -80 °C, and storage for up to 3 months did not result in significant loss of [³H]spiperone binding activity. Protein concentration was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard and employing a precipitation step with trichloroethanoic acid (10 %).

[³H]Spiperone binding assays

[³H]Spiperone binding to brain D₂ receptors was determined essentially as described in Withy *et al.* (1981) and Simmonds *et al.* (1986). Briefly, bovine brain membranes (see below for protein concentration) were incubated at 25 °C for 30 min in triplicate in a final volume of 1 ml of Hepes-buffered physiological medium (Golds *et al.*, 1980) with [³H]spiperone [approx. 0.25 nM for displacement (competition) studies; 25 pM–5 nM for saturation analyses] and other drugs where indicated. Competing substances were used at the concentrations shown in the Figures. The solutions were supplemented additionally with dithiothreitol (0.1 mM) and pargyline (10 µM). Assays were terminated in one of two ways, as follows.

(i) 5 ml of ice-cold buffer was added to the assay tube, and the contents were filtered under vacuum through a 2.5 cm glass fibre GF/B filter disc on a 12-place Millipore filtration manifold. The filters were washed by two further 5 ml washes of buffer before radioactivity trapped on the filter was determined by scintillation counting in 3 ml of Optiphase (Fisons) containing approx. 10 % water.

(ii) The contents of the assay tube were filtered through Whatman 934 AH glass-fibre filter sheets on a Dynatech Automash 2000 cell harvester and the filter was washed with ice-cold 150 mM-NaCl/10 mM-sodium phosphate, pH 7.4 (15 ml). Filters were dried by a stream of air (30 s) and radioactivity was determined as before.

Results obtained using either technique were essentially identical, except that for method (i) a protein concentration of 0.1–0.25 mg/ml was used whereas for method (ii) protein concentrations up to 0.15 mg/ml were used. Total [³H]spiperone binding showed a linear dependence on protein concentration up to 0.5 mg/ml (manifold apparatus) or up to 0.2 mg/ml (cell harvester) so that the protein concentrations used were both in the linear range. Specific [³H]spiperone binding was defined as that binding displaced by 3 µM-(+)-butaclamol and amounted to 80 % (manifold) and 90 % (cell harvester) of the total binding (free [³H]spiperone 0.25 nM). Bound [³H]spiperone was always less than 10 % of the total [³H]spiperone in an assay and for saturation analyses a minor correction to the data was made to take account of this as described in Golds *et al.* (1980). When bound [³H]spiperone was determined by centrifugation (with

only superficial washing of the pellet) as opposed to filtration (when extensive washing occurs) the bound [³H]spiperone was still less than 10 % of the total [³H]spiperone so that there was no major component of non-specific binding which could have been removed by washing in the filtration assays.

Data analysis

Saturation binding and displacement data were analysed by non-linear least squares computer curve fitting as described in Withy *et al.* (1981). Briefly, competition data were first assessed by fitting to eqn. (1), evaluating the pseudo Hill coefficient (*n*):

$$Y = \frac{L^n}{L^n + (IC_{50})^n} \quad (1)$$

where *Y* is the fractional occupancy of binding sites by competing ligand (concentration *L*) as determined from inhibition of specific radioligand binding, and *IC*₅₀ is the concentration of competing ligand giving half-maximal inhibition of specific radioligand binding. Where the pseudo Hill coefficient was close to 1 the data were also fitted to eqn. (1) with *n* = 1. The goodness of fit was assessed by determining the nearest neighbour residual correlation coefficient, which shows whether there were systematic deviations of the data from the fitted curve. Where the pseudo Hill coefficient was less than 1, eqn. (1) (*n* = 1) and a two-site model (eqn. 2) were used for fitting:

$$Y = \frac{\alpha L}{L + (IC_{50})_1} + \frac{(1 - \alpha)L}{L + (IC_{50})_2} \quad (2)$$

where α is the fraction of binding sites with (*IC*₅₀)₁ and (1 - α) is the fraction of binding sites with (*IC*₅₀)₂.

Goodness of fit was determined as above. *K*_i values were determined from *IC*₅₀ values as in Withy *et al.* (1981). Saturation binding data were analysed as above, but using eqn. (3):

$$\frac{B}{B_{\max}} = \frac{L}{L + K_d} \quad (3)$$

where *B* is specifically bound radioligand at free radioligand concentration *L*, *B*_{max} is total number of binding sites and *K*_d is dissociation constant for binding of radioligand.

RESULTS

Nature of [³H]spiperone binding in different brain regions

Specific [³H]spiperone binding, defined as that displaceable by 3 µM-(+)-butaclamol, could be observed in all brain regions tested but at different levels. Displacement of specific [³H]spiperone binding by domperidone, mianserin and prazosin was performed in order to investigate the relative contributions of D₂ dopamine, 5HT₂ serotonin and α_1 -adrenergic receptors in the different regions. Prazosin displacement occurred only at high concentrations (> 10 µM), indicating the absence of α_1 -adrenoceptor binding. Domperidone and mianserin displacements were biphasic in caudate nucleus, nucleus accumbens, olfactory tubercle and putamen, indicating the presence of mixtures of D₂ dopamine and 5HT₂ serotonin receptor binding. In all cases the 5HT₂ serotonin receptor component was a minor one at the [³H]spiperone concentration used. The apparent

Fig. 1. Displacement of [³H]spiperone binding by various substances

Total [³H]spiperone binding to bovine olfactory tubercle was determined in the presence of different concentrations of displacing substances [(a): (+)-butaclamol (O) and (-)-butaclamol (Δ); (b): domperidone (□), haloperidol (■), α-flupenthixol (○) and mianserin (▲)]. The data shown are the mean of triplicate determinations from a single representative experiment replicated as in Table 1. The standard errors were less than 10%. In order to analyse the data, non-specific [³H]spiperone binding [defined by 3 μM-(+)-butaclamol] was subtracted from total [³H]spiperone binding, and this specific [³H]spiperone binding was analysed by non-linear least squares curve fitting as described. The curves shown were then obtained by adding back non-specific binding to the specific binding at each point. The best fit curves were one-site models (eqn. 1, $n = 1$) for (+)-butaclamol (IC₅₀ 29 nM), haloperidol (IC₅₀ 65 nM) and α-flupenthixol (IC₅₀ 69 nM) or two-site models (eqn. 2) for domperidone [IC₅₀¹ 13 nM (77%), IC₅₀² 1.6 μM (23%)] and mianserin [IC₅₀¹ 11 nM (17%), IC₅₀² 6 μM (83%)] (the percentage values are of the specific binding). For (-)-butaclamol the data were fitted by eye.

affinities of mianserin and domperidone for the two classes of sites were very similar in these brain regions. In cingulate cortex domperidone (low affinity) and mianserin (high affinity) displacements were consistent with the presence of a single class of sites which were presumably 5HT₂ serotonin receptors. This region was not examined in any detail owing to the lack of detectable D₂ dopamine receptors.

In caudate nucleus, nucleus accumbens, olfactory

tubercle and putamen displacement studies with a series of substances were carried out (Fig. 1, Table 1). For (+)-butaclamol, α-flupenthixol and haloperidol data for displacement of specific [³H]spiperone binding were described best by a one-binding-site model (eqn. 1, $n = 1$) and displacement occurred in each case to the same plateau level. Displacements for domperidone and mianserin were best described by the two-binding-site model (eqn. 2) and displacement at high concentrations approached the plateau level seen with (+)-butaclamol, α-flupenthixol and haloperidol. Therefore all the substances tested are binding to the same total number of receptor sites in each region and in each region 3 μM-(+)-butaclamol was a satisfactory definition of this specific [³H]spiperone binding. These observations also preclude the presence of any spirodecane components of [³H]spiperone binding. For each region [³H]spiperone binding was at equilibrium within 20 min and assays were performed for 30 min.

In order therefore to examine D₂ dopamine receptor binding of [³H]spiperone alone it was necessary to eliminate [³H]spiperone binding at serotonin 5HT₂ receptors. This has previously been accomplished for bovine caudate nucleus by including 0.3 μM-mianserin (Withy *et al.*, 1981). Examination of mianserin displacement curves in other brain regions indicated that this would be a suitable blocking concentration. Ketanserin was also tested in this context but proved less selective than mianserin in bovine caudate nucleus (IC₅₀ for D₂ dopamine receptor: mianserin, 5.7 ± 1.5 μM; ketanserin, 2.7 ± 1.9 μM; IC₅₀ for 5HT₂ serotonin receptor: mianserin, 3.0 ± 1.0 nM; ketanserin, 61 ± 31 nM; mean ± S.D., three experiments).

D₂ dopamine receptor binding of [³H]spiperone in the different brain regions

[³H]Spiperone binding was determined in caudate nucleus, olfactory tubercle and putamen in the presence of 0.3 μM-mianserin in order to examine D₂ dopamine receptors alone. [³H]Spiperone binding was saturable and of high affinity in each of these brain regions (Table 2). The saturation binding data obtained fitted well to a model of a single class of site (eqn. 3) in each case, emphasizing that only D₂ dopamine receptors were being examined. K_d values for the three regions were very similar.

Displacement of [³H]spiperone binding (in the presence of 0.3 μM-mianserin) from D₂ dopamine receptors by classical dopamine antagonists [(+)-butaclamol, domperidone, haloperidol, α-flupenthixol] was carried out using full displacement curves and was found to be exclusively to a single class of sites (as shown by the good fit of data to eqn. 1, $n = 1$). In addition displacement by the isomers of butaclamol showed a high degree of stereoselectivity. K_i values for displacing substances were very similar in the different brain regions.

Displacement of [³H]spiperone binding by four substituted benzamide drugs (clebopride, DO710, remoxipride, sulpiride) and the dibenzazepines clozapine and loxapine was also well described by a one-binding-site model (eqn. 1, $n = 1$) and the drugs showed similar affinities in the different brain regions tested (Table 3 and Fig. 2).

Agonist displacement of [³H]spiperone binding

Agonist displacement of [³H]spiperone binding was carried out in caudate nucleus, olfactory tubercle and

Table 1. [³H]Spiperone binding in different regions of bovine brain

Specific [³H]spiperone binding was determined in the different brain regions as described. Displacement experiments were performed and $-\log(IC_{50})$ (concentration of substance that displaces half the specific [³H]spiperone binding) values were determined from full displacement curves. Data fitted one-site models (eqn. 1, $n = 1$) well except for domperidone and mianserin where a two-site fit (eqn. 2) was a better description and the parameters derived from these analyses [$-\log(IC_{50})$ values at higher (H) and lower (L) affinity sites and percentage of lower affinity sites (%L)] are given. Specific [³H]spiperone binding was defined as that binding displaced by 3 μM -(+)-butaclamol. Data are expressed as means $\pm$ S.D. (three or more experiments) or mean $\pm$ range (two experiments). Limited displacement data were obtained for cingulate cortex: $-\log(IC_{50})$ values (M) were (+)-butaclamol 7.25 $\pm$ 0.13 (2); (-)-butaclamol, < 6; prazosin, < 5; domperidone, 6.13 (1); mianserin, 7.70 (1).

Tissue	$-\log(IC_{50})$ (M) for displacing substances									
	(+)-Butaclamol	(-)-Butaclamol	Haloperidol	α -Flupenthixol	Prazosin	H	Domperidone		Mianserin	
							L	%L	L	%L
Caudate nucleus	7.58 $\pm$ 0.05 (2)	< 6	7.20 $\pm$ 0.02 (2)	7.24 $\pm$ 0.05 (2)	< 5	7.66 $\pm$ 0.05 (2)	5.54 $\pm$ 0.05	22 $\pm$ 4	5.25 $\pm$ 0.10	81 $\pm$ 2
Nucleus accumbens	7.79 $\pm$ 0.18 (2)	< 6	7.26 (1)	6.35 (1)	< 5	7.57 $\pm$ 0.32 (3)	5.30 $\pm$ 0.17	14 $\pm$ 2	4.96 $\pm$ 0.01	86 $\pm$ 6
Olfactory tubercle	7.69 $\pm$ 0.01 (2)	< 6	7.26 $\pm$ 0.05 (3)	7.32 $\pm$ 0.04 (3)	< 5	7.92 $\pm$ 0.03 (3)	5.82 $\pm$ 0.02	18 $\pm$ 8	5.95 $\pm$ 0.09	76 $\pm$ 1
Putamen	7.77 $\pm$ 0.03 (2)	< 6	6.92 $\pm$ 0.04 (2)	7.50 $\pm$ 0.02 (2)	ND	7.89 $\pm$ 0.03 (2)	5.96 $\pm$ 0.11	13 $\pm$ 1	5.89 $\pm$ 0.09	83 $\pm$ 1

Table 2. Saturation analysis of [³H]spiperone binding to D₂ dopamine receptors in different regions of bovine brain

Specific [³H]spiperone binding to D₂ dopamine receptors was determined for a range of [³H]spiperone concentrations. Specific binding was defined as that displaceable by 3 μM -(+)-butaclamol, and 0.3 μM -mianserin was present throughout to mask 5HT₂ receptors. Data were analysed by non-linear least squares computer curve fitting as in Withy *et al.* (1981) and a one-binding-site model (eqn. 3) was a good description of the data in all cases. Parameters are given as mean $\pm$ range (two experiments) or mean $\pm$ S.D.

Brain region	K_d (pM)	B_{max} (fmol/mg of protein)	No. of experiments
Caudate nucleus	168 $\pm$ 43	410 $\pm$ 46	4
Olfactory tubercle	163 $\pm$ 42	243 $\pm$ 8	3
Putamen	253 $\pm$ 86	259 $\pm$ 4	2

putamen and agonist displacement curves were characterised by pseudo Hill coefficients less than 1. The displacement curves could be described well by a two-binding-site model (eqn. 2) with contributions from two classes of sites (40% higher affinity, 60% lower affinity) (Table 4 and Fig. 3). The non-hydrolysable guanine nucleotide Gpp[NH]p caused a shift of the agonist displacement curves to lower affinities. The effects of Gpp[NH]p were maximal at 100 μM ; similar effects were obtained with GTP and there was no effect of these nucleotides on antagonist binding (results not shown).

Agonist displacement in the presence of 100 μM -Gpp[NH]p in olfactory tubercle and putamen could be described well by a one-binding-site model (eqn. 1, $n = 1$) and the apparent IC_{50} values under these conditions were similar to the IC_{50} values of the lower affinity sites observed in the absence of guanine nucleotides. This pattern was not observed for caudate nucleus, where a two-binding-site model was still the better description of the data in the presence of 100 μM -Gpp[NH]p. The IC_{50} values of higher and lower affinity sites in the absence and presence of Gpp[NH]p agreed, suggesting that the effects of Gpp[NH]p could be described as interconversion of higher and lower affinity sites with a proportion of higher affinity sites being insensitive to Gpp[NH]p.

It has been reported by others (Grigoriadis & Seeman, 1985) that a temperature of 37 °C was necessary to observe full interconversion of higher and lower affinity sites in such an experiment, but at 37 °C similar data to those described above were observed (Table 4). Also, in case the differences in behaviour in caudate nucleus were in some way associated with interaction of dopamine with D₁ dopamine receptors these receptors were blocked with SCH 23390 (0.1 μM), but similar data were still obtained (Table 4). SCH 23390 did not affect [³H]-spiperone binding at the concentration used.

DISCUSSION

In this paper we have examined the ligand-binding properties of D₂ dopamine receptors in different regions

Table 3. Displacement of [³H]spiperone binding from D₂ dopamine receptors in different regions of bovine brain by various substances

Specific [^3H]spiperone (approx. 0.25 nM) binding was determined in the presence of different concentrations of displacing substances. Specific binding was defined using 3 μM -(+)-butaclamol, and 0.3 μM -mianserin was present throughout in order to mask 5HT $_2$ serotonin receptors. Data were analysed using non-linear least squares computer curve fitting and a one-site model (eqn. 1, $n = 1$) was a good description of the data in all cases. IC $_{50}$ values (concentration of substance that inhibits half the specific [^3H]spiperone binding) were determined and converted to K_i values as in Withy *et al.* (1981). Data are given as mean $\pm$ range (two experiments) or mean $\pm$ S.D. (three or more experiments).

Tissue	(+)-Buta-clamol	(-)-Buta-clamol	Domperidone	Cleboipride	DO 710	Remoxipride	Sulpiride	Clozapine	Loxapine
Caudate nucleus	8.00 $\pm$ 0.10 (3)	< 6	8.15 $\pm$ 0.07 (3)	8.00 $\pm$ 0.07 (2)	7.19 $\pm$ 0.03 (3)	5.65 $\pm$ 0.05 (3)	6.91 $\pm$ 0.06 (4)	6.34 $\pm$ 0.08 (2)	7.24 $\pm$ 0.10 (3)
Olfactory tubercle	7.90 $\pm$ 0.01 (4)	< 6	8.25 $\pm$ 0.03 (3)	7.88 $\pm$ 0.07 (3)	7.17 $\pm$ 0.02 (2)	5.50 $\pm$ 0.03 (3)	6.78 $\pm$ 0.08 (5)	6.35 $\pm$ 0.10 (4)	7.27 $\pm$ 0.08 (3)
Putamen	7.85 $\pm$ 0.12 (3)	< 6	8.30 $\pm$ 0.03 (3)	7.88 $\pm$ 0.07 (3)	7.21 $\pm$ 0.11 (3)	5.67 $\pm$ 0.05 (3)	6.77 $\pm$ 0.07 (3)	6.40 $\pm$ 0.02 (3)	7.30 $\pm$ 0.02 (3)

Fig. 2. Displacement of [³H]spiperone binding from D₂ dopamine receptors of bovine olfactory tubercle by various substances

Specific [^3H]spiperone binding was determined in the presence of different concentrations of displacing ligand [clebopride (●), DO 710 (▽), sulpiride (□), remoxipride (▲), loxapine (△) and clozapine (○)]. Specific [^3H]spiperone binding was defined using 3 μM -(+)-butaclamol, and 0.3 μM -mianserin was included throughout to mask 5HT₂ serotonin receptors. The data are the means of triplicate determinations from single representative experiments replicated as in Table 3. Standard errors were less than 10%. The curves shown are the best-fit curves obtained by fitting the data to eqn. (1), $n = 1$; IC₅₀ values were clebopride, 20 nM; DO 710, 140 nM; sulpiride, 250 nM; remoxipride, 10 μM ; loxapine, 125 nM; clozapine, 1.2 μM .

of bovine brain. D₂ dopamine receptors have been assayed in this study using [³H]spiperone binding under conditions where binding of this radioligand to other receptors was absent. D₂ dopamine receptors were found to varying degrees in caudate nucleus, nucleus accumbens, olfactory tubercle and putamen but could not be detected in cingulate cortex. This may be because there are too few D₂ dopamine receptors in cingulate cortex relative to non-specific binding of [³H]spiperone. Indeed, Martres *et al.* (1985) reported that, in rat brain, D₂ dopamine receptor binding in cortical regions was present at about 2% of the level in striatum and this would be difficult to detect with the present assay system.

Displacement of [³H]spiperone binding from D₂ dopamine receptors in the different brain regions by classical antagonists, by dibenzazepines and by substituted benzamides has indicated that no major differences exist between the receptors in the different regions. Similar affinities were observed for the displacing ligands in all cases and these agreed with values reported previously for caudate nucleus (Withy *et al.*, 1981) and anterior pituitary (Simmonds *et al.*, 1986). Also there was no evidence for heterogeneity among receptor species within a brain region, so that the bovine dopamine D₂ receptors seem to be a uniform species as defined by antagonist binding.

Thus we can find no evidence for heterogeneity of D₂ dopamine receptors at the level of antagonist binding. The findings of Sokoloff and colleagues (Sokoloff *et al.*, 1984, 1985) of a benzamide-selective receptor subtype (D₄) in striatum are not supported by these studies.

Table 4. Dopamine displacement of [³H]spiperone binding from D₂ dopamine receptors in different regions of bovine brain and the effect of guanine nucleotides

Specific [³H]spiperone (approx. 0.25 nM) binding was determined in the presence of different concentrations of dopamine in the absence and presence of 0.1 mM guanine nucleotide. Specific binding was defined using 3 μ M-(+)-butaclamol, and 0.3 μ M-mianserin was present throughout in order to mask 5HT₂ serotonin receptors. The standard experimental temperature is 25 °C and data are also given for experiments at 37 °C. In addition data obtained in the presence of SCH 23390 (0.1 μ M) are given. Data were analysed by non-linear least squares computer curve fitting and, except for olfactory tubercle and putamen in the presence of guanine nucleotides [where a one-site model (eqn. 1, $n = 1$) was the best description (and no improvement in fit was obtained by using a two-site model)], the two-site model (eqn. 2) was the best description of the data [the parameters given are the $-\log(\text{IC}_{50})$ values for the higher (H) and lower (L) affinity sites and the percentage of lower affinity sites (%L)]. Data are the means $\pm$ s.d. of three or more experiments

Tissue	$-\log[\text{IC}_{50} \text{ (M)}]$						
	Control			Guanine nucleotide	+ 0.1 mM-guanine nucleotide		
	H	L	%L		H	L	%L
Caudate nucleus	6.60 $\pm$ 0.14	4.62 $\pm$ 0.13	59 $\pm$ 3	Gpp[NH]p	6.38 $\pm$ 0.18	4.36 $\pm$ 0.10	78 $\pm$ 14
Caudate nucleus (37 °C)	6.22 $\pm$ 0.06	4.45 $\pm$ 0.09	47 $\pm$ 1	GTP	5.85 $\pm$ 0.22	4.10 $\pm$ 0.07	61 $\pm$ 7
Caudate nucleus (+ SCH 23390)	6.29 $\pm$ 0.19	4.24 $\pm$ 0.02	57 $\pm$ 1	GTP	5.94 $\pm$ 0.23	4.30 $\pm$ 0.06	71 $\pm$ 5
Olfactory tubercle	6.64 $\pm$ 0.63	4.63 $\pm$ 0.29	57 $\pm$ 11	Gpp[NH]p	—	4.68 $\pm$ 0.08	100
Putamen	6.41 $\pm$ 0.37	4.53 $\pm$ 0.21	61 $\pm$ 10	Gpp[NH]p	—	4.62 $\pm$ 0.09	100

Although this could be due to species differences (the current work used bovine tissue whereas previous reports were with rat tissue) Hamblin *et al.* (1984) and Grigoriadis & Seeman (1986) also failed to find any deviation from a one-binding-site model in sulpiride displacement of [³H]domperidone binding from rat striatum.

The results also do not support the suggestions of Ogren *et al.* (1984) or of Gower & Marriott (1982) of D₂ dopamine receptors in limbic brain areas with differential sensitivity to benzamides or dibenzazepines. It seems likely that any differences observed may be due to differential distribution of drugs in different regions. Binding of dibenzazepines in limbic and striatal regions has been examined by Reynolds *et al.* (1982), Seeman &

Fig. 3. Effect of guanine nucleotides on the displacement of [³H]spiperone binding from D₂ dopamine receptors by dopamine

Specific [³H]spiperone binding was determined in the presence of different concentrations of dopamine in the absence (■) and presence (▲) of 0.1 mM-Gpp[NH]p in bovine olfactory tubercle (a) or bovine caudate nucleus (b). Specific [³H]spiperone binding was defined using 3 μ M-(+)-butaclamol, and 0.3 μ M-mianserin was included throughout to mask 5HT₂ serotonin receptors. The data are the means of triplicate determinations from single representative experiments replicated as in Table 4. Standard errors were less than 10 %. The curves shown are the best-fit curves. (a) Control (eqn. 2) IC_{50}^1 0.94 μ M (54 %), IC_{50}^2 67 μ M (46 %); + Gpp[NH]p, (eqn. 1, $n = 1$) IC_{50}^1 0.31 μ M (62 %). (b) Control (eqn. 2) IC_{50}^1 0.46 μ M (38 %), IC_{50}^2 23 μ M (62 %); + Gpp[NH]p (eqn. 2) IC_{50}^1 0.35 μ M (82 %), IC_{50}^2 47 μ M (82 %).

Ulpian (1983) and Richelson & Nelson (1983), again indicating no major differences between receptors in different regions.

There do appear, however, to be differences in the behaviour of D₂ dopamine receptors in the different brain regions as assessed by agonist binding. Agonist displacement curves in the three brain regions studied are similar in the absence of guanine nucleotides. Pseudo Hill coefficients are less than 1 and the curves may be analysed in terms of a two-binding-site model with similar affinities in the different regions. These results are in agreement with our previous observations in caudate nucleus (Withy *et al.*, 1981). In olfactory tubercle and putamen in the presence of guanine nucleotides, agonist displacement curves conformed to a one-binding-site model with the effects of guanine nucleotides appearing to be a conversion of higher and lower affinity populations of sites into a uniformly lower affinity population. This pattern has been described extensively for D₂ dopamine receptors in other tissues (Sibley *et al.*, 1982; De Lean *et al.*, 1982; George *et al.*, 1985).

In caudate nucleus, however, although there was an apparent interconversion of higher and lower affinity sites caused by guanine nucleotides, a proportion of higher affinity sites remained in the presence of these substances. Similar observations have been reported for bovine caudate nucleus by De Keyser *et al.* (1985). It has been suggested by Grigoriadis & Seeman (1985) that full conversion of higher to lower affinity sites requires a temperature of 37 °C and NaCl (100 mM approx.). Since the conditions used for the present experiments already included NaCl we repeated experiments at 37 °C, but incomplete conversion of sites was still observed. The incomplete conversion for caudate nucleus receptors is unlikely in fact to be due to the assay conditions, as complete conversion is seen for olfactory tubercle and putamen receptors. It seems more likely that the observations reflect real differences between brain regions, and this is supported by the findings of De Keyser *et al.* (1985) in globus pallidus of guanine nucleotide insensitive receptors, Simmonds *et al.* (1986) reported a different pattern again in bovine pituitary where the affinities of both higher and lower affinity sites were reduced by guanine nucleotides and other partial or mixed effects have been reported (De Lean *et al.*, 1982; Wreggett & Seeman, 1984; Wreggett & de Lean, 1984). Thus there may be a spectrum of behaviour in different tissues. Similar differential sensitivities of agonist displacement curves to guanine nucleotides have been reported for muscarinic receptors (Dunlap & Brown, 1984).

Regulation of agonist binding to D₂ dopamine receptors is related to receptor coupling to a guanine nucleotide regulatory protein (N protein) (see for example Simmonds *et al.*, 1986, and references therein). R–N coupling has been extensively described in terms of a ternary complex model (see for example Wreggett & de Lean, 1984) but as we have pointed out (Simmonds *et al.*, 1986; Strange, 1987) this model may be an oversimplification given the emerging knowledge on the multiplicity and structural complexity of N proteins. Nevertheless the results presented here may point to differences in R–N coupling in different brain regions and this in turn may be reflected in different receptor mechanisms. The recent report of apparent mechanistic differences between D₂ dopamine receptors in limbic and

striatal regions (Stoof & Verheijden, 1986) supports this idea. It cannot be excluded, however, that the apparent differences in R–N coupling in different brain regions reflect different levels of protease activity which lead to guanine nucleotide resistant agonist-binding states in one brain region but not in another.

In summary, therefore, in different brain regions a similar pattern of D₂ dopamine receptor ligand binding is observed with homogeneous antagonist binding and heterogeneous agonist binding, as established by Withy *et al.* (1981) for caudate nucleus receptors. Differences between receptors in the different regions of bovine brain may be manifest at the level of R–N coupling.

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