We thank the Pharmaceutical Society of Great Britain for the award of a postgraduate scholarship to D. A. W.

April 24, 1979

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Specific binding of [^{3}H]sulpiride to rat striatal preparations

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Sulpiride is an antipsychotic agent belonging to the substituted benzamide group of compounds (Justin-Besancon et al 1967). Sulpiride also is an anti-emetic (Corsini et al 1976) and increases serum prolactin concentrations in man (Mancini et al 1976). All these effects are associated with dopamine receptor antagonism, so sulpiride appears to act like neuroleptics of the phenothiazine, butyrophenone and thioxanthene classes.

Animal experiments support this hypothesis. Sulpiride blocks locomotor activity induced by apomorphine, inhibits apomorphine- and amphetamine-induced circling behaviour and increases both striatal and mesolimbic dopamine turnover (Elliott et al 1977). However, sulpiride has little cataleptic activity, does not inhibit stereotyped behaviour induced by apomorphine and does not inhibit dopamine-stimulated adenylate cyclase activity either in vitro or in vivo (Laville 1972; Trabucchi et al 1975; Elliott et al 1977; Jenner et al 1978a). In addition, sulpiride only weakly inhibits [^{3}H]haloperidol or [^{3}H]spiperone receptor binding in striatal preparations (Jenner et al 1978a; Jenner et al 1978b) despite being equivalent in activity to haloperidol or fluphenazine on direct intracerebral injection (Hondo et al 1977; Costall et al 1978).

These differences in the mode of action of sulpiride (and other benzamide drugs in general) has formed one of the essential pieces of data taken by some workers to indicate the presence of multiple dopamine receptors in brain (see Kebabian & Calne 1979 and references therein). Two major classes of dopamine receptors are currently conceived, namely those dependent on adenylate cyclase for impulse transmission and those which are not. Substituted benzamide drugs are thought to act as specific antagonists on this latter receptor population.

It is of obvious importance to be able to specifically characterize each of these receptor sub-populations. *cis*-Flupenthixol provides a means of examining adenylate cyclase-dependent receptors. There is a correlation between the ability of neuroleptics to displace [^{3}H]*cis*-flupenthixol from its striatal binding sites and their ability to inhibit dopamine stimulation of striatal adenylate cyclase (Hyttel 1978). However, no means of examining the adenylate cyclase-independent dopamine receptor population has been available until now. We report the specific binding of [^{3}H]-sulpiride to rat striatal preparations.

(±)-[^{3}H]Sulpiride (26·2 Ci mmol^{-1}) was custom synthesized by The Radiochemical Centre, Amersham by the catalytic dehydrogenation of the brominated derivative *N*-1-ethyl-2-pyrrolidyl-methyl-2-methoxy-4-bromo-5-sulphamoylbenzamide (SESIF, France). Purity was 98% as judged by thin layer and paper chromatography.

Female Wistar rats (150 ± 10 g; Olac International) were killed by cervical dislocation and decapitation. The brain was rapidly removed onto ice and the striata dissected into ice-cold 50 mM Tris-HCl buffer (pH 7·7). Striatal tissue was prepared according to the method of Leysen et al (1978). Aliquots of the final ice-cold tissue preparation (1 ml containing 12·5 mg striatal tissue) were placed in small glass tubes and 0·05 ml 0·1% ascorbic acid solution or displacing drugs dissolved in this volume of ascorbic acid solution was added. (±)-Sulpiride or its enantiomers, (+)- and (—)-sultopride (SESIF, France), (+)- and (−)-butaclamol hydrochloride (Ayerst Laboratories), *cis*- and *trans*-flupenthixol hydrochloride (Lundbeck, Copenhagen), dopamine hydrochloride, noradrenaline hydrochloride or 5-hydroxytryptamine creatinine sulphate (Sigma Chemical Co.) all at concentrations between $10^{-10} - 5 \times 10^{-3}$ M, or 0·05 ml 0·1% ascorbic acid solution were added to the incubates in displacement experiments. Incubations were started by the addition of [^{3}H]sulpiride (5–40 nM) in 0·05 ml 0·1% ascorbic acid solution. Incubation was carried out at 37 °C for 1 h after which the incubates were immediately transferred to plastic micro-centrifuge tubes (capacity 1·5 ml; Alpha Laboratories) and centrifuged in a Beckmann Microfuge B at approximately 8000 *g* for 1 min. Samples were placed in ice, the supernatant

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aspirated and the surface of the pellet washed twice with 0·25 ml ice-cold 50 mM Tris-HCl buffer pH 7·7. The bottoms of the centrifuge tubes containing the pellet were cut into scintillation vials; 10 ml Instagel scintillation cocktail (Packard Instruments) was added and the vials were shaken vigorously for 30 min. Counting was carried out in a Packard 2425 liquid scintillation spectrometer at an efficiency of approximately 40%. Full details of the development of the methodology for [^{3}H]sulpiride binding will be published elsewhere (Theodorou, Crockett et al, in preparation).

Addition of [^{3}H]sulpiride (5–40 nM) to striatal preparations produced a linear increase in total tissue binding (Fig. 1A). Total binding at 40 nM [^{3}H]sulpiride was 51·8 ± 2·11 p mol g^{-1} wet weight of tissue which represented only 1·6% of the total ligand concentration. Unlabelled (±)-sulpiride (5×10^{-6} M) decreased [^{3}H]sulpiride binding to tissue revealing the presence of a saturable displaceable component (specific binding; Fig. 1A). Maximum binding at 40 nM [^{3}H]sulpiride of this saturable component was 15·18 ± 2·11 p mol g^{-1} wet weight of tissue, which represented approximately 29% of total tissue binding. The residual non-displaceable binding of [^{3}H]sulpiride to tissue increased linearly over the substrate range used. Analysis of the specific binding component by Scatchard analysis revealed a maximum density of [^{3}H]sulpiride binding sites of 23·5 ± 5·3 p mol g^{-1} wet weight of tissue and a dissociation constant (K_D) of 26·9 ± 10·3 nM (Fig. 1B). For displacement experiments a non-saturating concentration of [^{3}H]sulpiride of 15 nM was used. At this concentration total binding was 5330 ± 110 counts min^{-1} whereas residual binding in the presence of unlabelled (±)-sulpiride was 3229 ± 69 counts min^{-1} providing a gate for specific binding of 2101 ± 110 counts min^{-1}.

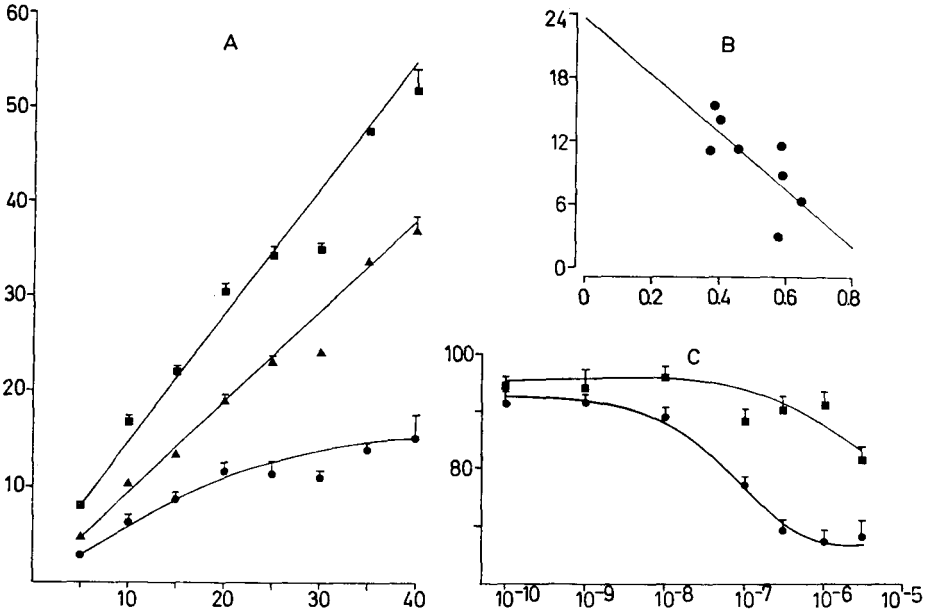

FIG. 1. Characteristics of [^{3}H]sulpiride binding to rat striatal preparations: A. Total (■), residual (▲) and specific binding (●) of [^{3}H]sulpiride (5–400 nM) as judged by displacement with (±)-sulpiride (5×10^{-6} M) Ordinate: [^{3}H]sulpiride binding (pmol g^{-1} wet weight of tissue. Abscissa: [^{3}H]sulpiride concentration (nM). B. Scatchard analysis of specific binding of [^{3}H]sulpiride (5–40 nM). K_D = 26·9 ± 10·3 mM, B_{max} = 23·5 ± 5·3 pmol g^{-1}. Ordinate: bound (pmol g^{-1}). Abscissa: bound/free $\left(\frac{\text{pmol g}^{-1}}{\text{nM}}\right)$. r = 0·727, $P < 0{\cdot}05$. C. Displacement of [^{3}H]sulpiride (15 nM) by (+)- (■) or (−)- (●) sulpiride ($10^{-10} - 5 \times 10^{-6}$ M). Ordinate: % total binding of [^{3}H]sulpiride. Abscissa: concentration (M).

The specific binding of [^{3}H]sulpiride (15 nM) was displaced in a concentration-dependent manner by the incorporation of dopamine ($10^{-10} - 5 \times 10^{-3}$ M) into incubates (Table 1). No concentration dependent displacement of [^{3}H]sulpiride binding was observed in the presence of noradrenaline or 5-HT (10^{-10}–5×10^{-6} M). Binding of [^{3}H]sulpiride (15 nM) was stereoselectivity displaced by (+)- and (−)-sulpiride in a concentration-dependent manner (Fig. 1C). The (−)-enantiomer of sulpiride was approximately 10 times more active than (+)-sulpiride. (+)-Sulpiride only displaced [^{3}H]sulpiride at concentrations exceeding 10^{-7} M. In a similar manner the isomers of sultopride caused a concentration-dependent stereoselective displacement of [^{3}H]sulpiride (Table 1), the (−)-isomer

Table 1. IC50 values for the displacement of [^{3}H]-sulpiride (15 nM) from rat striatal preparations by dopamine, noradrenaline, 5-hydroxytryptamine, (±)-, (+)- and (−)-sulpiride, (+)- and (−)-sultopride, (+)- and (−)-butaclamol, and *cis*- and *trans*-flupenthixol.

Displacing drug	Concn. drug (M)	IC50 (M)*	Max. displacement (pmol g^{-1} wet of tissue)
Dopamine	10^{-10}–5×10^{-3}	$1{\cdot}2 \times 10^{-6}$	7·1
Noradrenaline	10^{-10}–5×10^{-6}	$>5 \times 10^{-6}$	—
5-Hydroxytryptamine	10^{-10}–5×10^{-6}	$>5 \times 10^{-6}$	—
(±)-Sulpiride	10^{-10}–5×10^{-5}	$9{\cdot}0 \times 10^{-8}$	3·0
(+)-Sulpiride	10^{-10}–5×10^{-6}	$9{\cdot}8 \times 10^{-7}$	2·5
(−)-Sulpiride	10^{-10}–5×10^{-6}	$9{\cdot}3 \times 10^{-8}$	4·5
(+)-Sultopride	10^{-10}–5×10^{-6}	$1{\cdot}0 \times 10^{-6}$	3·2
(−)-Sultopride	10^{-10}–5×10^{-6}	$1{\cdot}9 \times 10^{-8}$	5·4
(+)-Butaclamol	10^{-10}–5×10^{-6}	$2{\cdot}0 \times 10^{-9}$	4·2
(−)-Butaclamol	10^{-10}–5×10^{-6}	$9{\cdot}0 \times 10^{-7}$	2·2
trans-Flupenthixol	10^{-10}–5×10^{-6}	$>5 \times 10^{-6}$	—
cis-Flupenthixol	10^{-10}–5×10^{-6}	$>5 \times 10^{-6}$	—

* IC50 values were assessed as the concentration of displacing agent causing a half-maximal displacement of [^{3}H]sulpiride from the level obtained with the lowest concentration of added displacing agent or, in the case of isomeric drugs, by the difference between the minimum concentration causing no stereoselective displacement and the maximum displacement observed.

being approximately 53 times more potent than (+)-sultopride. While the (+)-enantiomer of butaclamol was more potent than (−)-butaclamol in causing a stereoselective concentration-dependent displacement of [^{3}H]sulpiride, no such displacement was observed in the presence of *cis*- and *trans*-flupenthixol (10^{-10} – 5×10^{-6} M).

The specific binding of [^{3}H]sulpiride to rat striatal membranes would indicate the presence of a limited number of binding sites which may be associated with an action of this compound at neurotransmitter receptors. Since dopamine, but not noradrenaline or 5-HT, displaces [^{3}H]sulpiride, it would appear that such receptors may be dopaminergic in origin. That these receptors are of pharmacological importance is suggested by the greater activity of the more pharmacologically active isomers of sulpiride, sultopride and butaclamol (Andrews & Woodruff 1978; Jenner, Clow et al, in preparation) in causing a stereoselective displacement of [^{3}H]sulpiride from its binding sites. The failure of *cis*- and *trans*-flupenthixol to cause a concentration-dependent displacement of [^{3}H]sulpiride in the range of 10^{-9}–5×10^{-6} M was unexpected. This may be related to the close relationship between *cis*-flupenthixol binding and dopamine-stimulated adenylate cyclase. [^{3}H]Sulpiride may label cyclase independent receptors only. The relationship between [^{3}H]sulpiride binding sites and adenylate cyclase-independent receptors now needs to be fully explored. In particular, the action of sulpiride on adenylate cyclase-independent dopamine receptors on the glutamate fibres from cortex to striatum (Schwarcz et al 1978) may provide the clue to the anomalous action of the substituted benzamide drugs as a whole.

This study was supported by the Wellcome Trust and the Research Funds of King's College Hospital and the Bethlem Royal and Maudsley Hospitals. We thank SESIF, France Ltd., Lundbeck & Co. A/S and Ayerst Laboratories Ltd. for generous supplies of compounds.

April 5, 1979

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