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Definition of the In-vivo Binding of [^{3}H]Spiperone in Rat Brain Using Substituted Benzamide Drugs

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Abstract—The ability of some substituted benzamide drugs to define in-vivo the binding of [^{3}H]spiperone to brain dopamine receptors in rats was assessed using behaviourally effective doses in comparison with haloperidol. As judged using haloperidol, [^{3}H]spiperone identified dopamine receptors in the substantia nigra, striatum, tuberculum olfactorium and hypothalamus, but not in frontal cortex or nucleus accumbens. The substituted benzamide compounds alizapride, metoclopramide, clebopride and YM 09151-2 prevented the accumulation of [^{3}H]spiperone in the substantia nigra, striatum, tuberculum olfactorium and hypothalamus. However, YM 09151-2 also caused displacement of [^{3}H]spiperone accumulation in the nucleus accumbens and frontal cortex. (±)-Sulpiride, (±)-sultopride, amisulpiride and prosulpride all prevented the accumulation of [^{3}H]spiperone in the hypothalamus but were ineffective in one or more of the other regions containing dopamine receptors defined by [^{3}H]spiperone. The isomers of sulpiride and sultopride stereoselectively defined the accumulation of [^{3}H]spiperone in dopamine containing brain regions. The (−)-isomers of both drugs prevented the accumulation of [^{3}H]spiperone in the substantia nigra, striatum, tuberculum olfactorium and hypothalamus. In contrast, (+)-sulpiride and (+)-sultopride were ineffective. Selected substituted benzamide drugs can be used to define the interaction of ligands with dopamine receptors in-vivo. These substances may be useful in PET studies in man. The isomers of some substituted benzamine drugs may be used to define dopamine receptors in-vivo by enantiomeric selectivity.

Studies involving positron emission tomography (PET) are being increasingly used in man to define neuronal loss in degenerative disorders such as Parkinson's disease. A range of ligands for identifying brain dopamine receptors is now available and these include ^{76}Br-spiperone (Mazière et al 1985), ^{11}C-spiperone (Arnett et al 1983), ^{11}C-*N*-methylspiperone (Wagner et al 1984), ^{18}F-spiperone, ^{18}F-*N*-methylspiperone (Arnett et al 1985) and ^{11}C-raclopride (Farde et al 1985, 1986). However, it is necessary to define in-vivo the sites with which such compounds interact since none is specific for dopamine receptor sites in the brain. A bolus of an unlabelled neuroleptic drug can be used to displace, or prevent the accumulation of, the radioactive ligand from its binding sites, but most neuroleptic drugs are also not specific for dopamine receptors alone. To overcome such problems, compounds which in-vivo will selectively label brain dopamine receptors, or which will selectively define the binding of ligands to such sites have been sought.

We, and others, have utilized [^{3}H]spiperone in-vivo in rats to label brain dopamine receptors (Laduron et al 1978; Kohler et al 1979, 1981; Saelens et al 1980; Chivers et al 1987). Spiperone also interacts with 5-hydroxytryptamine (5-HT) and noradrenaline sites so the ligand is not specific for dopamine receptors (List & Seeman 1981). Indeed, we found [^{3}H]spiperone only to identify dopamine receptors in the substantia nigra, striatum, tuberculum olfactorium and hypothalamus. Labelling of other brain areas such as nucleus accumbens and frontal cortex involved 5-HT and noradrenaline sites. This emphasises the problems to be encountered in PET studies in man.

Previously we have investigated the receptor selectivity of substituted benzamide drugs, using in-vitro ligand binding techniques (Chivers et al 1988). This is one group of dopamine antagonist drugs which might be of use in PET studies. Some members of this series, for example clebopride and YM 09151-2, potently interact with brain dopamine receptors but are not selective for these sites, also acting on 5-HT and noradrenaline sites. Others are less potent, for example (±)-sultopride, amisulpiride, metoclopramide and prosulpride, but show good differentiation (10-100 fold) between activity on dopamine sites compared with other neuronal receptors. However, some drugs such as (±)-sulpiride and alizapride are highly selective for dopamine receptors.

In-vitro studies do not necessarily reflect the in-vivo action of substituted benzamide drugs since they do not take into account factors such as metabolism, absorption and brain penetration. For example, (±)-sulpiride does not readily penetrate into brain (Herberg & Wishart 1980) and it may selectively accumulate in only some dopaminergic brain regions. Other compounds of the same class may be more appropriate in-vivo to define dopamine receptors in brain. Alternatively, for an optically active compound, such as sulpiride, the use of the active (−)-isomer might be advantageous and allow definition of the nature of the receptor population labelled selectively by enantiomers. For these reasons we have assessed in-vitro the ability of various members of the substituted benzamide drug series to define the specific interaction of [^{3}H]spiperone with brain dopamine receptors, in comparison with haloperidol which have been shown previously to identify dopamine receptors in some brain areas. The drugs studied were given in a single

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pharmacologically active dose based on their ability to inhibit apomorphine-induced stereotyped behaviours.

Methods

Apomorphine-induced stereotyped behaviour

Stereotyped behaviour was assessed 15 min following the administration of apomorphine hydrochloride (0·5 mg kg^{-1} s.c.; Sigma Chemical Co.) using the following scoring system: 0 = animals indistinguishable from control rats; 1 = discontinuous sniffing with locomotor activity; 2 = continuous sniffing accompanied by locomotor activity; 3 = occasional licking, gnawing or biting with spasmodic locomotor activity; 4 = continuous licking, gnawing or biting with only occasional locomotor episodes.

Animals (200–250 g female Wistar rats; Bantin & Kingman Ltd.) were injected 1 h before apomorphine with either haloperidol (0·05–0·5 mg kg^{-1} i.p.), (±)-, (−)- or (+)-sulpiride (8–128 mg kg^{-1} i.p.), (±)- or (+)-sultopride (8–128 mg kg^{-1} i.p.), (−)-sultopride (8–64 mg kg^{-1} i.p.), alizapride (8–128 mg kg^{-1} i.p.), amisulpiride (8-128 mg kg^{-1} i.p.), metoclopramide (1–16 mg kg^{-1} i.p.), prosulpride (16–128 mg kg^{-1} i.p.), clebopride (0·125–2·0 mg kg^{-1} i.p.), YM 09151-2 (0·010–0·160 mg kg^{-1} i.p.).

ID50 values were obtained by calculating the dose of drug required to inhibit apomorphine-induced stereotyped behaviour in control animals by 50%. At least six animals were used at each dosage level.

In-vivo binding displacement of [^{3}H]spiperone by haloperidol and a series of substituted benzamide drugs

Female Wistar rats (125–150 g; Bantin & Kingman) were used. Animals were manually restrained and [^{3}H]spiperone (25 μCi in 250 μL 0·9% saline 15·6–17·0 Ci mmol^{-1}; Amersham International) was administered via the tail vein. Immediately, the animals were also injected intraperitoneally with either saline (0·9%), haloperidol (0·16 mg kg^{-1}), (±)- or (−)-, or (+)-sulpiride (80 mg kg^{-1}), (±)- or (+)-sultopride (80 mg kg^{-1}), (−)-sultopride (10·5 mg kg^{-1}), alizapride (46 mg kg^{-1}), amisulpiride (81 mg kg^{-1}), metoclopramide (5·6 mg kg^{-1}), prosulpride (88 mg kg^{-1}), clebopride (0·34 kg^{-1}) or YM 09151-2 (0·017 mg kg^{-1}).

One hour later animals were anaesthetized using chloral hydrate (500 mg kg^{-1} i.p., in 0·6 mL 0·9% saline, BDH). The thoracic cavity was opened and a cannula (1 mm diam.) was introduced into the aorta and the animals perfused with 0·9% saline (50 mL) to remove all blood; the jugular veins were cut to allow removal of overflow fluid. This procedure was carried out to reduce the non-specific/specific binding ratio (Niehoff et al 1979). Animals were then decapitated and the brain rapidly removed onto ice and the various brain areas dissected out based on the method of Glowinski & Iversen (1966).

Tissue samples were then oxidized using a Packard 306 Tri-Carb Tissue Oxidizer (98–99% recovery) with the addition of Monophase-40 (10 mL; Packard). Radioactive content of the samples was assessed by scintillation spectrometry using a Packard 460C scintillation counter (efficiency 43–45%). Correction for counting efficiency was made and the radioactivity contained in each area expressed in d min^{-1} wet weight of tissue.

The accumulation of radioactivity in brain areas was determined by comparison with the cerebellum. The radioactivity contained in the cerebellum was chosen as a blank value, since this brain area does not appear to contain dopamine receptors. There was little accumulation of [^{3}H]spiperone in this area, and administration of unlabelled drugs did not alter the radioactive content of the cerebellum. In addition, displacement of [^{3}H]spiperone with a high dose of unlabelled spiperone reduced the radioactivity in the other brain areas studied to approximately cerebellar levels (Chivers et al 1987).

The prevention of accumulation of radioactivity derived from [^{3}H]spiperone following drug displacement is represented either as total binding in control animals compared with binding in the presence of displacing agent, or is expressed as a percentage of the total binding after subtraction of the radioactivity present in the cerebellum in control animals remaining after administration of the displacing agent. In all experiments the data used in statistical analysis were the binding measurements above cerebellar levels. Results were analysed using a Mann Whitney U-test, and probability of less than 5% was considered to indicate a significant difference. Ten animals were used in each group.

Drugs

Drugs were obtained from the following sources: haloperidol: Janssen Pharmaceutica; (±)-, (−)- and (+)-sulpiride, (±)-, (−)- and (+)-sultopride, alizapride HCl, amisulpiride, prosulpride: Delagrange Laboratories; Metoclopramide HCl: Beecham Pharmaceuticals; clebopride: Almirall Laboratories; YM 09151-2 (*N*-(1-benzyl-2-methyl-3-pyrrolidinyl)-4-chloro-2-methoxy-4-methylaminobenzamide): Yamanouchi, Japan. All substituted benzamide drugs were dissolved in a minimum quantity of 2% sulphuric acid then brought back to pH 6·0–7·0. Haloperidol was dissolved in a minimum quantity of glacial acetic acid and then brought back to pH 6·0-6·5. [^{3}H]Spiperone was obtained from Amersham Internatiomal, specific activity ranging from 15·6–17·0 Ci mmol^{-1}, since two different batches were used in the course of the experiments.

Results

Inhibition of apomorphine-induced stereotypy by substituted benzamide drugs (Table 1)

Administration of apomorphine hydrochloride (0·5 mg kg^{-1} s.c., 15 min previously) caused a stereotyped response in control rats consisting of continuous sniffing, occasional licking, gnawing or biting accompanied by some locomotor activity (average score range for control groups 2·5–3·0). Haloperidol (0·05-0·5 mg kg^{-1} 1 h previously) potently inhibited the stereotyped response produced by apomorphine. Administration of (±)-sulpiride, (−)-sulpiride or (+)-sulpiride (8–128 mg kg^{-1} 1 h previously) did not inhibit apomorphine-induced stereotypy. Administration of (±)-sultopride (4–64 mg kg^{-1} 1 h previously) and (−)-sultopride (8–64 mg kg^{-1} 1 h previously), but not (+)-sultopride (16-128 mg kg^{-1} 1 h previously), inhibited apomorphine-induced stereotypy.

Metoclopramide (1-16 mg kg^{-1} 1 h previously), clebopride (0·125–1·0 mg kg^{-1} 1 h previously) and YM 09151-2 (0·010–0·160 mg kg^{-1} 1 h previously) potently inhibited apomorphine-induced stereotypy. In contrast, prosulpride (16–128 mg kg^{-1} 1 h previously), alizapride (8–128 mg kg^{-1} 1 h previously) and amisulpiride (8-128 mg kg^{-1} 1 h previously) only weakly inhibited the stereotyped response.

Table 1. ID50 values for inhibition of apomorphine-induced stereotyped behaviour by a range of substituted benzamide drugs and haloperidol. Apomorphine hydrochloride (0·5 mg kg^{-1} s.c.) was injected 15 min before the assessment of stereotyped behaviour. Drugs were administered (i.p.) 1 h before apomorphine administration. ID_{50} values were calculated as the dose required to inhibit control response by 50%.

Drug	Dose range (mg kg^{-1})	ID50 (mg kg^{-1})
Haloperidol	0·05–0·50	0·16
Clebopride	0·125–2·0	0·34
YM 09151-2	0·010–0·160	0·017
Metoclopramide	1·0–16	5·6
(±)-Sultopride	4·0–64	41
Amisulpiride	8·0–128	81
Prosulpride	16–128	88
Alizapride	8–128	46
(±)-Sulpiride	8–128	> 128
(−)-Sulpiride	8–128	> 128
(+)-Sulpiride	8–128	> 128
(−)-Sultopride	8–64	10·5
(+)-Sultopride	16–128	> 128

For active drugs the ID50 dose for inhibition of apomorphine-induced stereotypy was chosen as a pharmacologically effective dosage for subsequent study of in-vivo displacement of [^{3}H]spiperone. For non-active compounds ((±)-sulpiride, (+)- and (−)-sulpiride and (+)-sultopride) a dose of 80 mg kg^{-1} was used as a high dosage tolerated by animals without producing toxic effects. Although the data are not shown, these doses of (±)-sulpiride and (−)-sulpiride are sufficient to cause total inhibition of apomorphine-induced climbing in the rat. The inactive isomers, namely (+)-sulpiride and (+)-sultopride, are obviously ineffective in inhibiting any such behaviour in the doses employed.

Effect of haloperidol and a series of substituted benzamide drugs on the accumulation of radioactivity derived from [^{3}H]spiperone (Figs 1, 2, 3 and Table 2)

Administration of haloperidol (0·16 mg kg^{-1} i.p., 1 h before death) prevented the accumulation of [^{3}H]spiperone (25 μCi) in substantia nigra, striatum, tuberculum olfactorium and hypothalamus, but not in nucleus accumbens or frontal cortex (Figs 1–3). YM 09151-2 also displaced [^{3}H]spiperone from those areas but also from nucleus accumbens and frontal cortex.

Overall, other drugs examined prevented the accumulation of radioactivity in hypothalamus, but not nucleus accumbens or frontal cortex and varied in their effects on other brain areas. Alizapride, metoclopramide and clebopride prevented the accumulation of [^{3}H]spiperone in substantia nigra, striatum and tuberculum olfactorium. (±)-Sultopride (41 mg kg^{-1}) and amisulpiride (81 mg kg^{-1}) prevented accumulation of [^{3}H]spiperone in substantia nigra and tuberculum olfactorium but not striatum. (±)-Sulpiride (80 mg kg^{-1}) prevented accumulation of radioactivity in substantia nigra. Prosulpride (88 mg kg^{-1}) did not affect the accumulation of [^{3}H]spiperone in substantia nigra or tuberculum olfactorium.

It should be noted, however, that some drugs in some brain areas produced non-significant displacements equal to or greater than those shown by compounds producing a significant decrease in [^{3}H]spiperone accumulation. Thus,

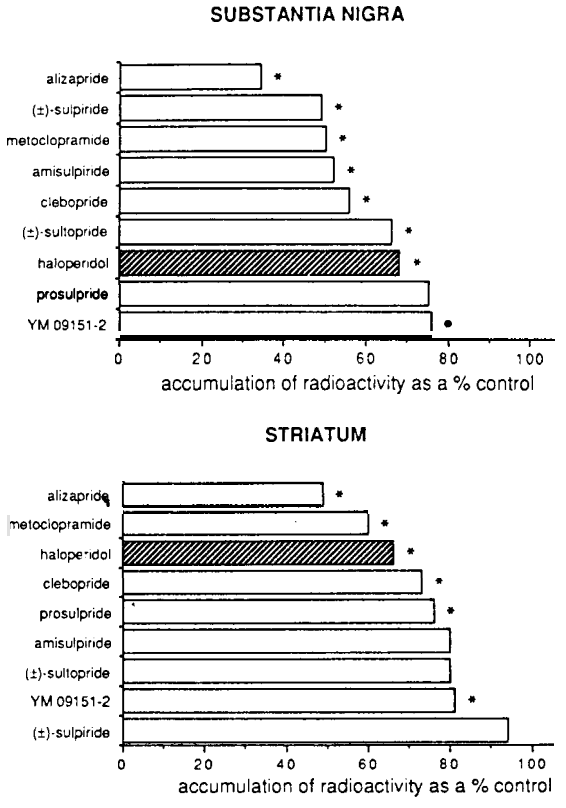

FIG. 1. Ability of a series of substituted benzamide drugs and haloperidol to prevent accumulation of radioactivity derived from [^{3}H]spiperone in substantia nigra and striatum. Data presented as the percentage accumulation of [^{3}H]spiperone (25 μCi per rat, n = 10) compared with controls, in the presence of haloperidol (0·16 mg kg^{-1}), (±)-sulpiride (80 mg kg^{-1}), alizapride (46 mg kg^{-1}), amisulpiride (81 mg kg^{-1}), metoclopramide (5·4 mg kg^{-1}), sultopride (41 mg kg^{-1}), prosulpride (88 mg kg^{-1}), clebopride (0·34 mg kg^{-1}), YM 09151-2 (0·017 mg kg^{-1}). In each area the amount of binding above cerebellar levels was calculated for both control and drug-treated groups. In the control group this was taken to represent total binding in that area and the amount of binding found in the presence of displacing drug was calculated as a percentage of total binding. The standard error of the mean was never greater than 15%. * $P < 0·05$ for displacement of [^{3}H]spiperone accumulation. Results analysed using a Mann-Whitney U-test.

considerable effects were produced by (±)-sultopride in striatum, prosulpride in the substantia nigra, metoclopramide in the nucleus accumbens and alizapride in the frontal cortex.

Effect of isomers of sulpiride and sultopride on accumulation of radioactivity derived from [^{3}H]-spiperone

Administration of (−)-sulpiride (80 mg kg^{-1} i.p. 1 h before death) prevented the accumulation of radioactivity derived from [^{3}H]spiperone in substantia nigra, striatum, tuberculum olfactorium and hypothalamus, but not in nucleus accumbens or frontal cortex (Fig. 4A). The administration of (+)-sulpiride (80 mg kg^{-1} i.p. 1 h before death) did not prevent the accumulation of [^{3}H]spiperone in any brain area studied (Fig. 4B).

Administration of (−)-sultopride (10·5 mg kg i.p. 1 h before death) also prevented the accumulation of [^{3}H]spiperone in substantia nigra, striatum, tuberculum olfactorium and hypothalamus but not in nucleus accumbens or frontal cortex (Fig. 5A). In contrast, administration of (+)-sulto-

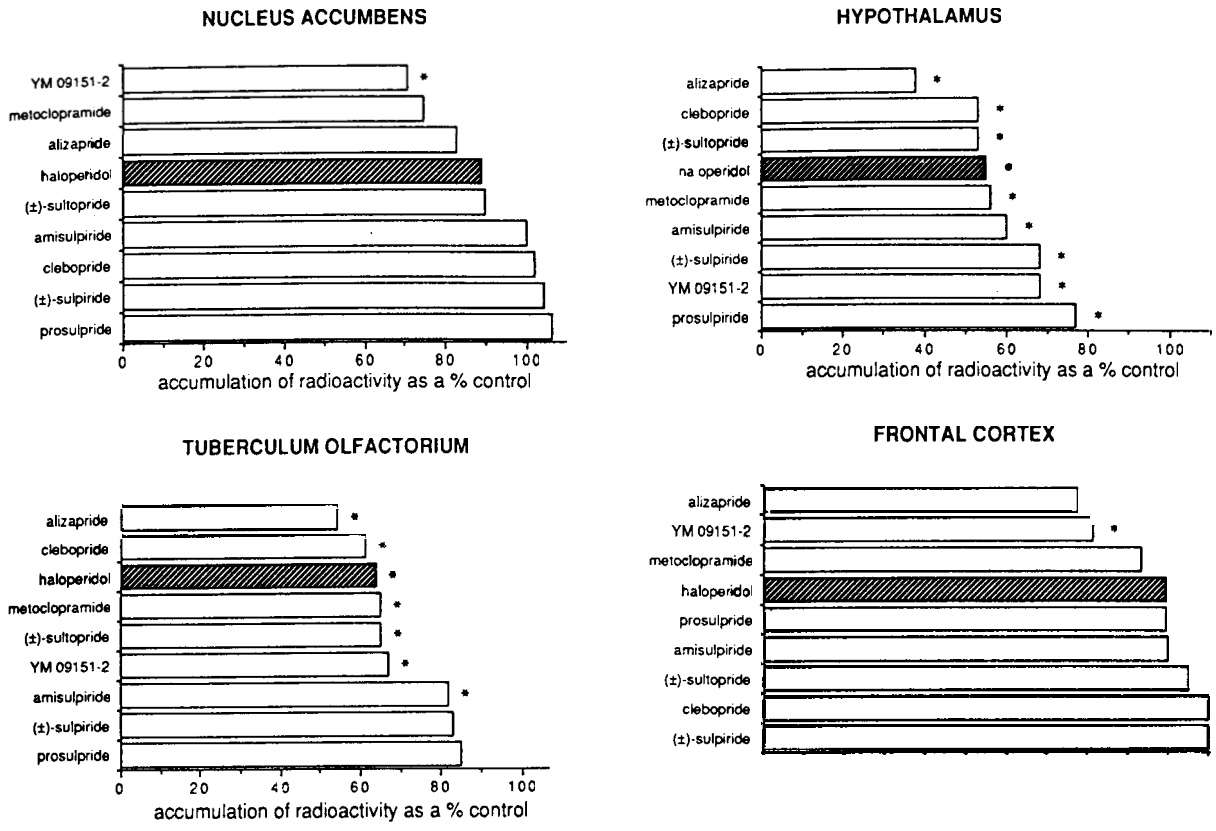

FIG. 2. Ability of a series of substituted benzamide drugs and haloperidol to prevent accumulation of radioactivity derived from [^{3}H]spiperone in nucleus accumbens and tuberculum olfactorium. Data presented as the percentage accumulation of [^{3}H]spiperone (25 μCi per rat, n = 10) compared with controls, in the presence of haloperidol (0·16 mg kg^{-1}), (±)-sulpiride (80 mg kg^{-1}), alizapride (46 mg kg^{-1}), amisulpiride (81 mg kg^{-1}), metoclopramide (5·4 mg kg^{-1}), (±)-sultopride (41 mg kg^{-1}), prosulpride (88 mg kg^{-1}), clebopride (0·34 mg kg^{-1}), YM 09151-2 (0·017 mg kg^{-1}). In each area the amount of binding above cerebellar levels was calculated for both control and drug-treated groups. In the control group this was taken to represent total binding in that area and the amount of binding found in the presence of displacing drug was calculated as a percentage of total binding. The standard error of the mean was never greater than 15%. * $P < 0.05$ for displacement of [^{3}H]spiperone accumulation. Results analysed using a Mann–Whitney U-test.

Table 2. Summary of the prevention of the in-vitro accumulation of [^{3}H]spiperone (25 μCi) by drugs in selected regions of rat brain.

	% displacement					
Drug	SN	STRI	NA	TO	HYPO	FC
Alizapride	66*	51*	17	46*	62*	23
Clebopride	44*	27*	−2	39*	47*	−10
(±)-Sultopride	34*	20	10	39*	47*	− 5
Haloperidol	32*	34*	11	36*	45*	1
Metoclopramide	50*	40*	25	35*	44*	7
Amisulpiride	48*	20	0	18*	40*	0
(±)-Sulpiride	51*	6	−4	17	32*	−10
YM 09151-2	24*	19*	29*	33*	32*	19*
Prosulpride	25	24*	−6	15	23*	1

The order of drugs is based on the rank order of potency in displacing [^{3}H]spiperone from the hypothalamus, an area in which all the compounds were effective.

SN—substantia nigra; STRI—striatum; NA—nucleus accumbens; TO—tuberculum olfactorium; HYPO—hypothalamus; FC—frontal cortex.

* $P < 0.05$ for displacement of [^{3}H]spiperone accumulation. Results analysed using a Mann-Whitney U-test.

pride (80 mg kg^{-1} i.p. 1 h before death) did not prevent the accumulation of radioactivity in any area studied (Fig. 5B).

Discussion

The difficulties in investigating in-vivo dopamine receptor binding in rats with [^{3}H]spiperone are similar to those experienced in PET scanning studies in man (Chivers et al 1987). Usually, only a single ligand concentration can be employed with definition of binding sites being made with a single pharmacological dose of an unlabelled displacing drug. In addition, in in-vivo binding studies the difference between extent of accumulation of radioactivity in brain in individual animals are considerable. As a consequence, displacement of approximately 50% of accumulated radioactivity above cerebellar levels by an unlabelled drug is necessary to reliably define a receptor population. This is particularly relevant to drugs such as the substituted benzamides where penetration into brain may be poor and variable (Herberg & Wishart 1980).

In these studies it was not feasible to administer a range of

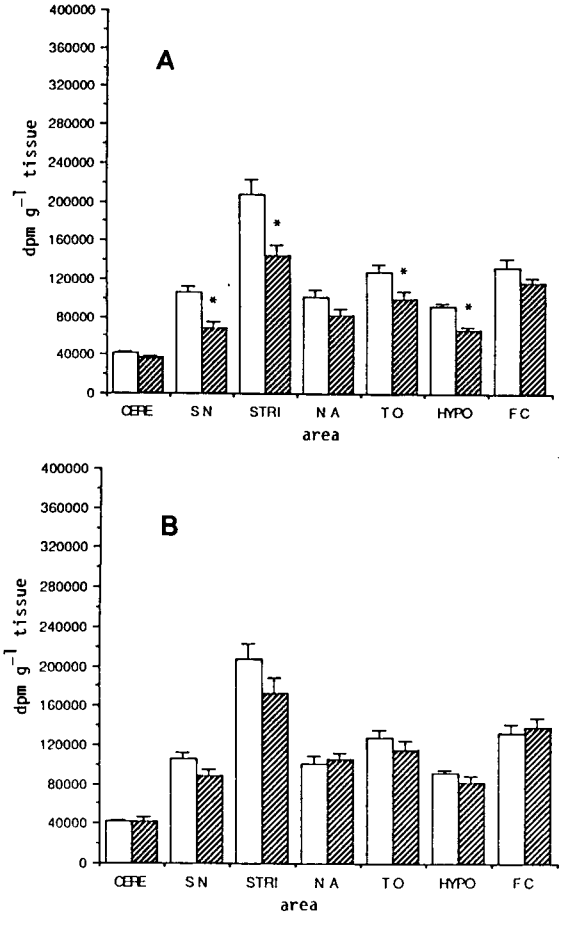

FIG. 4. A.B. Accumulation of radioactivity derived from [^{3}H]spiperone (25 μCi) in selected areas of rat brain (open bars) and its displacement following administration of (A) (−)- or (B) (+)-sulpiride (80 mg kg^{-1}) (hatched bars). [^{3}H]Spiperone (25 μCi per rat) was injected via the tail vein 1 h before death. Some animals also received (A) (−)- or (B) (+)-sulpiride (80 mg kg^{-1} i.p.) immediately following [^{3}H]spiperone administration. Statistical analysis was carried out using a Mann-Whitney U-test and results expressed as the mean ± s.e.m. for 10 animals in each treatment group. * < 0·05 for displacement of radioactivity by (−)-sulpiride. CERE—cerebellum, SN—substantia nigra, STRI—striatum, NA—nucleus accumbens, TO—tuberculum olfactorium, HYPO—hypothalamus, FC—frontal cortex.

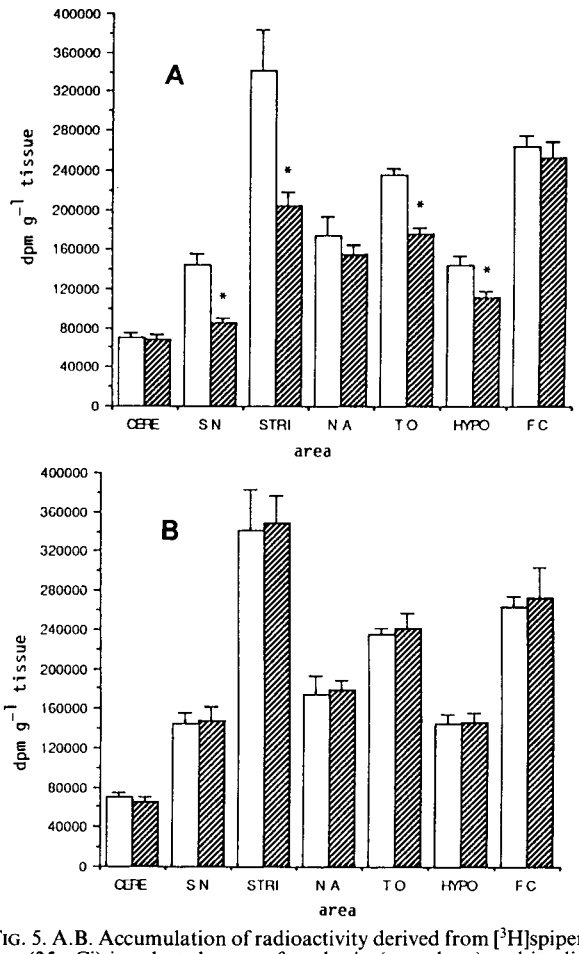

FIG. 5. A.B. Accumulation of radioactivity derived from [^{3}H]spiperone (25 μCi) in selected areas of rat brain (open bars) and its displacement following administration of (A) (−)- or (B) (+)-sultopride (10·5 or 80 mg kg^{-1} respectively) (hatched bars). [^{3}H]Spiperone (25 μCi per rat) was injected via the tail vein 1 h before death. Some animals also received (A) (−)- or (B) (+)-sultopride (10·5 or 80 mg kg^{-1} i.p., respectively) immediately following [^{3}H]spiperone administration. Statistical analysis was carried out using a Mann-Whitney U-test and results are expressed as the mean ± s.e.m. for 10 animals in each treatment group. * $P < 0·05$ for displacement of radioactivity by (−)-sultopride. CERE—cerebellum, SN—substantia nigra, STRI—striatum, NA—nucleus accumbens, TO—tuberculum olfactorium, HYPO—hypothalamus, FC—frontal cortex.

doses of each potential displacing agent so we used a single pharmacologically active dose. Drug doses were chosen as those capable of producing 50% inhibition of apomorphine-induced stereotypy. Rather than administering drugs in dose ratios equivalent to their in-vitro ability to displace [^{3}H]-spiperone, we allowed for differences in pharmacokinetic handling and in particular brain penetration, by using a functional test of dopamine receptor occupation. Some substituted benzamide drugs, for example sulpiride, are not active against apomorphine stereotypy. In this case we chose to use 80 mg kg^{-1} as a dose known to be totally effective in other paradigms, for example apomorphine-induced climbing.

As a standard displacing agent we employed haloperidol, since we have shown previously that in-vivo it only displaces [^{3}H]-spiperone from dopamine receptors in striatum, substantia nigra, tuberculum olfactorium and nucleus accumbens (Chivers et al 1987). Indeed haloperidol in this investigation displaced [^{3}H]spiperone from the substantia nigra, striatum, tuberculum olfactorium and hypothalamus, but not from nucleus accumbens or frontal cortex. Consequently, it was possible to use the effectiveness of haloperidol in the dose employed as a standard for the effectiveness of substituted benzamide drugs.

Of the substituted benzamide drugs examined, we previously showed only (±)-sulpiride and alizapride to be entirely selective for dopamine receptors as judged by in-vitro ligand binding experiments (Chivers et al 1988). The other compounds used showed varying affinities in-vitro for either 5-HT or noradrenaline sites. In the present in-vivo experiments, (±)-sulpiride was not effective in preventing the accumulation of [^{3}H]spiperone in striatum or tuberculum olfactorium. Although a selective agent in-vitro, its actions in-vivo would not be sufficient to identify all dopamine containing areas. Similarly, (±)-sultopride, prosulpride and amisulpiride did not prevent the accumulation of [^{3}H]spiperone in all areas where the ligand identified brain dopamine

receptors. Only alizapride, metoclopramide, clebopride and YM 09151-2 produced displacement of [^{3}H]spiperone from substantia nigra, striatum, tuberculum olfactorium and hypothalamus. However, YM 09151-2 also produced displacement in the nucleus accumbens and frontal cortex, reflecting its wide range of action on other receptors as determined by in-vitro ligand binding experiments. In addition, while alizapride, metoclopramide and clebopride were more, or equally, effective as haloperidol, YM 09151-2 was one of the least effective displacing drugs used. Of the three remaining compounds both metoclopramide and clebopride showed activity at α-1 and 5HT-2 sites in-vitro. So, although these were active only on dopamine-containing regions identified by [^{3}H]spiperone in-vivo, some question remains as to their specificity for dopamine receptors under other conditions. Only alizapride fulfilled the criteria of a selective D-2 action in-vitro, coupled with the ability to identify dopamine receptors labelled by [^{3}H]spiperone and defined by haloperidol in-vitro. Indeed, the greatest displacements produced by any drug were invariably produced by alizapride.

Although the use of raclopride to identify brain dopamine receptors as defined by [^{3}H]spiperone accumulation was not investigated in this study, Kohler et al (1985) have determined specific in-vivo binding of [^{3}H]raclopride. They suggest [^{3}H]raclopride would be a useful tool for in-vivo studies since it penetrates into brain readily and binds with high specificity to dopamine receptors.

Two of the most commonly used substituted benzamide drugs, (±)-sulpiride and (±)-sultopride, were employed in this investigation. (±)-Sulpiride was selected on the basis of its in-vitro selectivity for dopamine receptors, while (±)-sultopride showed in-vitro potency for dopamine receptors at concentrations allowing good separation from its effect on noradrenaline sites. However, neither drug produced a consistent identification of dopamine receptors determined by [^{3}H]spiperone in the striatum. This may relate to the in-vivo potency of these agents or more likely to their variable penetration into different brain regions. Both sulpiride and sultopride exist in enantiomeric forms; in each case the (−)-isomer is far more active than the (+)-form (Jenner et al 1980). Consequently, it is possible to use the enantiomers to obtain greater pharmacological activity and selectivity by comparison of the effects of the (+)- and (−)-isomers. Indeed, compared with the racemic drugs, (−)-sulpiride and (−)-sultopride clearly defined the binding of [^{3}H]spiperone to dopamine receptors in striatum, substantia nigra, tuberculum olfactorium and hypothalamus. In contrast, (+)-sulpiride and (+)-sultopride were without effect on the accumulation of [^{3}H]spiperone in any brain area examined. In conclusion, not all substituted benzamide drugs are suitable for defining the in-vivo binding of dopamine ligands in all brain regions. However, selected drugs, such as alizapride and raclopride, may be appropriately employed in PET studies in man. Alternatively, the use of the optical isomers of compounds, such as sulpiride or sultopride, may allow characterization of dopamine receptors by the classical technique of enantiomeric selectivity.

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