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Definition of the In-vivo Accumulation of [^{3}H]spiperone in Brain using Haloperidol and Sulpiride to Determine Functional Dopamine Receptor Occupation

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Abstract—The in-vivo administration of [^{3}H]spiperone caused an accumulation of radioactivity in the substantia nigra, tuberculum olfactorium, nucleus accumbens, striatum and frontal cortex when compared with cerebellar levels. Haloperidol (0·01–1·0 mg kg^{-1} i.p.) dose-dependently prevented the accumulation of [^{3}H]spiperone in the substantia nigra, tuberculum olfactorium, striatum and nucleus accumbens. Sulpiride (10–160 mg kg^{-1} i.p.) dose-dependently prevented the accumulation of [^{3}H]spiperone only in the substantia nigra. The effects of sulpiride on other areas were not consistent; there was a suggestion of a reduction in the accumulation of [^{3}H]spiperone in tuberculum olfactorium and striatum, but not in nucleus accumbens. Neither haloperidol (0·01–1·0 mg kg^{-1} i.p.) nor sulpiride (10–160 mg kg^{-1} i.p.) caused displacement of [^{3}H]spiperone from the frontal cortex. Both haloperidol (0·01–0·5 mg kg^{-1}) and sulpiride (10–80 mg kg^{-1}) increased striatal and mesolimbic HVA concentrations. Haloperidol potently blocked apomorphine-induced stereotypy but sulpiride was only effective at the highest dose employed. The functional effect produced by haloperidol correlated with its ability to define [^{3}H]spiperone binding in-vivo to dopamine receptors in the substantia nigra, striatum and tuberculum olfactorium. In contrast, there was no correlation between functional effect of sulpiride and its ability to define [^{3}H]spiperone binding in-vivo.

Position emission tomography (PET) scanning is being increasingly used to examine abnormalities of brain function in-vivo in man. Compounds such as [^{18}F]spiperone (Arnett et al 1985a), [^{18}F]methylspiperone (Arnett et al 1985b), [^{11}C]methylspiperone (Wagner et al 1984), [^{77}Br]bromospiperone (Maziere et al 1984) and [^{11}C]raclopride (Farde et al 1986) are potential candidates for the identification of dopamine receptors in the basal ganglia in diseases such as Parkinson's disease and schizophrenia. However, many of these ligands are not specific for brain dopamine receptors; they may also identify noradrenaline and 5-HT sites (List & Seeman 1981). Indeed, in rodents the in-vivo binding of [^{3}H]spiperone has been shown to identify dopamine receptors alone, only in the striatum, substantia nigra and tuberculum olfactorium (Chivers et al 1987 and references contained therein). In the frontal cortex [^{3}H]spiperone identified mainly 5-HT sites whereas in nucleus accumbens the ligand interacted with both 5-HT and dopamine receptors.

In relation to PET studies in man, the lack of specificity of spiperone and its derivatives raises the problem of interpretation of the images produced. The nature of the receptor population can be assessed by prevention of the accumulation of the ligand within a given brain area by prior administration of a high dose of another dopamine receptor antagonist. However, this adds increased complexity, for the unlabelled neuroleptic drug may act on a variety of neuronal receptors in brain, and may itself cause unwanted pharmacological effects. For example, haloperidol may identify dopamine and 5-HT receptors (Leysen et al 1978a; Helmeste & Seeman 1983), and may induce acute dystonic reactions or worsen parkinsonism. However, drugs of the substituted benzamide group, such as sulpiride, only identify dopamine receptors in brain (Jenner & Marsden 1981) but such drugs may not equally affect all brain dopamine containing areas (Costall et al 1978). Thus, it has been claimed that sulpiride selectively displaces [^{3}H]spiperone in-vivo from mesolimbic rather than striatal dopamine receptors (Kohler et al 1979, 1981).

It remains unknown whether the radioactivity detected in brain during PET studies reflects an interaction with neuronal receptors or an accumulation within the extracellular space. Indeed, recently Barone and colleagues (1985) have shown the accumulation of [^{3}H]spiperone in rat brain to involve a considerable amount of drug present in soluble fractions compared with that specifically associated with membrane fractions. So it is necessary in human studies to determine the extent to which ligand accumulation reflects the identification of pharmacologically relevant receptor sites.

In the present study we have compared the use of haloperidol and sulpiride in defining the interaction of [^{3}H]spiperone with dopamine receptors in rat basal ganglia. We wished to assess which drug class might be the most appropriate for defining the interaction of ligands with brain dopamine receptors during PET investigations. We have also compared the functional interaction of these drugs with cerebral dopamine receptors as judged by alterations in brain dopamine turnover and the inhibition of dopamine mediated behaviours with their ability to cause regional displacement of [^{3}H]spiperone.

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Materials and Methods

Administration of [^{3}H]spiperone

Female Wistar rats (125–150 g; Bantin & Kingman) were used. Animals were manually restrained and [^{3}H]spiperone (3·75 μg kg^{-1}; 25 μCi in 250 μL 0·9% saline; 21 Ci $mmol^{-1}$; Amersham International) was administered via the tail vein. The dose of [^{3}H]spiperone used was previously shown to provide an adequate level of radioactivity in brain that readily allowed the detection of changes in drug displacement experiments (see Chivers et al 1987). After 1 h the animals were anaesthetized using chloral hydrate (500 mg kg^{-1} i.p. in 0·6 mL 0·9% saline; British Drug Houses Ltd). The thoracic cavity was opened and blood was removed by cardiac puncture and placed in lithium heparin tubes. 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 severed to accommodate the overflow of fluid. This procedure was carried out to reduce the non-specific/specific binding ratio (Niehoff et al 1979).

Tissue radioactivity determinations

Animals were decapitated, the brain rapidly removed onto ice and dissected according to Glowinski & Iversen (1966).

The brain was positioned with its dorsal surface uppermost. The rhombencephalon was separated by removing the cerebellum using iris forceps, and making a transverse 30° angled cut from the dorsal surface of the medulla oblongata to the ventral surface of the brain, exposing the caudal edge of the substantia nigra. The brain was turned to expose the ventral surface and the substantia nigra was removed using micro-dissecting forceps. Using iris forceps the tuberculum olfactorium and nucleus accumbens were dissected out. The brain was turned again to expose the dorsal surface and the two hemispheres parted using iris forceps to expose the striata. The striatum was removed using iris forceps. A portion of frontal cortex was removed using a scalpel blade. The areas dissected weighed (mean ± 1 s.e.m.); cerebellum 54·3 ± 3·2 mg; substantia nigra 6·9 ± 0·3 mg; olfactory tubercules 34·1 ± 1·7 mg; nucleus accumbens 6·4 ± 0·5 mg; striata 51·8 ± 1·7 mg; frontal cortex 32·8 ± 1·9 mg.

The tissue samples were placed in Soluene (1·0 mL; Packard) and dissolved. Following the addition of Instafluor II (10 mL; Packard) the samples were left in darkness for a minimun of 6 h to eliminate chemiluminescence. Subsequently radioactivity was assessed by scintillation spectroscopy using a Packard 460C scintillation spectrometer. The efficiency of counting varied between 36 and 39% dependent on tissue weight and nature. Correction for counting efficiency was made and the radioactivity contained in each area was expressed in d min^{-1} g^{-1} wet weight of tissue.

Drug competition experiments

In drug competition experiments animals received 0·9% saline (0·5 mL) or haloperidol (0·01–1·0 mg kg^{-1} in 0·5 mL 0·9% saline 1 h before death; Janssen Pharmaceutica, Belgium) or sulpiride (10–160 mg kg^{-1} i.p. on 0·5 mL 0·9% saline 3 h before death; Delagrange, France). Haloperidol was initially dissolved in a minimum quantity of glacial acetic acid diluted to volume and the pH adjusted to between 6·0 and 6·5 using 2 M sodium hydroxide. Sulpiride was dissolved in a minimum quantity of 2% sulphuric acid diluted to volume, and the pH adjusted to between 6·0–6·5 using 2M sodium hydroxide. The doses and timings were those found in separate experiments to be effective against the dopamine receptor stimulation produced by apomorphine as exhibited by stereotyped behaviour (see below).

The prevention of accumulation of radioactivity derived from [^{3}H]spiperone by sulpiride or haloperidol was expressed as a percentage of the total binding occurring in rats treated with saline alone following subtraction of radioactivity present in cerebellum. The cerebellum was chosen for blank determination as a region not containing dopamine innervation. To determine the maximum displacement occurring in each area, unlabelled spiperone (5·0 mg kg^{-1} i.p. 1 h previously; Janssen Pharmaceutica, Belgium) and (+)-butaclamol (5·0 mg kg^{-1} 1 h previously; Ayerst Laboratories) was administered. Total binding and drug displacement was carried out using 10 animals at each dosage level.

Determination of striatal and mesolimbic HVA concentrations

Female Wistar rats (125–150 g) received either 0·9% saline (0·5 mL), haloperidol (0·01–0·5 mg kg^{-1} i.p. 1 h before death) or sulpiride (10–80 mg kg^{-1} i.p. 3 h before death). Rats were killed by cervical dislocation and decapitation and the brains rapidly removed onto ice. The mesolimbic area (nucleus accumbens plus tuberculum olfactorium) and the striatum (caudate putamen) were dissected out and homogenized in 0·4 M perchloric acid (1·0 mL). HVA was separated using G10 Sephadex columns by a modification of the method of Earley & Leonard (1978) and measured using the semi-automated fluorimetric technique of Westerink & Korf (1977).

Apomorphine-induced stereotyped behaviour

Stereotyped behaviour was assessed 15 min following administration of apomorphine hydrochloride (0·5 mg kg^{-1} s.c. in 0·1 mL 0·9% saline; MacFarlan Smith) 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.

Haloperidol (0·05–0·5 mg kg^{-1} i.p.) and sulpiride (10–160 mg kg^{-1} i.p.) were administered 1 h and 3 h before the administration of apomorphine, respectively. Stereotypy was assessed in 8 animals at each dosage level.

Statistics

In experiments to determine the maximum displacement of [^{3}H]spiperone by unlabelled spiperone and (+)-butaclamol, statistical analysis was carried out using a Mann-Whitney U test. In drug competition experiments, dose-response curves were obtained using linear regression analysis. The difference in the slopes of the dose-response curves in each area compared with the slope of the dose-response curves in the cerebellum was compared using analysis of variance. Striatal and mesolimbic HVA determinations dose-response curves were analysed using linear-regression analysis and ED100 values calculated. Inhibition of apomorphine-induced stereotyped behaviour was analysed using a Chi-squared test.

Results

Accumulation of [^{3}H]spiperone in rat brain

Following administration of [^{3}H]spiperone (25 μCi i.v.; 1 h before death) radioactivity derived from the ligand accumulated in substantia nigra, tuberculum olfactorium, nucleus accumbens, striatum and frontal cortex when compared with levels in the cerebellum (Fig. 1). Accumulation was most marked in the striatum, followed by the tuberculum olfactorium and frontal cortex and less marked in substantia nigra and nucleus accumbens. Administration of unlabelled spiperone (5·0 mg kg^{-1} i.p. 1 h previously) caused a reduction of radioactivity to approximately cerebellar levels in these areas. Similarly (+)-butaclamol (5 mg kg^{-1} i.p. 30 min previously) also caused displacement in areas where [^{3}H]spiperone accumulated.

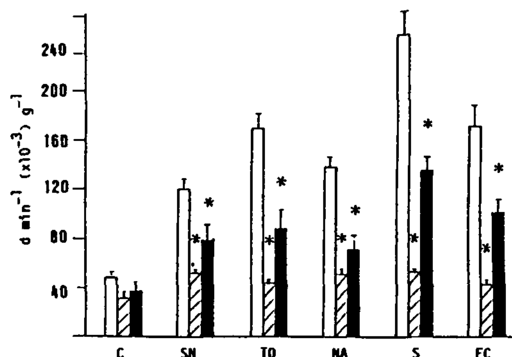

FIG. 1. Accumulation of radioactivity derived from [^{3}H]spiperone (25 μCi i.v.) in selected areas of rat brain and its displacement following administration of unlabelled spiperone and (+)-butaclamol. The open bars represent the total accumulation of radioactivity in that area after administration of [^{3}H]spiperone (25 μCi per rat, i.v., 1 h previously). The hatched bars represent the amount of [^{3}H]spiperone accumulating in the presence of 5 mg kg^{-1} unlabelled [^{3}H]spiperone (i.p. 1 h before death). The solid bars represent the amount of [^{3}H]spiperone accumulating in the presence of 5 mg kg^{-1} (+)-butaclamol (i.p., 30 min before death). The values are the mean $\pm$ 1 s.e.m. of 10 rats per group. *$P<0·05$ for displacement of radioactivity compared with totals. Statistical analysis was carried out using a Mann-Whitney U-test. C = cerebellum, SN = substantia nigra, TO = tuberculum olfactorum, NA = nucleus accumbens, S = striatum, FC = frontal cortex.

Prevention of accumulation of [^{3}H]spiperone by haloperidol or sulpiride (Figs 2, 3)

In control animals there was an accumulation of radioactivity in all brain areas studied compared with cerebellum. In the control group for haloperidol experiments radioactivity levels were; cerebellum—71 719 $\pm$ 3581, substantia nigra—152 770 $\pm$ 9115, tuberculum olfactorium—206 993 $\pm$ 11 706, nucleus accumbens—149 709 $\pm$ 9305, striatum—300 026 $\pm$ 19 345 and frontal cortex—199 390 $\pm$ 12 015 d min^{-1} g^{-1} tissue. In the control group for the sulpiride experiments radioactivity levels were; cerebellum—63 513 $\pm$ 6388, substantia nigra—147 766 $\pm$ 18 910, tuberculum olfactorium—206 562 $\pm$ 31 526, nucleus accumbens—133 901 $\pm$ 15 955 striatum—296 497 $\pm$ 32 795 and frontal cortex—203 264 $\pm$ 12 015 d min^{-1} g^{-1} tissue. In the substantia nigra, administration of haloperidol (0·01–1·0 mg kg^{-1} i.p., 1 h previously) caused a dose-dependent decrease in the accumulation of radioactivity derived from [^{3}H]spiperone. Sulpiride (10–160 mg kg^{-1} i.p., 3 h previously) also caused a dose-dependent decrease in the accumulation of [^{3}H]spiperone in the substantia nigra. In the tuberculum olfactorium, haloperidol (0·01–1·0 mg kg^{-1} i.p., 1 h previously) decreased [^{3}H]spiperone accumulation in a dose-dependent fashion, but sulpiride had no significant effect. In

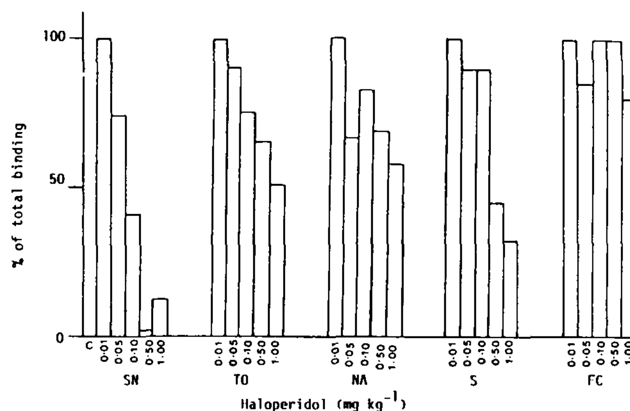

FIG. 2. Accumulation of radioactivity derived from [^{3}H]spiperone (25 μCi i.v.) in selected areas of rat brain and its displacement following administration of haloperidol. Control rats received [^{3}H]spiperone (25 μCi per rat) via the tail vein, 1 h before death. This gives a value for the total amount of [^{3}H]spiperone accumulating in each area. Other rats also received haloperidol (0·01–1·0 mg kg^{-1} i.p., 1 h before death). The bars represent the amount of [^{3}H]spiperone accumulating in the presence of haloperidol (0·01–1·0 mg kg^{-1}) and this is calculated as a percentage of the total accumulation following subtraction of radioactivity present in cerebellum. The effect of haloperidol was assessed by comparing the difference in slopes of the dose response curves in each brain area with that in the cerebellum using analysis of variance. The cerebellum was previously shown to be an area where [^{3}H]spiperone did not interact with brain dopamine receptors (see Chivers et al 1987). See Fig. 1 for abbreviations.

	F ratio	*P* value
Substantia nigra	$F_{1,50}=67·51$	$<0·01$
Tuberculum olfactorium	$F_{1,50}=34·47$	$<0·01$
Nucleus accumbens	$F_{1,50}=5·45$	$<0·05$
Striatum	$F_{1,50}=61·49$	$<0·01$

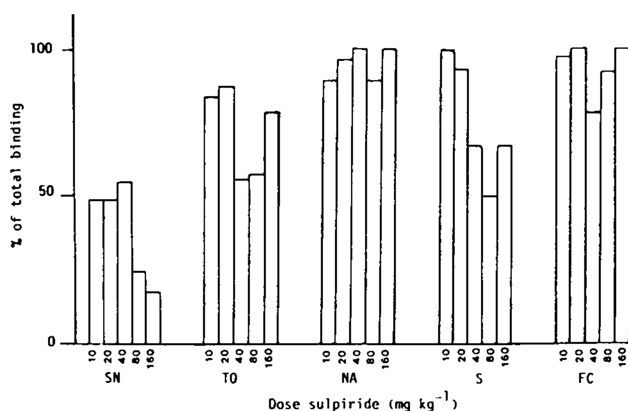

Fig. 3. Accumulation of radioactivity derived from [^{3}H]spiperone (25 μCi i.v.) in selected areas of rat brain and its displacement following administration of sulpiride. Control rats received [^{3}H]spiperone (25 μCi per rat) via the tail vein, 1 h before death. This gives a value for the total amount of spiperone accumulating in each area. Other rats also received sulpiride (10–160 mg kg^{-1} i.p., 3 h before death). The bars represent the amount of [^{3}H]spiperone accumulating in the presence of sulpiride (10–160 mg kg^{-1}) and this is calculated as a percentage of the total accumulation following subtraction of radioactivity present in cerebellum. The values are the mean of those obtained for 10 individual rats at each dosage level. The effect of sulpiride was assessed by comparing the difference in the slope of the dose response curves in each brain area with that in the cerebellum using analysis of variance. The cerebellum was previously shown to be an area where [^{3}H]spiperone did not interact with brain dopamine receptors (see Chivers et al 1987). See Fig. 1 for abbreviations.

	F ratio	*P* value
Substantia nigra	$F_{1,47}=25{\cdot}67$	$<0{\cdot}01$
Tuberculum olfactorium	$F_{1,50}=3{\cdot}54$	$>0{\cdot}05$
Nucleus accumbens	$F_{1,50}=0{\cdot}81$	$>0{\cdot}05$
Striatum	$F_{1,50}=3{\cdot}84$	$>0{\cdot}05$

the nucleus accumbens, haloperidol (0·01–1·0 mg kg^{-1} i.p., 1 h previously) decreased the accumulation of [^{3}H]spiperone in a dose-dependent manner; sulpiride had no dose-dependent effect. In the striatum, administration of haloperidol (0·01–1·0 mg kg^{-1} i.p., 1 h previously) prevented the accumulation of [^{3}H]spiperone in a dose-dependent manner; sulpiride had no significant dose-dependent effect. In the frontal cortex, neither haloperidol nor sulpiride had a significant effect on [^{3}H]spiperone accumulation.

Plasma and red blood cell levels of [^{3}H]spiperone were unaffected by the administration of haloperidol (0·01–1·0 mg kg^{-1} i.p., 1 h previously) or sulpiride (10–160 mg kg^{-1} i.p., 3 h previously) (data not shown).

Striatal and mesolimbic HVA concentrations

In saline-treated control animals HVA concentrations were 1001 ± 58 ng g^{-1} in striatum and 282 ± 43 ng g^{-1} in mesolimbic tissue. Administration of haloperidol (0·01–0·50 mg kg^{-1} i.p., 1 h previously) caused elevation of HVA concentrations in striatum and mesolimbic tissue in a dose-dependent fashion with ED100 values of 0·018 and 0·013 mg kg^{-1}, respectively (Fig. 4).

Administration of sulpiride (10–80 mg kg^{-1} i.p. 3 h previously) caused elevation of HVA concentrations in striatum and mesolimbic tissue in a dose-dependent fashion, ED100 values of 51·0 and 25·5 mg kg^{-1}, respectively (Fig. 4).

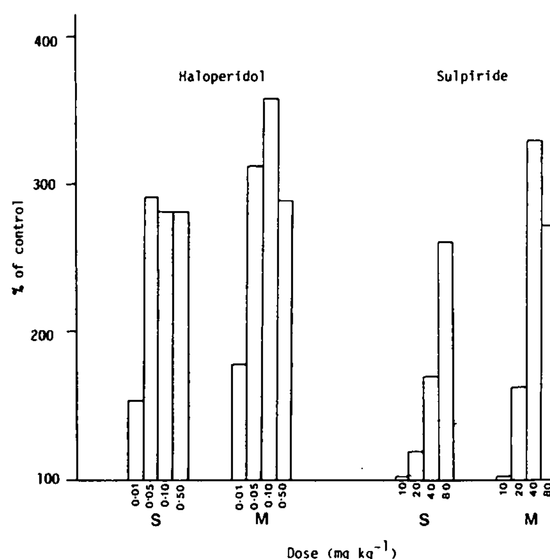

Fig. 4. Effect of haloperidol (0·01–0·50 mg kg^{-1}) and sulpiride (10–80 mg kg^{-1}) on HVA levels in rat striatum (S) and mesolimbic (M) area. Some animals received haloperidol (0·01–0·50 mg kg^{-1} i.p., 1 h before death). Some animals received sulpiride (10–80 mg kg^{-1} i.p. 3 h before death). Each value is the mean of 3 experiments and is expressed as a percentage of the related control (= 100%). Statistical analysis was carried out using linear regression analysis.

Apomorphine-induced stereotypy

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 = 2·9). Administration of haloperidol (0·01 and 0·05 mg kg^{-1} i.p., 1 h previously) did not inhibit apomorphine-induced stereotyped behaviour (Fig. 5). Haloperidol (0·1, 0·25 and 0·50 mg kg^{-1}) inhibited apomorphine-induced response (ID50 value 0·16 mg kg^{-1}). Administration of sulpiride (10–160 mg kg^{-1} i.p., 3 h previously) did not

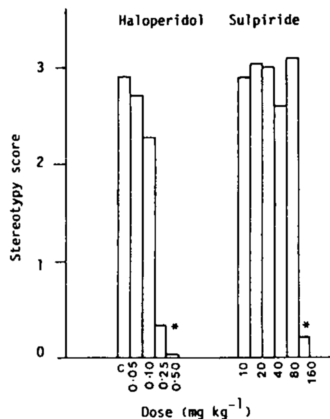

FIG. 5. Effect of haloperidol (0·05–0·5 mg kg^{-1}) and sulpiride (10–160 mg kg^{-1}) on apomorphine-induced stereotyped behaviour. For the stereotypy experiments control animals (C) received apomorphine hydrochloride (0·5 mg kg^{-1} s.c. in 0·1 mL 0·9% saline). Some animals received haloperidol (0·05–0·5 mg kg^{-1} i.p. 1 h prior to apomorphine administration) and some received sulpiride (10–160 mg kg^{-1} i.p., 3 h prior to apomorphine administration). Stereotyped behaviour was assessed 15 min following administration of apomorphine. Eight animals were examined at each dosage level. $^*P < 0·05$ for inhibition of apomorphine-induced stereotypy compared with controls. Statistical analysis was performed using a Chi-squared test.

inhibit the stereotyped response to apomorphine except at the highest dose used (160 mg kg^{-1}) (ID50 126 mg kg^{-1}).

Discussion

In this study we have compared the ability of two neuroleptic drugs, haloperidol and sulpiride, to define the interaction of [^{3}H]spiperone in-vivo with dopamine receptors in selected areas of rat brain. As previously demonstrated we found [^{3}H]spiperone to accumulate to differing extents in the brain regions studied (Chivers et al 1987). In all areas except cerebellum the accumulation of [^{3}H]spiperone could be prevented by the prior administration of unlabelled spiperone or (+)-butaclamol. Such results validate the approach used in PET scanning studies where ligand accumulation is measured relative to that occurring in cerebellum (Ferrero et al 1983; Wagner et al 1983; Maziere et al 1984).

The typical neuroleptic drug haloperidol, in the doses used, did not prevent the accumulation of [^{3}H]spiperone in frontal cortex, where the latter largely identifies 5-HT sites (Creese & Snyder 1978; List & Seeman 1981). In the substantia nigra, striatum, tuberculum olfactorium and nucleus accumbens, haloperidol caused a dose related decrease of [^{3}H]spiperone accumulation in agreement with previous studies (Kohler et al 1979; 1981). However, the prevention of [^{3}H]spiperone accumulation by haloperidol was more marked in the substantia nigra than in other areas.

The results of experiments employing haloperidol suggest that this drug may be usefully employed to assess the extent of ligand binding to dopamine receptors. It should be remarked, however, that haloperidol does interact with 5-HT receptors and that in other brain areas this may compromise the interpretation of in-vivo binding data. In contrast to haloperidol, sulpiride appeared less effective in defining the interaction of [^{3}H]spiperone to brain dopamine receptors in many brain regions.

As expected, sulpiride caused no displacement in the frontal cortex since it does not interact with 5-HT sites (Jenner & Marsden 1981). In the striatum (and to a less convincing degree in the tuberculum olfactorium) sulpiride in doses of 40–80 mg kg^{-1} appeared to partially prevent [^{3}H]spiperone accumulation. But sulpiride at a dose of 160 mg kg^{-1} caused less displacement of [^{3}H]spiperone than observed at lower doses. Overall there was no dose-dependent effect of sulpiride on [^{3}H]spiperone accumulation in the striatum or tuberculum olfactorium even if the data obtained at 160 mg kg^{-1} sulpiride were omitted from the analyses. In a previous study, we did observe a statistically significant displacement of [^{3}H]spiperone from the tuberculum olfactorium following administration of sulpiride (40 mg kg^{-1} i.p.) (Chivers et al 1987). This emphasizes one of the difficulties of in-vivo binding studies where it is necessary to produce > 50% displacement of [^{3}H]spiperone to measure consistent effects. Indeed for a drug such as sulpiride, which is weakly lipophilic and enters into brain poorly, small differences in drug penetration in individual animals result in a large variance so making small effects statistically difficult to assess. In these experiments 10 animals were used at each dosage level but still significance was difficult to achieve. With a large increase in group size such differences may become significant.

Among those areas where [^{3}H]spiperone clearly identifies dopamine receptors alone (namely the substantia nigra, tuberculum olfactorium and striatum), sulpiride only caused marked displacement in the substantia nigra. Surprisingly, sulpiride did not prevent the accumulation of [^{3}H]spiperone in the nucleus accumbens, an area previously associated with a selective action of the drug. Kohler et al (1979, 1981) also showed that sulpiride did not displace [^{3}H]spiperone from the nucleus accumbens in low doses (20 mg kg^{-1} i.p.). However, higher doses (150 and 250 mg kg^{-1}) were effective in their study, although not in ours. Those authors also found an effect of sulpiride on other limbic areas, including the tuberculum olfactorium, although we could not consistently demonstrate that effect. Again, such discrepancies emphasize the difficulties of using a drug of low lipophilicity and poor brain penetration, such as sulpiride, to consistently define the in-vivo binding of ligand to brain dopamine receptors.

The critical issue remains as to how the displacements of [^{3}H]spiperone caused by haloperidol and sulpiride relates to their pharmacological action. This issue has previously been discussed (Kohler et al 1979, 1981, Magnusson et al 1986) for both haloperidol and sulpiride. In these studies, the ability of haloperidol and sulpiride to block stereotyped behaviour was correlated with displacement of [^{3}H]spiperone in the striatum. In contrast, it was found that the ability of low doses of sulpiride to inhibit apomorphine-induced hyperactivity correlated with a selective effect on limbic brain regions (tuberculum olfactorium and septum, but not the nucleus accumbens). It was also claimed that the ability to increase striatal dopamine turnover was related to the displacement of [^{3}H]spiperone from striatum caused by the same drug doses. Those studies concluded that there was a good relationship between in-vivo [^{3}H]spiperone binding and drug effect.

In our study this was also at least partially true for

haloperidol. Thus, the ED100 values for haloperidol to elevate striatal dopamine turnover (as judged by HVA content), and to inhibit apomorphine-induced stereotypy were qualitatively consistent with the ED50 values for displacement of [^{3}H]spiperone (Table 1). However, in contrast to previous studies no absolute correlation between doses preventing [^{3}H]spiperone accumulation and those having behavioural or biochemical effects was observed, with the possible exception of the changes in the substantia nigra. Such exact relationships should not be expected since higher concentrations of haloperidol may be required to prevent accumulation of [^{3}H]spiperone than to inhibit the effects of apomorphine. Indeed, ligands such as [^{3}H]spiperone are employed for their high affinity for dopamine receptors, an affinity greater than that of either haloperidol or apomorphine (Leysen et al 1978b). As far as sulpiride is concerned, the inconsistent displacement of [^{3}H]spiperone in many brain areas caused by that drug shows little correlation between drug effect and displacement of [^{3}H]spiperone binding in-vivo. So another reason for not employing sulpiride to define the specific interaction of [^{3}H]spiperone is the apparent lack of correlation with functional effect.

Interestingly for both haloperidol and sulpiride, the ED50 values for inhibition of apomorphine-induced climbing behaviour (unpublished data; Table 1) showed a better correlation with displacement of [^{3}H]spiperone from striatum or tuberculum olfactorium than observed for stereotyped behaviour. So the correlation achieved between in-vivo [^{3}H]spiperone binding and functional effects may be dependent on the motor behaviour studied. Indeed, in a recent commentary (Swart & Korf 1987) it was suggested that behavioural responses should not necessarily correlate with biochemical response since "behaviour is a reflection of a more integral function of an organism and often cannot be directly deduced by the activity of a class of receptors". In human PET scanning studies limited numbers of observations can be made on any individual. It usually is necessary to employ limited time points, single ligand concentration and single doses of competing drugs. Such circumstances are not ideal for studying drug receptor interactions. However, useful information can be obtained by careful definition of the receptor population identified. We have previously shown that [^{3}H]spiperone can be used to identify dopamine receptors in some brain areas. We have now shown that its interaction with these sites can be defined by prior administration of haloperidol and that the receptors characterized correlate to some extent with functional responses of dopamine receptor occupation. In contrast, sulpiride does not appear to be a drug of choice for defining in-vivo [^{3}H]spiperone binding.

Table 1. Comparison of ED50 and ED100 values for displacement of [^{3}H]spiperone binding by haloperidol and sulpiride with inhibition of apomorphine-induced stereotypy and climbing behaviour and elevation of HVA concentrations.

	mg kg^{-1}	
	Haloperidol	Sulpiride
In-vivo [^{3}H]spiperone binding:		
Substantia nigra	0·082	<10
Striatum	0·42	NS
Tuberculum olfactorium	$>1·0$	NS
Nucleus accumbens	$>1·0$	NS
Frontal cortex	NS	NS
Stereotyped behaviour	0·16	126
Climbing behaviour[a,c]	0·14	36·4
Striatal HVA concentration[b]	0·018	51
Mesolimbic HVA concentration[b]	0·013	25·5

[a] ED50 values were obtained from log dose response curves as the effective dose required to reduce the control response by 50%.
[b] ED100 values were obtained from log dose response curves as the effective dose required to increase the control HVA by 100%.
[c] Unpublished observations.
NS = not significant.

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