Biochemical Pharmacology, Vol. 31, No. 3, pp. 325–328, 1982.
Printed in Great Britain.

0006-2952/82/030325-04 $03.00/0
Pergamon Press Ltd.

REPEATED ADMINISTRATION OF SULPIRIDE FOR THREE WEEKS PRODUCES BEHAVIOURAL AND BIOCHEMICAL EVIDENCE FOR CEREBRAL DOPAMINE RECEPTOR SUPERSENSITIVITY

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(*Received* 5 *June* 1981; *accepted* 27 *July* 1981)

Abstract—Administration of sulpiride (2 × 100 mg/kg i.p.) or haloperidol (5 mg/kg i.p.) to rats for 3 weeks with subsequent withdrawal for 3 or 4 days induced cerebral dopamine receptor supersensitivity. Apomorphine-induced stereotyped behaviour after drug withdrawal was enhanced by pretreatment with either haloperidol or sulpiride both of which increased the number of specific striatal binding sites (B_{max}) for [^{3}H]spiperone, [^{3}H]*N*,*n*-propylnorapomorphine and [^{3}H]sulpiride. Neither drug altered the dissociation constant (K_D) for the ligand binding assays. Striatal dopamine sensitive adenylate cyclase activity was unaltered by such a pretreatment with either haloperidol or sulpiride. The data show that sulpiride, like haloperidol, is capable of inducing behavioural and biochemical supersensitivity of cerebral dopamine receptors.

Sulpiride [(±)-*N*(1′-ethyl-2′-pyrrolidinylmethyl)-2-methoxy-sulphamoyl benzamide] is a cerebral dopamine receptor antagonist in both animals and man [1]. However, it differs from most other neuroleptics in that it only weakly inhibits dopamine-mediated motor phenomena, such as apomorphine-induced stereotypy or circling behaviour in rats, and in that it usually causes little or no catalepsy. In addition, sulpiride does not antagonise dopamine stimulation of striatal adenylate cyclase *in vitro* [2, 3], a fact responsible for its classification as a selective antagonist of adenylate cyclase independent receptors [4]. We have recently demonstrated a specific striatal binding site for [^{3}H]sulpiride [5]. [^{3}H]Sulpiride binding is distinguished from that of [^{3}H]spiperone, which also labels adenylate cyclase independent receptors, by the interaction of sulpiride with the receptor having an absolute requirement for sodium ions [6].

The repeated administration of neuroleptic drugs for some weeks, and their subsequent withdrawal for a few days, induce cerebral dopamine receptor supersensitivity [7–9]. Animals treated thus exhibit increased spontaneous activity and enhanced stereotyped behaviour to apomorphine. Neuroleptic pretreatment increases the number of striatal [^{3}H]spiperone or [^{3}H]haloperidol binding sites [10, 11] but, apparently, not the number of [^{3}H]*N*,*n*-propylnorapomorphine binding sites [12]. Evidence for such treatment increasing dopamine-sensitive adenylate cyclase activity is equivocal [13–15].

The present study was designed to determine whether sulpiride, although not inhibiting motor behaviour on acute administration, would, like haloperidol after repeated administration, cause an enhanced stereotyped response to apomorphine. In addition, we have compared the effect of pretreatment with haloperidol and sulpiride on the specific striatal binding of the antagonist ligands [^{3}H]spiperone and [^{3}H]sulpiride, and on the agonist binding site for [^{3}H]*N*,*n*-propylnorapomorphine. We have also examined whether the failure of sulpiride to alter dopamine stimulation of adenylate cyclase activity is maintained after repeated administration and whether haloperidol, a compound inhibiting adenylate cyclase stimulation albeit weakly, induces altered enzyme activity.

MATERIALS AND METHODS

Drug administration. Male Wistar rats (150 ± 10 g at the start of the experiment; Olac Southern Ltd.) were housed in groups of 8 under conditions of standard lighting (12 hr light-dark cycle) and temperature (23 ± 3°), and allowed free access to food and water. The animals were treated for 21 days with either saline (0.5 ml i.p. twice daily), haloperidol (5 mg/kg i.p. daily in 0.15 ml of vehicle; Haldol, Janssen Pharmaceutica) or sulpiride (100 mg/kg i.p. twice daily in 0.5 ml vehicle; Delagrange). Sulpiride was dissolved in the minimum quantity of 2% v/v sulphuric acid and diluted to volume, with the pH adjusted to 7.0 by addition of dilute sodium hydroxide solution. After the period of drug administration the animals were allowed a 3 or 4 day drug-washout period prior to behavioural and biochemical examination. The experiment was repeated on 3 separate occasions using different batches of rats from the same supplier.

Apomorphine-induced stereotypy. Animals were housed in individual perspex cages (20 × 18 × 18 cm). Stereotyped behaviour was assessed 15 min following administration of apomorphine hydrochloride (0.125–2.0 mg/kg s.c.; Macfarlan Smith Ltd.). The following scoring system was utilised: 0 = animals indistinguishable from control animals; 1 = continuous locomotor activity, discontinuous

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sniffing; 2 = discontinuous locomotor activity, continuous sniffing; 3 = occasional locomotor activity, discontinuous licking, gnawing or biting; 4 = continuous licking, gnawing or biting; 5 = picking up and chewing of faeces.

Ligand binding assays. Ligand binding assays were carried out on the pooled striatal tissue from 7 or 8 rats. Each individual experiment was carried out in triplicate at each ligand concentration using at least 3 separate tissue pools for each of the 3 individual groups of drug-treated and control animals. Tissue from control and drug treated animals was compared on the same occasion and assays were run in strict parallel to negate day-to-day variation in assay procedure.

Preparation of striatal tissue and the determination of specific [^{3}H]spiperone and [^{3}H]sulpiride binding was carried out according to the technique of Leysen, Gommeren and Laduron [16], but using as the final incubation buffer 50 mM Tris–HCl (pH 7.7) containing 0.1% ascorbic acid, 10 μM pargyline hydrochloride and 120 mM sodium chloride. [^{3}H]Spiperone (26 Ci/mmole; Radiochemical Centre) was incorporated into the incubates at concentrations between 0.125 and 4.0 nM and specific binding defined by the incorporation of (+)-butaclamol (Ayerst Laboratories Ltd; 5×10^{-6} M). The specific binding of [^{3}H](±)-sulpiride (26.2 Ci/mmole; custom synthesised by the Radiochemical Centre) was carried out as previously described [5]. Specific binding of [^{3}H]sulpiride (5–40 nM) was defined by the incorporation of (−)-sulpiride (5×10^{-6} M). Specific binding of [^{3}H]*N*,*n*-propylnorapomorphine (50.2 Ci/mmole; New England Nuclear) was defined using a modification (unpublished data) of the procedure employed for [^{3}H]apomorphine binding [17] utilising EDTA (1 mM) in tissue buffers to optimise specific binding. [^{3}H]*N*,*n*-propylnorapomorphine was employed in concentrations between 0.0625 and 2.0 nM and specific binding defined by the incorporation of (+)-butaclamol (10^{-6} M).

The data for each individual experiment was subjected to Scatchard analysis using linear regression analysis to determine the number of binding sites (B_{max}; pmoles/g wet wt. of tissue) and the dissociation constant (K_D; nM). The individual values for each treatment group were then pooled and averaged to obtain the overall change in binding parameters.

Adenylate cyclase assay. Basal and dopamine (1–1000 μM) stimulated adenylate cyclase activity were assayed according to the technique of Miller, Horn and Iversen [18] using the saturation assay of Brown, Ekins and Albano [19]. After decapitation the brain was rapidly removed onto ice and the paired striata from three animals from each group dissected out. Basal and dopamine-stimulated adenylate cyclase were determined in duplicate in the tissue homogenate from each pair of striata and on at least two separate occasions.

The results for each group of animals were pooled and the concentration response curve for cyclic AMP formation was examined by linear regression analysis; the stimulation of cyclic AMP formation produced by 50 μM dopamine was determined.

Statistical analysis. Differences between control and drug-treated groups were analysed using Mann Whitney U test for the scores from non-parametric apomorphine-induced stereotyped behaviour and a two-tailed Student's *t* test for the biochemical determinations which were normally distributed.

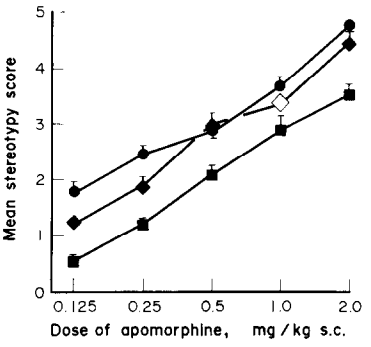

Fig. 1. Stereotyped response to apomorphine (0.125–2.0 mg/kg s.c. 15 min previously) in animals treated with saline, haloperidol (5 mg/kg i.p.) or sulpiride (2 × 100 mg/kg i.p.) for 21 days and then withdrawn for 3 or 4 days. The results are the mean stereotypy scores (± 1 S.E.M.) for drug treated animals compared to saline treated animals obtained from different groups of 6 animals on three separate occasions. Animals were scored 15 min after the administration of apomorphine in doses ranging from 0.125–2.0 mg/kg s.c. Open symbols represent stereotypy scores not significantly different from saline treated animals. Closed symbols represent stereotypy scores significantly different from those obtained from saline treated animals as judged using the Mann Whitney U test ($P < 0.05$). -■- Saline, -●- haloperidol, -◊--◆- sulpiride.

RESULTS

Apomorphine-induced stereotyped behaviour. Apomorphine hydrochloride (0.125–2.0 mg/kg s.c. 15 min previously) induced dose-dependent stereotyped behaviour in saline-treated animals (Fig. 1). Repeated administration of haloperidol (5 mg/kg/day i.p.) or sulpiride (2 × 100 mg/kg/day i.p.) for 21 days and subsequent withdrawal for 3 or 4 days, caused a shift of the dose–response curve for apomorphine-induced stereotypy to the left, indicating the development of post-synaptic dopamine receptor supersensitivity. There was no difference between the change induced by haloperidol compared to that produced by sulpiride.

Ligand binding assays. Repeated administration of haloperidol (5 mg/kg/day i.p.) or of sulpiride (2 × 100 mg/kg/day), followed by 3–4 days drug withdrawal, caused an increase in specific striatal binding sites (B_{max}) for [^{3}H]spiperone, [^{3}H]sulpiride and [^{3}H]*N*,*n*-propylnorapomorphine (Table 1) compared to saline treated animals. For each ligand no alteration in the dissociation constant (K_D) was observed.

The increase in B_{max} for all these ligands was slightly greater in animals receiving haloperidol compared to animals receiving sulpiride, but none of the differences reached statistical significance, even employing the non-parametric Wilcoxon or Mann Whitney U test.

Adenylate cyclase assay. Basal adenylate cyclase activity in straital homogenates from control animals

Table 1. The effect of repeated administration of haloperidol (5 mg/kg/day i.p.) or sulpiride (2 × 100 mg/kg/day i.p.) for 21 days and subsequent withdrawal (3 or 4 days) on specific [^{3}H]spiperone (0.125–4.0 nM), [^{3}H]sulpiride (5–40 nM) or [^{3}H]*N*,*n*-propylnorapomorphine (0.0625–2.0 nM) binding to striatal membranes compared to saline-treated control animals

Treatment	[^{3}H]Spiperone		[^{3}H]Sulpiride		[^{3}H]*N*,*n*-Propylnorapomorphine	
	B_{max} (pmoles/g tissue)	K_D (nM)	B_{max} (pmoles/g tissue)	K_D (nM)	B_{max} (pmoles/g tissue)	K_D (nM)
Saline	24.0 ± 1.8	0.22 ± 0.03	33.9 ± 2.6	19.6 ± 2.6	6.8 ± 0.5	1.1 ± 0.1
Haloperidol	32.3 ± 2.3* (135%)	0.23 ± 0.02	51.1 ± 2.2* (151%)	22.6 ± 3.7	12.7 ± 1.8* (187%)	1.2 ± 0.2
Sulpiride	29.5 ± 2.4* (123%)	0.27 ± 0.04	47.3 ± 2.7* (140%)	22.4 ± 3.3	9.7 ± 1.2* (143%)	1.3 ± 0.2

* $P < 0.05$ compared to saline treated animals.

The results are expressed as the mean (± 1 S.E.M.) of the values obtained from Scatchard analysis of data from 3 separate tissue pools for each treatment group, each ligand concentration being examined in triplicate.

Specific binding of [^{3}H]spiperone was defined using (+)-butaclamol (5×10^{-6} M) and that of [^{3}H]*N*,*n*-propylnorapomorphine defined using (+)-butaclamol (10^{-6} M). Specific binding of [^{3}H]sulpiride was defined using (−)-sulpiride (5×10^{-6} M). The per cent values in parentheses are the values for B_{max} in drug-treated animals expressed as a percentage of those values for saline treated animals.

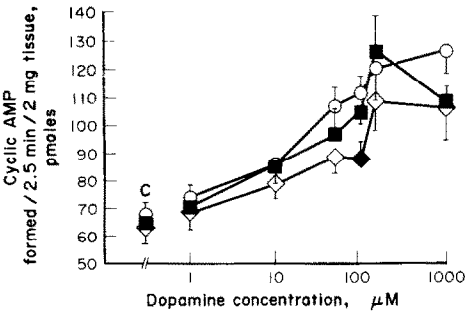

Fig. 2. Dopamine (1–1000 μM)-induced stimulation of cyclic AMP formation in striatal preparations from rats treated with saline, haloperidol (5 mg/kg i.p.) or sulpiride (2 × 100 mg/kg i.p.) for 21 days and then withdrawn for 3 or 4 days. The results are the mean (± 1 S.E.M.) of 2 separate determinations on the paired striata from 3 individual rats on each occasion, each concentration of dopamine being examined in sextuplicate. Open symbols represent cyclic AMP levels not different from those for tissue from control animals at the same dopamine concentration. Closed symbols represent significantly different values as judged by Student's *t* test ($P < 0.05$). -■- Saline, -○- haloperidol, -◊--♦- sulpiride.

was 67 ± 17 pmoles cyclic AMP/2.5 min/2.0 mg tissue wet wt.). Basal adenylate cyclase activity in animals 3 or 4 days after withdrawal from a pretreatment with sulpiride (2 × 100 mg/kg/day i.p.) or haloperidol (5 mg/kg/day i.p.) for 21 days was the same as in control animals. In tissue from control animals the incorporation of dopamine 1–1000 μM caused a concentration related increase in cyclic AMP formation.

Pretreatment of animals with sulpiride or haloperidol did not alter the dopamine stimulation of cyclic AMP formation (Fig. 2). There was no difference between the slopes of the regression lines, and the formation of cyclic AMP produced by 50 μM dopamine did not alter with drug pretreatment (% increase ± 1 S.E.M. over basal level produced by 50 μM dopamine: saline group 59.6 ± 6.5; haloperidol group 53.1 ± 2.4; sulpiride group 44.9 ± 4.2).

DISCUSSION

Repeated administration of neuroleptic drugs to rodents is associated with the development of tolerance to the acute cerebral dopamine receptor antagonist action of these drugs [20, 21], and the emergence of dopamine receptor supersensitivity. Sulpiride, another proven antipsychotic agent [22, 23], induced the same effects after administration for three weeks. Sulpiride, in this regime, caused an increase in apomorphine-induced stereotyped behaviour, and an increase in striatal dopamine receptors, as measured using three ligand binding assays. These effects of sulpiride were similar to those of haloperidol given for the same period of time. This suggests that sulpiride, at least, on repeated administration, acts in a manner typical of neuroleptic compounds. The acute administration of sulpiride, however, does not antagonise the stereotyped response to apomorphine [24]. Yet

apomorphine-induced stereotypy was enhanced by repeated administration of the drug as utilised in the present study, in agreement with the finding of Fuxe *et al.* [25]. This suggests that the acute pharmacological profile of sulpiride may not truly reflect its *in vivo* activity on repeated administration.

Repeated administration of both sulpiride and haloperidol enhanced [^{3}H]spiperone and [^{3}H]sulpiride binding to adenylate cyclase independent receptors to the same extent in striatal preparations. This suggests that despite the differentiation of these ligands based on absolute dependence of [^{3}H]sulpiride binding on sodium ions [6], these ligands either label the same site, or label different receptor sites which respond in the same way to repeated neuroleptic administration.

The present findings contrast with those of Trabucchi *et al.* [26] who claimed that sulpiride (20 mg/kg i.p. for 16 days and withdrawn for 4 days) did not enhance [^{3}H]spiperone binding (as defined using (+)-butaclamol 10^{-7} M), and that haloperidol (1 mg/kg i.p. for 16 days and withdrawn for 4 days) did not increase the high affinity binding of [^{3}H]sulpiride (as defined using (+)- and (−)-sulpiride 10^{-6} M) measured by their own method. However, these authors used lower doses of sulpiride (20 mg/kg i.p.) and haloperidol (1 mg/kg i.p.) for a shorter period (16 days), so perhaps the differentiation between [^{3}H]sulpiride and [^{3}H]spiperone sites is only apparent after lower neuroleptic intake. However, as judged by their capacity to increase brain dopamine turnover, or to inhibit the binding of [^{3}H]spiperone, haloperidol 1 mg/kg i.p. must be equivalent to a dose of sulpiride (given as the racemate, as both we and Trabucchi *et al.* have done) of about 100 mg/kg or more. Indeed, Fuxe *et al.* [25] found neither (−)-sulpiride (2 × 20 mg/kg i.p.), nor haloperidol (2 × 0.2 mg/kg i.p.) for 14 days (plus 2 days withdrawal) to enhance either [^{3}H]spiperone or [^{3}H]ADTN binding (both defined using (+)-butaclamol 10^{-6} M).

It has been claimed also that repeated neuroleptic administration differentially alters dopamine receptors labelled by agonist and antagonist ligands. Thus, Goldstein *et al,* [12] found repeated haloperidol (5 mg/kg i.p.) for 21 days (plus 4 days withdrawal) to increase the number of striatal [^{3}H]spiperone binding sites (defined by (+)-butaclamol 10^{-7} M), but not those for [^{3}H]*N*,*n*-propylnorapomorphine (defined by apomorphine 10^{-6} M). However, in our study, agonist binding was increased by both sulpiride and haloperidol pretreatment. This agrees with the findings of Leysen [27] for [^{3}H]apomorphine binding (as defined by (+)-butaclamol 10^{-5} M) following repeated haloperidol (0.2 mg/kg for 21 days plus 4 days withdrawal) administration.

Sulpiride is only one of a number of drugs of the substituted benzamide series, nearly all of which are characterised by an inability to inhibit dopamine stimulation of striatal adenylate cyclase. Our findings on sub-acute administration of sulpiride parallel the independent findings of Lautin *et al.* [28] with another substituted benzamide, metoclopramide. These authors showed that a 39 day period of administration of metoclopramide in drinking water, followed by 5 days withdrawal, enhanced the stereotyped response to apomorphine and increased the binding of [^{3}H]spiperone (1 nM) to rat striatal homogenates.

Acknowledgements—This study was supported by the Wellcome Trust, the Medical Research Council and the Research Funds of King's College Hospital and the Bethlem Royal and Maudsley Hospitals. We are grateful to Delagrange Laboratories for supplies of sulpiride and Janssen Pharmaceutica for supplies of Haldol.

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