Effects of B-HT 920 on nigrostriatal and mesolimbic dopamine systems in normosensitive and supersensitive rats

¹Paul B.S. Clarke, Kathleen J. Wyder, Alexander Jakubovic & Hans C. Fibiger

Division of Neurological Sciences, Department of Psychiatry, University of British Columbia, 2255 Wesbrook Mall, Vancouver, B.C., Canada V6T 2A1

- 1 B-HT 920, a D₂ dopamine receptor agonist, was tested for its ability to exert presynaptic actions in normosensitive rats, and for possible postsynaptic actions in rats made 'supersensitive' to apomorphine.
- 2 In normosensitive rats, B-HT 920 (0.01–0.3 mg kg⁻¹, i.p.) increased dopamine concentrations and lowered metabolite levels to a similar extent in all four terminal regions examined (medial prefrontal cortex, olfactory tubercle, nucleus accumbens, caudate-putamen). Analogous effects were seen for 5-hydroxytryptamine and its metabolite 5-hydroxyindoleacetic acid.
- 3 Rats which received bilateral 6-hydroxydopamine (6-OHDA) infusions into the caudate-putamen showed signs of postsynaptic dopamine receptor activation (stereotyped behaviour) in response to B-HT 920 (0.1 and 1.0 mg kg⁻¹, i.p.) and to apomorphine (0.2 mg kg⁻¹, s.c.). Similarly, B-HT 920 (0.1 mg kg⁻¹) induced contralateral circling in rats that had received unilateral 6-OHDA infusions into the medial forebrain bundle; the rate of circling increased gradually over several weeks.
- 4 In contrast, bilateral 6-OHDA infusions into the nucleus accumbens resulted in a supersensitive (locomotor stimulant) response to a low dose of apomorphine (0.1 mg kg⁻¹, s.c.), but not to B-HT 920 (0.01 and 0.1 mg kg⁻¹).
- 5 In intact rats, withdrawal of chronic haloperidol treatment induced behavioural supersensitivity to apomorphine but not to B-HT 920.

Introduction

B-HT 920 (6-allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo- [4, 5-d]azepine) was originally identified as an α_2 -adrenoceptor agonist (Kobinger & Pichler, 1981). However, at low doses it displays selective dopamine autoreceptor agonist properties, with a pharmacology resembling that of 3(–)PPP, TL-99 and EMD 23,448 (Anden *et al.*, 1982; 1983a,c). Thus, in normosensitive animals, systemic administration of B-HT 920 reduces A9 and A10 cell firing (Eriksson *et al.*, 1985; Clark & Chiodo, 1988) and attenuates dopamine synthesis and release in terminal areas (Anden *et al.*, 1982; 1983a,b,c). Possible pharmacological actions of B-HT 920 on non-catecholaminergic systems have received little attention.

Anden *et al.*, (1983b) have reported that B-HT 920 reduces dopamine synthesis with greater potency and efficacy in mesolimbic terminal regions (particularly in olfactory tubercle) than in caudate-putamen. A preferential mesolimbic action was also suggested by behavioural experiments, in which B-HT 920 potently inhibited spontaneous locomotor activity but did not induce catalepsy (Ahlenius & Hillegart, 1986). However, two recent reports cast doubt on this conclusion. Dopamine release, assessed by brain dialysis in freely moving rats, was reduced by B-HT 920 with similar potency and effectiveness in nucleus accumbens and dorsal striatum (Imperato *et al.*, 1988), and in addition, neuronal activity was reduced with similar potency though somewhat slower recovery in mesolimbic than in nigrostriatal dopaminergic neurones (Clark & Chiodo, 1988). Thus, at present, it is unclear whether the presynaptic actions of B-HT 920 are selective for mesolimbic neurones.

B-HT 920 has little if any activity at D₁ dopamine receptors but acts potently at D₂ receptors (Jennewein *et al.*, 1986; Hinzen *et al.*, 1986). When given alone to normosensitive animals, B-HT 920 does not produce behavioural signs of

postsynaptic dopamine receptor activation; thus, it fails to induce stereotypy or locomotor stimulation (Anden *et al.*, 1982; 1983c), and does not induce circling in rats with unilateral striatal ibotenate lesions or in hemitranssected animals (Hinzen *et al.*, 1986; Arnt & Perregaard, 1987). However, when coadministered with a selective D₁ agonist (SKF 38393), B-HT 920 produces intense stereotyped behaviour, consistent with a postsynaptic dopamine action within the striatum (Meltzer *et al.*, 1988; Johansen *et al.*, 1988). In the presence of SKF 38393, B-HT 920 does not appear to increase place-to-place locomotor activity (Meltzer *et al.*, 1988), suggesting that an analogous activation of postsynaptic mesolimbic dopamine receptors does not occur, even though D₁-enabled electrophysiological actions of B-HT 920 have been demonstrated on neurones within the nucleus accumbens (Johansen *et al.*, 1988). Taken together, these findings suggest that the postsynaptic dopaminergic behavioural actions of B-HT 920 in normosensitive animals, when enabled by concurrent D₁ receptor activation, are preferentially expressed via the nigrostriatal system.

Following the destruction of dopaminergic neurones with 6-OHDA, B-HT 920 readily displays behavioural signs of postsynaptic activation when given alone (Hinzen *et al.*, 1986). This has been shown in the unilateral 6-OHDA lesion model in rats, and also in a primate model of Parkinson's disease (MPTP-treated rhesus monkey), where administration of B-HT 920 reversed parkinson-like symptoms and restored normal behaviour (Hinzen *et al.*, 1986). Thus B-HT 920 appears to act preferentially at dopamine receptors made supersensitive through loss of dopaminergic input. At present, there is little or no experimental data bearing on the possible postsynaptic effects of B-HT 920 at 'supersensitive' dopamine receptors in the mesolimbic system, which is also frequently compromised in Parkinson's disease (Fibiger, 1984).

The mechanism whereby postsynaptic effects of B-HT 920 become unmasked following denervation are unknown, but is believed to reflect an uncoupling of D₁ and D₂ stimulus-effector mechanisms following the loss of dopaminergic tone (Clark & White, 1987). It was therefore of interest to examine

¹ Author for correspondence at present address, Dept of Pharmacology and Therapeutics, McGill University, McIntyre Building, Room 1325, 3655 Drummond St, Montreal, Canada H3G 1Y6.

the effects of chronic D₂ receptor blockade on subsequent responses to B-HT 920, in behavioural tests designed to examine nigrostriatal and mesolimbic activation.

The experiments described below had several objectives: (1) to compare the effects of B-HT 920 on nigrostriatal and mesolimbic dopamine metabolism in normosensitive, freely-moving rats; (2) to compare the effects of B-HT 920 on dopamine and 5-hydroxytryptamine (5-HT) metabolism, as a test of pharmacological selectivity; (3) to examine the behavioural effects of B-HT 920 following nigrostriatal and/or mesolimbic denervation; and (4) to study the effects of B-HT 920 in animals made supersensitive to dopamine agonists after withdrawal of chronic neuroleptic treatment.

Methods

Male Long-Evans hooded rats (Charles River) were housed two or four per cage (depending on the experiment) in a room illuminated from 08 h 00 min to 20 h 00 min, with free access to food and water. Rats were adapted to laboratory conditions for at least two weeks prior to behavioural testing.

Surgery

Rats were pretreated with pargyline HCl 50 mg kg⁻¹ i.p., 30 min before surgery. In Experiment 2, rats also received desmethylinipramine (DMI) 25 mg kg⁻¹ i.p., 30 min before surgery. Rats were anaesthetized with chloral hydrate 400 mg kg⁻¹ i.p. and placed in a stereotaxic frame (David Kopf, Tujunga, CA) with the tooth bar positioned 3.9 mm below the interaural line. A small burr hole was made in the skull above each injection site. Injection of 6-OHDA solution or vehicle (control subjects) was made into the brain via 30 gauge stainless steel cannulae attached by polyethylene tubing to 5 µl Hamilton syringes driven by a syringe pump (Sage Instruments, Cambridge, MA). In Experiment 2, injections were given at 1 µl min⁻¹, with 1 min delay before cannula withdrawal. To improve anatomical specificity, slower injections were used subsequently (2 µl in 10 min plus 5 min delay in Experiment 3). After cannula removal, the wound was closed with stainless steel clips (Autoclips, Becton, Dickinson and Co., USA).

For caudate-putamen infusions, 6-OHDA was administered bilaterally at two sites per side (interaural A +10.4, L ±2.2, V +4.9, and A +8.9, L ±3.3, V +4.7). Infusions into the medial forebrain bundle were at interaural A +5.9, L +2.3, V +2.2. For greater accuracy at nucleus accumbens sites, stereotaxic coordinates were derived from the mean of two systems based respectively on interaural (IA) zero and bregma; coordinates were A_{IA} +10.9/A_{Bregma} +1.7, L ±1.5, V_{IA} +2.7/V_{Bregma} -7.3. 6-Hydroxydopamine HBr was made up in vehicle consisting of 0.1 mg ml⁻¹ ascorbic acid in 0.9% saline solution, kept on ice, and shielded from light. 6-OHDA was given as 8 µg base in 2 µl per site, except into the MFB (Experiment 3, 4 µg in 2 µl). Control rats received vehicle.

Locomotor activity

Five circular (61 cm diameter) activity cages (BRS/LVE), each transected by two perpendicular coplanar arrays of three infrared photobeams, were used to measure locomotor activity. Photocell beam interruptions occurring more than 500 ms apart were recorded with a NOVA IV (Data General) mini-computer equipped with MANX (GC Controls) software and interface. During testing, a light-proof lid was placed over each activity cage. Prior to each test session, rats were placed in a holding cage for 50 min, in order to habituate to the test room. Unless otherwise stated, each rat then received an injection of drug or saline, was replaced in the holding cage, and then introduced to the test cage 60 s after injection. The test session was started 60 s later and was of 20–120 min duration, depending on the experiment. As far as possible, equal

numbers of rats from each experimental condition were tested in each cage. All behavioural tests were carried out between 09 h 00 min and 17 h 00 min.

Behavioural observation and stereotypy rating

Observation cages comprised a wire grid floor (40 × 45 cm) with plexiglass sides (45 × 45 cm). An oblique mirror permitted viewing from below the cage floor. Rats were kept in a holding cage for 15 min before injection and then placed individually in observation cages immediately after injection. General observations of behaviour and stereotypy ratings were made for 2 min at 10, 20 and 30 min after injection. The observer was blind with respect to surgical treatment and drug treatment. During this time, a brief description of posture and movements was recorded. Stereotypy was continuously rated during the observation period, as follows (modified from Kelly *et al.*, 1975): 0 – asleep or stationary; 1 – active; 2 – predominantly active with bursts of stereotyped sniffing and/or rearing; 3 – stereotyped activity, predominantly sniffing and rearing, over a large area of the cage; 4 – stereotyped behaviour maintained in one location; 5 – stereotyped behaviour in one location with bursts of gnawing and/or licking; 6 – continual gnawing or licking of grid floor. During each observation period, one stereotypy score was allocated to each rat, corresponding to the behavioural category occupying the most time.

Circling behaviour

The locomotor test cages were used (see above). Subjects were kept in a holding cage for 15 min before drug injection. For tests with B-HT 920, rats were placed individually in observation cages immediately after injection, and rotational behaviour (complete 360 degree rotations to left or right) scored for 2 min periods at 20, 40 and 60 min (Experiment 3).

Determination of brain amines

Rats were killed by cervical dislocation and the brain was rapidly removed and frozen. One millimetre thick coronal sections of forebrain were taken with a freezing microtome, and major dopaminergic terminal areas (medial prefrontal cortex – mPFCx, olfactory tubercle – OT, nucleus accumbens – NACC, caudate-putamen – CP) were dissected, as previously described (Clarke *et al.*, 1988a). For the determination of amines and metabolites, tissue samples from left and right sides were combined, weighed, and disrupted by sonication. Samples were analyzed by high pressure liquid chromatography with electrochemical detection, as described in detail elsewhere (Clarke *et al.*, 1988a). The following monoamines and metabolites were assayed: dopamine, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxytryptamine (5-HT), and 5-hydroxyindolacetic acid (5-HIAA).

Drugs

Drugs and suppliers were as follows: 6-hydroxydopamine HBr, pargyline HCl, and apomorphine HCl (Sigma Chemical Co., St Louis, MO), chloral hydrate (BDH, Toronto, Ont), desmethylinipramine (Merrel Dow Research Institute, Cincinnati, OH), haloperidol HCl (McNeil), B-HT 920 (Boehringer-Ingelheim KG, Ingelheim, FRG). Chloral hydrate, pargyline, B-HT 920 and desmethylinipramine were given i.p.; apomorphine was administered s.c. into the flank. Unless otherwise stated, drugs were dissolved in 0.9% saline and given in a volume of 1 ml kg⁻¹. Ascorbic acid (0.1 mg ml⁻¹) was added to 6-OHDA and apomorphine to inhibit oxidation. For oral administration, haloperidol 10 mg ml⁻¹ was dissolved in 0.5% lactic acid solution and stored at 4°C in the dark. Aliquots of this stock solution were diluted to final concentrations of 0.02 mg ml⁻¹ (haloperidol) and 0.001% (lactate vehicle), and presented in opaque drinking bottles. Every other day, bottles

were weighed to determine fluid intake, and drinking solutions were changed. Drug doses refer to the salt, except for apomorphine and haloperidol (expressed as base).

Data analysis

Stereotypy scores occurring at a given dose of drug were analyzed for differences between experimental groups by the Mann-Whitney test (Tallarida & Murray 1981). Locomotor activity scores were assessed by univariate and multivariate analysis of variance (SPSS/PC+ software, SPSS Inc., Chicago). The following between-subjects factors were analyzed: Dose (for comparisons across different levels of dose, including saline), Lesion (6-OHDA vs. vehicle), and Group (haloperidol- vs. vehicle-withdrawn). Dose-dependent effects refer to linear trends across absolute values of dose. Each rat served as its own control, where appropriate. The within-subjects factors were: Drug, for comparisons across different drug treatments, and Time, referring to changes occurring within individual test sessions (divided into six equal periods of 10 or 20 min). In order to avoid positive biasing of the *F* test, which can occur in repeated measures designs, nonsignificant results refer to a conventional *F* test, whereas a significant result refers to a more conservative *F* test (Huyhn-Feldt; SPSS/PC+ software). As in previous studies (Clarke *et al.*, 1988a,b), the drug effect was defined as the difference between drug and saline test scores. Comparisons between control (i.e. saline-treated) and other drug groups were made with the Dunnett multiple range test. Other comparisons were made by Student's *t* test. Probability values are 2-tailed.

Procedures

Experiment 1: Acute effects of B-HT 920 on locomotor activity and on dopamine and 5-hydroxytryptamine metabolism in normosensitive rats Forty-four rats were used, weighing 260–330 g. They were randomly allocated to five groups for drug testing: saline (*n* = 12), B-HT 920 0.01, 0.03, 0.1, 0.3 mg kg⁻¹ (*n* = 8 for each dose). After injection, each rat was replaced in the holding cage, then tested for locomotor activity from 20–40 min post-injection. Each rat was killed for neurochemical analysis immediately after its locomotor test session.

Experiment 2: Behavioural effects of B-HT 920 following bilateral administration of 6-hydroxydopamine into caudate-putamen or nucleus accumbens Forty rats were used, weighing 270–335 g at surgery. Rats were randomly allocated to the following groups for surgery (treatment/site): 6-OHDA/CP (*n* = 12), Vehicle/CP (*n* = 8), 6-OHDA/NACC (*n* = 12), Vehicle/NACC (*n* = 8). Shortly after surgery, several rats died (3 in 6-OHDA/CP group, 2 in Vehicle/CP group). Rats in the 6-OHDA/CP group became aphagic and were tube-fed a mixture of powdered milk and sucrose water. From the day of surgery to the first behavioural test, this group gained 2.2 ± 9.4 g (mean ± s.e.mean), whereas the controls gained 26.8 ± 5.1 g.

Starting 10 days after surgery, and continuing every other day, rats in the two CP groups received four tests of stereotyped behaviour, once each with saline, apomorphine 0.2 mg kg⁻¹, B-HT 920 0.1 and 1.0 mg kg⁻¹, given in random order. During the same period of days, rats in the two NACC groups received four locomotor tests, following the administration of saline, apomorphine 0.1 mg kg⁻¹, B-HT 920 0.01 and 0.1 mg kg⁻¹, again presented in random order. Rats were habituated for 50 min in holding cages, then tested for 60 min after injection. The dose of apomorphine was selected so as to depress activity in intact rats but to increase activity in dopamine-depleted rats (Clarke *et al.*, 1988b). Five days after the final test, rats were killed for neurochemical analysis.

Experiment 3: Drug-induced circling behaviour following unilateral 6-hydroxydopamine injection into the medial forebrain bundle The above experiment was repeated with the follow-

ing modifications: (1) 6-OHDA was administered into the MFB, in order to deplete both nigrostriatal and mesolimbic terminal regions of dopamine; (2) tests were conducted for a longer period after B-HT 920 administration and post-surgery; and (3) the possible effects of repeated testing and repeated administration of B-HT 920 were examined.

Twenty rats (290–355 g) received an infusion of 6-OHDA (4 µg in 2 µl) into the MFB and were randomly allocated to one of three groups: the BHT/BHT group (*n* = 8) was tested with B-HT 920 0.1 mg kg⁻¹ on days 1, 3, 7, 14 and 35 after surgery; the Sal/BHT group (*n* = 8) was treated in the same way, except that saline replaced B-HT 920 on the first three tests; the third group (No test/BHT, *n* = 4) was tested with the same dose of B-HT 920, but only on the final day. Circling was counted at 20, 40 and 60 min after injection. Forty-two days after surgery, rats were killed for neurochemical analysis.

Experiment 4: Effects of chronic haloperidol treatment on subsequent behavioural responses to B-HT 920 and apomorphine Forty-eight rats were used, weighing 350–445 g on the first behavioural test. In broad outline, the experiment was conducted in two consecutive stages: (1) chronic treatment followed by tests of drug-induced stereotypy, then (2) the same chronic treatment, followed by tests of drug-induced locomotion. In stage 1, rats were randomly allocated to two groups, receiving chronic oral haloperidol (*n* = 24) or vehicle (*n* = 24) for a period of three weeks. Drinking fluids were then replaced with tap water, and 3 and 4 days later, rats received two stereotypy tests. Each group of 24 rats was randomly divided into three subgroups (*n* = 8), and each rat was then tested once with saline and once with either B-HT 920 0.1 mg kg⁻¹, B-HT 920 1.0 mg kg⁻¹, or apomorphine 0.2 mg kg⁻¹, depending on the subgroup. Saline and drug tests were conducted in a counterbalanced order within each subgroup. Stereotypy was rated at 10, 20 and 30 min after injection.

Stage 2 started three days later. The same groups were re-established on oral haloperidol or vehicle for 3 weeks and then withdrawn, as before. Three days later, each group was randomly divided into three subgroups (*n* = 8), and subjects were tested for locomotor activity with saline, or B-HT 920 0.025 mg kg⁻¹, or B-HT 920 0.1 mg kg⁻¹, depending on subgroup. On the next day, equal numbers of rats in each subgroup were randomly reassigned for testing with apomorphine 0.05 or 0.1 mg kg⁻¹. The locomotor testing procedure was intended to emphasize any stimulant effects present. Rats were therefore habituated to the testing apparatus for 40 min, removed for injection and immediately returned for a test session of 120 min.

Results

Experiment 1: Acute effects of B-HT 920 on locomotor activity and on dopamine and 5-hydroxytryptamine metabolism in normosensitive rats.

Locomotor activity was reduced by B-HT 920 in a dose-dependent way (mean effect of Dose: *F* = 39.9, d.f. 4, 38, *P* < 0.0001; linear trend of Dose: *F* = 96.3, d.f. 1, 38, *P* < 0.0001). Photocell counts (mean ± s.e.mean) in the groups treated with saline and B-HT 920 (0.01–0.3 mg kg⁻¹) were, respectively, 386.5 ± 18.1, 333.9 ± 28.8, 194.0 ± 16.4, 146.3 ± 16.2, and 112.5 ± 14.9. This depressant effect reached significance at 0.03 mg kg⁻¹ (Dunnett's test *P* < 0.01).

Regional concentrations of dopamine, 5-HT and metabolites in control (saline-treated) rats are shown in Table 1. B-HT 920 significantly increased dopamine concentrations in all regions except mPFCx (mean effect of Dose: *F* > 2.88, d.f. 4, 38, *P* < 0.05–0.005; Figure 1). This increase was significantly dose-related in NACC and CP (linear trend on dose: *F* > 16.0, d.f. 1, 39, *P* < 0.001; Figure 1), with a similar but non-significant trend in OT (*P* < 0.06). Significant effects were

Table 1 Regional concentrations* of dopamine (DA), 5-hydroxytryptamine (5-HT) and metabolites in control rats (Experiment 1)

Region	DA	HVA	DOPAC	5-HT	5-HIAA
mPFCx	0.70 ± 0.05	0.76 ± 0.04	0.50 ± 0.02	2.30 ± 0.08	1.75 ± 0.07
OT	39.7 ± 1.8	3.15 ± 0.10	11.3 ± 0.4	5.00 ± 0.15	2.41 ± 0.09
NACC	68.1 ± 3.4	7.86 ± 0.32	20.8 ± 0.9	2.73 ± 0.17	2.76 ± 0.06
CP	79.7 ± 2.4	8.17 ± 0.30	17.4 ± 0.6	1.64 ± 0.08	2.11 ± 0.07

* Concentrations (mean ± s.e.mean, $n = 12$) in picomoles per milligram of wet tissue.

HVA = homovanillic acid; DOPAC = dihydroxyphenylacetic acid; 5-HIAA = 5-hydroxyindolacetic acid; mPFCx = medial prefrontal cortex; OT = olfactory tubercle; NACC = nucleus accumbens; CP = caudate putamen.

reached at 0.03 to 0.1 mg kg⁻¹. B-HT 920 reduced dopamine metabolite concentrations in a dose-related way in all four areas (linear trend of Dose for DOPAC: $F > 6.33$, d.f. 1, 38, $P < 0.005$ – 0.0001 ; for HVA, $F > 29.7$, d.f. 1, 38, $P < 0.0001$), significant effects generally starting at the lowest dose (0.01 mg kg⁻¹). B-HT 920 appeared to alter concentrations of dopamine and its metabolites to a similar extent in all three subcortical areas examined.

As shown in Figure 1, B-HT 920 tended to elevate 5-HT concentrations, and this effect was significant in mPFCx, NACC and CP (main effect of Dose: $F = 2.61$, d.f. 4, 39, $P < 0.05$ – 0.005). B-HT 920 tended to reduce 5-HIAA concentrations in all four areas, but this was only significant in OT (main effect of Dose: $F = 3.88$, d.f. 4, 38, $P < 0.01$; linear trend $F = 9.94$, d.f. 1, 38, $P < 0.005$).

Experiment 2: Behavioural effects of B-HT 920 following bilateral administration of 6-hydroxydopamine into caudate-putamen or nucleus accumbens

Stereotypy Stereotypy scores did not vary appreciably between the three post-injection intervals, and only the observations from the middle period (20 min post-injection) are discussed. In saline tests, CP lesioned animals had significantly lower stereotypy scores than sham-operated controls ($P < 0.05$; Table 2), and they were generally less active. At the

doses tested, B-HT 920 produced appreciable stereotypy in CP lesioned rats but not in controls. Thus, significantly higher scores were observed in the lesioned group than in the control group at the lower dose of B-HT 920 ($P < 0.05$) and at the higher dose ($P < 0.02$). The higher dose (1.0 mg kg⁻¹) appeared more effective than the lower (0.1 mg kg⁻¹). In control subjects, the lower dose tended to induce frequent yawning ($n = 3/6$) and prostration ($n = 3/6$), whereas the higher dose produced prostration ($n = 6/6$) with a marked absence of sniffing. In CP lesioned rats, B-HT 920 tended to produce continuous sniffing, especially at the higher dose; by 30 min after injection, prostration was uncommon even at the high dose ($n = 2/9$). Apomorphine induced stereotypy in both groups, and this was more intense in lesioned rats, reflected by higher stereotypy ratings ($P < 0.02$; Table 2).

Infusion of 6-OHDA into the CP resulted in a widespread depletion of dopamine not only in CP (95%), but also in NACC (73%) and OT (56%), compared to sham-operated control subjects. In the control group, the absolute concentrations (mean ± s.e.mean) of dopamine in the CP, NACC and OT were, respectively: 101.7 ± 1.8, 89.3 ± 1.9, and 58.8 ± 2.5 pmol mg⁻¹ wet tissue. Concentrations of 5-HT were not significantly altered by the lesion; group mean tissue concentrations of 5-HT in lesioned rats ranged between 96% (OT) and 116% (CP) of those in the sham-operated group.

Locomotor activity Saline test activity did not differ between NACC lesion and control groups (main effect of Lesion:

Figure 1 Effects of B-HT 920 on dopamine (DA), 5-hydroxytryptamine (5-HT) and metabolite concentrations in medial prefrontal cortex, olfactory tubercle, nucleus accumbens and caudate-putamen (Experiment 1). Regional concentrations in rats receiving B-HT 920 ($n = 8$ per group) are expressed as a percentage of the corresponding mean value in control (saline-tested) subjects ($n = 12$): Open column, control; stippled column, B-HT 0.01 mg kg⁻¹; solid column, 0.03 mg kg⁻¹; hatched columns 0.1 mg kg⁻¹; horizontally lined columns 0.3 mg kg⁻¹. Columns show mean values with s.e.mean indicated by vertical bars. The control values are shown in Table 1. Rats were killed for analysis 40 min after i.p. injection.

Table 2 Stereotypy scores (Experiments 2 and 4)*

Rating	n	Saline					B-HT 920 (0.1 mg kg ⁻¹)					B-HT 920 (1.0 mg kg ⁻¹)					Apomorphine (0.2 mg kg ⁻¹)													
		0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5					
<i>Experiment 2</i>																														
Control	6			6				3	3					6												1	1	2	2	
CP 6-OHDA	9		6	3				1	2		4	2		2			2	4	1									1	4	4
<i>Experiment 4</i>																														
Control	24		2	19	3																									
Haloperidol	24		2	21	1																									
Control	8							5	3					8												7	1			
Haloperidol	8							1	5	1		1		6	2													4	4	

* Each rat was allocated a stereotypy score corresponding to the predominant category of behaviour displayed within the observation period (20–22 min after injection). The number of rats exhibiting a given stereotypy score is shown for each group. The rating scale is described in the text. CP = caudate-putamen; 6-OHDA = 6-hydroxydopamine.

$F = 1.25$, d.f. 1, 15, $P > 0.2$; Lesion $\times$ time: $F = 0.31$, d.f. 5, 75, $P > 0.9$). Mean ($\pm$ s.e.mean) saline activity scores (0–60 min) were 516.5 ± 34.5 and 583.9 ± 47.7 for control and NACC lesion groups, respectively. MANOVA revealed significant Lesion $\times$ Drug ($F = 6.86$, d.f. 2, 27, $P < 0.01$) and Lesion $\times$ Drug $\times$ Time interactions ($F = 4.66$, d.f. 6, 84, $P < 0.05$). Both doses of B-HT 920 reduced locomotor activity in lesioned rats as well as in controls; the higher dose was more effective. The depressant effect of the higher dose of B-HT 920 appeared to be attenuated by the lesion in the latter part of the session (Figure 2), but overall (0–60 min), the effect of this dose did not differ significantly between surgery groups ($t = 2.00$, d.f. 15, $P > 0.05$). In contrast, apomorphine depressed locomotor activity only in controls, and increased activity in lesioned animals (Figure 2).

Infusion of 6-OHDA into the NACC depleted dopamine in NACC by 62%, and modest losses were seen in CP (19%) and OT (35%). In the control group, the absolute concentrations (mean $\pm$ s.e.mean) of dopamine in the CP, NACC and OT were, respectively: 99.5 ± 2.3 , 80.3 ± 4.9 , and 47.9 ± 1.1 pmol mg⁻¹ wet tissue. Concentrations of 5-HT were not significantly altered by the lesion; group mean tissue concentrations of 5-HT in lesioned rats ranged between 98% (CP) and 111% (NACC) of those in the sham-operated group.

Experiment 3: Drug-induced circling behaviour following unilateral 6-hydroxydopamine injection into the medial forebrain bundle

Circling behaviour did not alter appreciably across the three observation periods (20, 40 and 60 min post-injection), and the

Figure 2 Effects of B-HT 920 (0.1 mg kg⁻¹ i.p.) and apomorphine (0.1 mg kg⁻¹ s.c.) on locomotor activity in sham-operated and 6-hydroxydopamine (6-OHDA) accumbens-lesioned rats (Experiment 2). The vertical axis shows the group mean (s.e.mean indicated by vertical bars) difference of photocell counts between drug and saline tests. (Δ) Sham operated, B-HT 0.1 mg kg⁻¹; ($\blacktriangle$) 6-OHDA, B-HT 0.1 mg kg⁻¹; ($\square$) sham operated, apomorphine 0.1 mg kg⁻¹; ($\blacksquare$) 6-OHDA, apomorphine 0.1 mg kg⁻¹. Saline test activity did not differ significantly between control and lesion groups (see Results). The effect of B-HT 920 was indistinguishable in the two groups of rats; apomorphine decreased activity in controls but increased activity in lesioned subjects.

data were pooled. Between 3 and 7 days after surgery, B-HT 920 began to have a measurable effect (Figure 3). The ipsiversion shown by rats tested with saline was reduced (day 3) or replaced by contraversion (day 7) in rats tested with B-HT 920. The response to B-HT 920 at 35 days did not appear to be significantly affected by repeated testing with or without the drug. The rate of contralateral turning in rats tested with B-HT 920 gradually increased over successive tests, averaging approximately 4 turns per min on the final test.

The extent of dopamine depletion induced by 6-OHDA was very similar in all three groups, the loss (compared to the unoperated side) ranging from 95–96% (OT), 96–99% (NACC), and 99% (CP). The mean tissue concentrations of dopamine on the unoperated side were similar across these three groups, ranging as follows: 98.9–103.0 (CP), 79.6–84.0 (NACC), and 46.9–50.2 pmol mg⁻¹. As in experiment 2, concentrations of 5-HT were not significantly altered by the lesion; group mean tissue concentrations of 5-HT on the lesioned side ranged between 89–95% compared to the unoperated side.

Experiment 4: Effects of chronic haloperidol treatment on subsequent behavioural responses to B-HT 920 and apomorphine

Haloperidol intake By the end of the first three-week period of chronic treatment, mean daily fluid intake was 42 ml in controls and 28 ml in rats drinking haloperidol solution, corresponding to a drug intake of approximately 1.4 mg kg⁻¹ day⁻¹. At the end of the second period, drug intake averaged 1.2 mg kg⁻¹ day⁻¹.

Figure 3 Emergence of ipsilateral circling in response to B-HT 920 (0.1 mg kg⁻¹ i.p.) in unilaterally medial forebrain bundle lesioned rats (Experiment 3). Rats received a unilateral infusion of 6-hydroxydopamine into the medial forebrain bundle. Group 1 (open columns) was tested on days 1, 3, 7, 14 and 35 days post-surgery with B-HT 920; Group 2 (solid columns) was tested with saline on days 1, 3 and 7, and subsequently with B-HT 920. Group 3 (hatched column) was tested only on day 35, with B-HT 920. S = saline test. The columns show the mean number of turns (ipsilateral minus contralateral) in a total observation period of 6 min; s.e.mean indicated by vertical bars.

Figure 4 Effects of B-HT 920 and apomorphine on locomotor activity in vehicle- and haloperidol-withdrawn rats (Experiment 6). Three days after a period of chronic haloperidol or vehicle (control) treatment, subjects were tested 0–120 min after injection of saline (open columns), B-HT 920 0.025 mg kg⁻¹ (solid columns) or 0.1 mg kg⁻¹ (stippled columns) i.p. ($n = 8$ per group). On the next day, rats were tested with apomorphine 0.05 mg kg⁻¹ (hatched columns) or 0.1 mg kg⁻¹ (cross-hatched columns) s.c. ($n = 12$ per group). Columns show mean values with s.e.mean indicated by vertical bars.

Stereotypy As in Experiment 2, stereotypy scores varied little between post-injection intervals, and data from 20 min post-injection are discussed. In saline tests, there were few signs of stereotypy, and the haloperidol-withdrawn group did not differ significantly from the control group that had not received haloperidol ($P > 0.1$; Table 2). In control subjects, neither dose of B-HT 920 produced stereotypy, and immobility was commonly seen (Table 2). When tested with the lower dose of B-HT 920, haloperidol-withdrawn rats were generally more mobile than the control subjects, and as a result, they obtained significantly higher stereotypy ratings than controls ($P = 0.025$). However, haloperidol-withdrawn rats displayed few signs of stereotypy when tested with either dose of B-HT 920. In contrast, withdrawal from chronic haloperidol treatment clearly increased apomorphine-induced stereotyped behaviour, and this is reflected by significantly higher stereotypy ratings ($P < 0.001$; Table 2).

Locomotor activity On the first test day, saline activity scores (0–120 min) did not differ significantly between control and haloperidol-withdrawn groups (mean $\pm$ s.e.mean: 540.4 $\pm$ 41.9, 514.8 $\pm$ 91.5, respectively). B-HT 920 reduced activity in both groups. This depressant effect appeared attenuated in haloperidol-withdrawn animals, a suggestion which was not supported by statistical analysis (Dose $\times$ Group: $F = 2.14$, d.f. 3, 29, $P > 0.1$; Dose $\times$ Group $\times$ Time: $F = 0.82$, d.f. 10, 195, $P > 0.5$; Figure 4). In contrast, the two chronic treatment groups differed significantly in locomotor scores during tests with apomorphine (main effect of Group: $F = 14.3$, d.f. 1, 42, $P < 0.001$), and there was no significant difference between the two doses (main effect of Dose: $F = 1.82$, d.f. 1, 42, $P > 0.1$; Dose $\times$ Group: $F = 0.46$, d.f. 1, 42, $P > 0.5$).

Discussion

In normosensitive rats, B-HT 920 increased regional concentrations of dopamine and decreased those of its major metabolites, consistent with previous evidence indicating autoreceptor agonist activity (Anden *et al.*, 1982; 1983a,b,c; Eriksson *et al.*, 1985; Clark & Chiodo, 1988). However, these effects were strikingly similar both in potency and efficacy in mesolimbic (olfactory tubercle, nucleus accumbens) and nigrostriatal terminal regions, contrary to previous evidence suggesting a preferential effect in mesolimbic areas (Anden *et al.*, 1983b). The present results support and extend a recent study employing intracranial microdialysis, where B-HT 920 was

found to reduce extracellular dopamine concentrations to the same extent in dorsal striatum as in nucleus accumbens (Imperato *et al.*, 1988). In addition, they suggest that B-HT 920 also potentially reduces dopamine outflow in the medial prefrontal cortex. Given the apparent lack of selectivity for the mesolimbic dopamine system, it is perhaps surprising that B-HT 920 markedly reduces locomotor activity but fails to induce catalepsy, an index of nigrostriatal dopaminergic transmission (Ahlenius & Hillegaart, 1986).

Analogous to its effects on dopaminergic indices, B-HT 920 tended to elevate concentrations of 5-HT whilst lowering those of its metabolite 5-HIAA. The similarity of effect in all four regions examined suggests that B-HT 920 may directly affect 5-hydroxytryptaminergic neurones, possibly acting as an autoreceptor agonist or by inhibiting monoamine oxidase.

B-HT 920 has D₂ but not D₁ dopamine receptor agonist properties (Jennewein *et al.*, 1986). Following prolonged depletion of dopamine, the behavioural effects of D₂ agonists no longer require an enabling action by concurrent D₁ receptor activation (Clark & White, 1987). Thus, in rats unilaterally lesioned with 6-OHDA, B-HT 920 induces contralateral rotation which is independent of D₁ receptor blockade (Hinzen *et al.*, 1986). In the present study, lesions of the nigrostriatal system induced by 6-OHDA produced clear evidence of postsynaptic dopamine receptor activation by B-HT 920 (stereotypy). In contrast, although 6-OHDA lesions directed at the mesolimbic system reduced the locomotor depressant effect of B-HT 920, presumably as a result of the removal of dopaminergic terminals, these lesions did not reveal signs of postsynaptic activation induced by B-HT 920 (locomotor stimulation). The basis for this difference is not clear, especially since both lesions resulted in supersensitive postsynaptic responses to apomorphine. It should be noted that in rats tested for stereotypy, residual dopamine in the target tissue (caudate-putamen) was depleted to a greater extent (95%) than was the target tissue in rats tested for locomotor activity (NACC, 62% loss). Since B-HT 920 markedly reduces dopamine release *in vivo* (Imperato *et al.*, 1988), it is possible that postsynaptic agonist effects of this drug may be masked in partially-lesioned animals by a concomitant reduction of presynaptically-released dopamine. The results of the rotation experiment (Experiment 3) are also consistent with this interpretation. Infusion of 6-OHDA into the medial forebrain bundle, which produced massive loss of dopamine in both nigrostriatal and mesolimbic terminal regions, revealed significant contraversive turning in response to B-HT 920; such behaviour requires postsynaptic dopamine receptor activation in both caudate-putamen and accumbens (Pycoc, 1980).

Experiments performed in unlesioned rats (Imperato *et al.*, 1988) suggest that the release of dopamine from presynaptic terminals is inhibited more profoundly and with shorter latency by B-HT 920 than by apomorphine at the doses used in the present experiments. Such a difference could possibly help to explain why apomorphine stimulated locomotor activity in 6-OHDA lesioned rats whereas B-HT 920 did not (Experiment 2), and would also account for the absence of B-HT 920-induced stereotypy or locomotor stimulation in haloperidol-withdrawn animals that were demonstrably supersensitive to low doses of apomorphine (Experiment 4).

Other explanations of the present findings should also be carefully considered. An uncoupling of D₁ and D₂ receptor mechanisms appears to occur in denervated animals (Clark & White, 1987). The failure of B-HT 920 to show postsynaptic dopamine agonist properties in the chronic haloperidol-treated animals suggests that this procedure for producing dopamine receptor supersensitivity possibly does not lead to an uncoupling of D₁ and D₂ receptors. Consistent with the present findings, others have concluded that denervation and dopamine receptor blockade produce receptor supersensitivity by fundamentally different mechanisms (e.g. Staunton *et al.*, 1982; Breese *et al.*, 1987).

When given alone, B-HT 920 produces few signs of postsynaptic dopamine receptor stimulation in normosensitive

animals. However, 'low level' stereotyped behaviour has been unmasked by coadministration of certain α_2 -noradrenoceptor antagonists (Johansen *et al.*, 1988; but see also Hinzen *et al.*, 1986). It therefore appears that the α_2 -agonist properties of B-HT 920 can inhibit the behavioural expression of postsynaptic dopamine receptor stimulation. The present experiments indicate that in rats lesioned with 6-OHDA, the expression of stereotyped behaviour does not require noradrenoceptor blockade.

Following unilateral nigrostriatal depletion with 6-OHDA, the mixed D_1/D_2 agonist apomorphine induces rapid contralateral turning within one or two days, and maximal circling rates are reached within a very few days (Ungerstedt, 1971; Staunton *et al.*, 1982). Locomotor stimulant effects of apomorphine following bilateral mesolimbic destruction have a similar rapid onset (Arnt & Hyttel, 1984; Clark *et al.*, 1985). In contrast, the D_2 -selective agonist pergolide, as well as compounds regarded as selective autoreceptor agonists (EMD

23448 and (-)-3-PPP) begin to show signs of postsynaptic activation over a longer period of time (Arnt & Hyttel, 1984; Arnt, 1985). In the present study, the contraversive tendency displayed by B-HT 920 emerged in a gradual fashion, consistent with the hypothesis (Arnt, 1985) that postsynaptic D_1 and D_2 receptors become uncoupled over a two week period following denervation in rats.

In summary, the conditions under which B-HT 920 displays postsynaptic dopamine receptor activation remain incompletely understood. The present results suggest that any therapeutic potential of B-HT in Parkinson's disease may be limited to cases of severe dopaminergic degeneration.

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