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INCREASED CENTRAL 5-HYDROXTRYPTAMINE RECEPTOR MECHANISMS IN RATS AFTER CHRONIC NEUROLEPTIC TREATMENT

D. DAWBARN, S.K. LONG & C.J. PYCOCK

Department of Pharmacology, Medical School, University of Bristol, Bristol BS8 1TD

1 The behavioural responses of drugs known to act through central 5-hydroxytryptamine (5-HT) mechanisms have been investigated in rats receiving a neuroleptic (trifluoperazine) in their drinking water for 4 to 6 months.

2 5-Hydroxytryptophan (5-HTP) induced 5-HT-dependent behaviours including head bobbing and lateral head weaving, reciprocal forepaw treading, tremor, backward walking, body writhing and 'wet-dog' shakes. In doses of 50 to 150 mg/kg, 5-HTP induced more intense behavioural effects in neuroleptic-treated rats than in the control animals.

3 Similarly the putative 5-HT agonist, quipazine (1 to 20 mg/kg) and the 5-HT releasing drug, fenfluramine (5 to 20 mg/kg), both induced significantly greater motor responses in the chronically neuroleptic-treated rats.

4 A 5-HT uptake inhibitor (femoxetine, 2.5 to 10 mg/kg) had little behavioural effect in either control or trifluoperazine-treated rats.

5 Total specific high-affinity binding of radiolabelled 5-HT was significantly increased in crude membrane fractions prepared from the cortex, striatum and substantia nigra of neuroleptic-treated rats compared to control animals.

6 High-affinity uptake of radiolabelled 5-HT into striatal slices was similar in experimental and control animals.

7 Behavioural and biochemical data would indicate that postsynaptic 5-HT mechanisms are enhanced in rats treated chronically with trifluoperazine. Chronic neuroleptic therapy may thereby induce cerebral 5-HT receptor supersensitivity in addition to the well-documented cerebral dopamine receptor supersensitivity.

Introduction

Rats which have been treated chronically with a neuroleptic drug in their drinking water show an enhanced behavioural response to the dopamine agonist, apomorphine (Clow, Jenner & Marsden, 1979a). Accompanying this exaggerated behavioural response there is an increased specific binding of radiolabelled neuroleptic drugs in striatal tissue taken from these animals (Schwartz, Baudry, Martres, Costentin & Protais, 1978; Clow, Jenner, Theodorou & Marsden, 1979b; Clow, Theodorou, Jenner & Marsden, 1980). Such results, together with an increased dopamine-sensitive adenylate cyclase activity, is taken as evidence for the development of supersensitive cerebral dopamine receptors caused by chronic blockade with dopamine antagonists (Schwartz *et al.*, 1978; Clow *et al.*, 1979b; 1980). Analysis of the binding data suggests that dopamine receptor supersensitivity after chronic blockade is probably due to both an enhanced affinity of the receptor for the ligand ([^{3}H]-spiroperidol) and also to an increase in the number of binding sites.

However, neuroleptic drugs also interact with central 5-hydroxytryptamine (5-HT) receptors, as has been demonstrated in *in vitro* binding studies (Creese & Snyder, 1978; Leysen, Niemegeers, Tollenaere & Laduron, 1978). It seemed pertinent therefore to investigate central 5-HT mechanisms in animals receiving chronic neuroleptic therapy. Rats were given trifluoperazine in their drinking water for 4 to 6 months, and cerebral 5-HT function estimated by behavioural and *in vitro* biochemical methods. Enhanced behavioural responses were observed in neuroleptic-treated rats to drugs believed to act through central 5-HT systems. In addition an increase in specific 5-HT receptor binding was measured, indicating enhanced 5-HT mechanisms in these animals.

Methods

Chronic neuroleptic administration

Female Porton rats (8 weeks old at the beginning of

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the experiment) were housed in groups of 8 under standard conditions of lighting (12 h light/dark cycle) and temperature 21 ± 2°C. For 4 to 6 months animals received trifluoperazine hydrochloride (Smith, Kline & French, Ltd.) in their drinking water. The drug was dissolved in tap water containing 0.1% ascorbic acid: age matched controls received ascorbic acid only in their drinking water. The concentration of trifluoperazine in the water was 12 μg/ml so as to achieve an approximate daily intake of 0.8 to 1.0 mg/kg. After the first few days the water intake in the drug-treated animals was similar to that of the control group, and remained constant throughout the duration of the experiment. After 4 to 6 months the animals were subjected to the behavioural and biochemical procedures described below. Neuroleptic administration was continued during these tests.

Behaviour testing

The following drugs were used: fenfluramine hydrochloride (Servier Laboratories) quipazine (Miles Laboratories); 5-hydroxytryptophan (5-HTP; Sigma); carbidopa (Merck, Sharp & Dohme Ltd.); femoxetine (Ferrosan). All drugs with the exception of 5-HTP and carbidopa were made up in 0.9% w/v NaCl solution (saline) and injected intraperitoneally in a volume of 1 ml/kg. 5-HTP was dissolved in saline containing a minimal amount of 2 N HCl and injected subcutaneously in a volume of 2 ml/kg. Carbidopa was suspended in 1% methylcellulose and injected intraperitoneally at a dose of 25 mg/kg 30 min before 5-HTP. 5-HTP was given in doses of 50, 100 and 150 mg/kg, and animals were scored 30, 60, 90 and 120 min after 5-HTP. The following doses of the other drugs as salts were used: fenfluramine, 5, 10, 20 mg/kg; quipazine, 1, 5, 10, 20 mg/kg; femoxetine, 2.5, 5, 10 mg/kg: all behaviours were scored after 15, 30 and 45 min.

Those components of the behaviours believed to be associated with cerebral 5-HT function were scored in an all-or-none fashion in a 1 min period at the time specified. Animals were placed in perspex boxes (25 cm × 25 cm) 15 min before drug injection and were injected in groups of 6 to 8 for each drug dose. Neuroleptic-treated animals and control rats were treated side by side. The following 5-HT-dependent behaviours were scored (see Grahame-Smith, 1971; Curzon, Fernando & Lees, 1979): head bobbing and lateral head weaving, Straub tail, reciprocal forepaw treading, tremor, backward walking and body writhing movements. 'Wet-dog' shakes (Bédard & Pycock, 1977) were counted in a 1 min period. In addition neuroleptic-treated animals often exhibited mouthing, 'munching' movements (as opposed to stereotyped biting and gnawing usually associated with cerebral dopamine receptor simulation) following administration of 5-HT stimulant drugs and these were also scored in an all-or-none fashion. Results are given as a mean of occurrence ± s.e.mean; statistical analyses were made by the Mann-Whitney U test, except for 'wet-dog' shake counts which were analysed with Student's *t* test. A period of at least 5 days was allowed between testing periods on the same animal: no one drug was given more than once to the same animal.

5-Hydroxytryptamine receptor binding

5-HT receptor binding was carried out in membrane fractions prepared from neuroleptic-treated and control rats using [^{3}H]-5-HT as ligand as in the method described by Fillion, Rousselle, Fillion, Beaudoin, Goiny, Deniau & Jacob, (1978). Animals were killed by cervical dislocation and the cerebral cortex, striata and substantia nigra were quickly removed and kept on ice. These were then homogenized in 20 vol 0.32 M sucrose and centrifuged at 1600 *g* for 10 min at 4°C. The supernatant layer was removed and respun at 40,000 *g*, 4°C for 30 min to produce a crude membrane fraction. The pellet was then rehomogenized in 20 vol 50 mM cold Tris HCl buffer, pH 7.4 (containing 0.1% ascorbic acid and 100 μM pargyline); 100 μl homogenate aliquots (containing approx. 5 mg wet wt. of original tissue) were incubated for 10 min at 37°C in tubes containing 0.5 to 25 nM [^{3}H]-5-HT (sp. act. 14 Ci/mmol; Radiochemical Centre, Amersham) in a final volume of 1 ml 50 mM Tris buffer pH 7.4. Specific 5-HT binding was defined as that which could be displaced by the presence of 10 μM nonradioactive 5-HT. Incubation was terminated by rapid filtration on to Whatman GF/B filters, washing with 2 × 10 ml cold 50 mM Tris HCl buffer and the radioactivity determined by liquid scintillation counting. Results for 5-HT binding to cortex and striatum were subjected to Scatchard analysis: for nigral tissue where only four concentrations of [^{3}H]-5-HT were used, specific binding in control and neuroleptic-treated rat membranes was compared directly. Protein was measured by the method of Lowry, Rosebrough, Farr & Randall (1951).

High affinity 5-hydroxytryptamine uptake

High affinity 5-HT uptake in striatal tissue was determined in control and neuroleptic-treated animals by incubating tissue slices in Krebs bicarbonate buffer, pH 7.4, containing 0.2 to 2 μM [^{3}H]-5-HT for 10 min at 37°C by the method as previously described (Kerwin & Pycock, 1979). Uptake was terminated by rapid filtration on to Whatman GF/B filters, and the results subjected to Lineweaver-Burk analysis for determination of the uptake affinity constant (K_m).

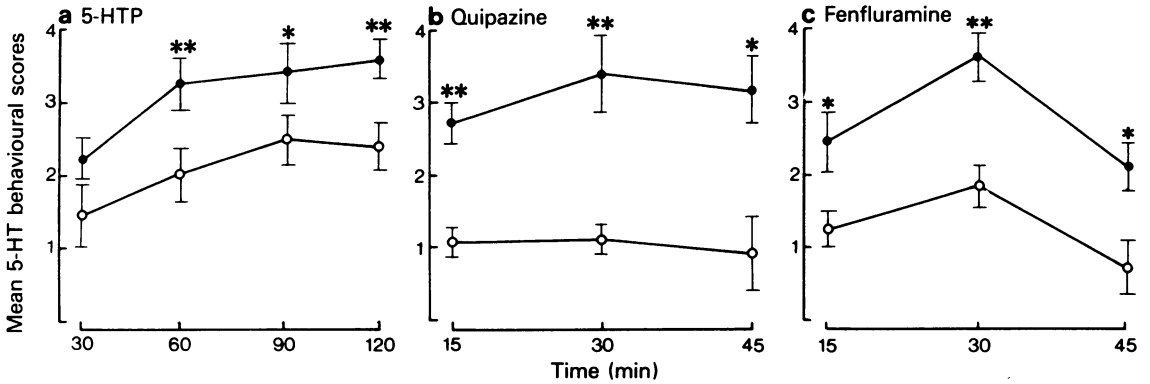

Figure 1 Behavioural scores of control (○) or neuroleptic-treated rats (●) at various times after receiving 5-hydroxytryptophan (5-HTP, 100 mg/kg, i.p., preceded by carbidopa, 25 mg/kg, 30 min, i.p.) (a), quipazine (5 mg/kg, i.p.) (b), or fenfluramine (10 mg/kg, i.p.) (c). The results are expressed as the mean of the number of behaviours being exhibited by an animal at the time of recording (n = 6–8); vertical lines show s.e.mean. Behaviours identified and included in the scoring system: forepaw treading, tremor, head bobbing, lateral head weaving, sniffing, backward walking, body writhing and 'munching' (i.e., a maximum score of 8 per animal). Statistical comparison between control and neuroleptic-treated groups: $*P < 0.05$; $**P < 0.01$ (Mann Whitney U test).

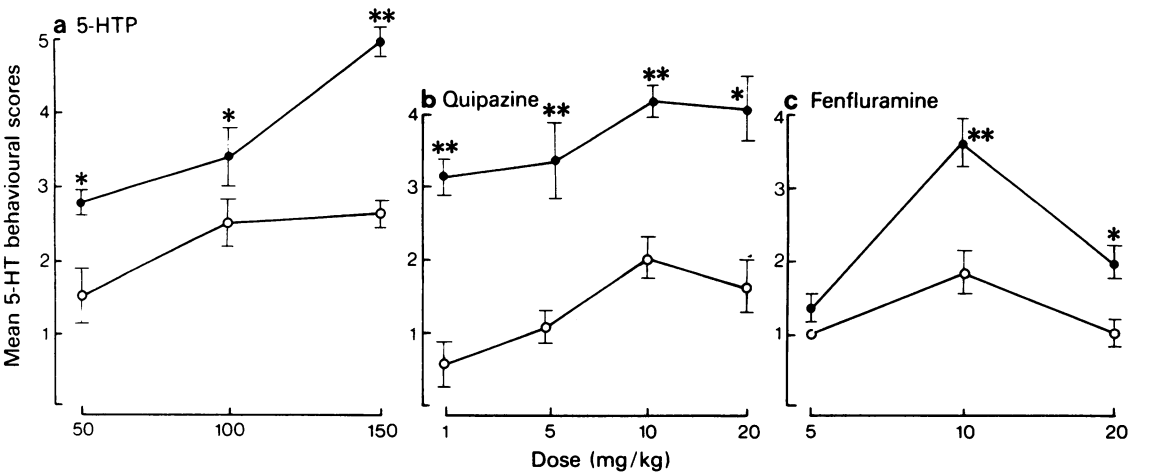

Figure 2 Behavioural scores of control (○) or neuroleptic-treated rats (●) after various doses of 5-hydroxytryptophan (5-HTP, 50 to 150 mg/kg, i.p., 90 min, preceded by carbidopa, 25 mg/kg, 30 min, i.p.) (a), quipazine (1 to 20 mg/kg, i.p., 30 min) (b), or fenfluramine (5 to 20 mg/kg, i.p., 30 min) (c). The results are expressed as mean number of behaviours per rat as explained in the legend to Figure 1; vertical lines show s.e.mean. Statistical comparison between control and neuroleptic-treated groups: $*P < 0.05$; $**P < 0.01$ (Mann Whitney U test) (n = 6–8).

Table 1 'Wet-dog' shakes induced in control or chronic neuroleptic-treated rats by centrally-active 5-hydroxytryptamine drugs

Drug	*Dose*		*Control*	*Neuroleptic*
5-HTP	50 mg/kg	90 min	2.3 ± 0.9 (6)	5.8 ± 2.0 (7)*
		120 min	4.5 ± 1.6 (6)	7.0 ± 1.4 (7)
	100 mg/kg	90 min	7.7 ± 1.9 (7)	11.4 ± 1.9 (8)*
		120 min	10.4 ± 1.8 (7)	14.2 ± 1.9 (8)**
	150 mg/kg	90 min	7.0 ± 1.7 (6)	12.8 ± 2.0 (6)*
		120 min	9.8 ± 1.4 (6)	19.0 ± 2.9 (6)**
Quipazine	5 mg/kg	30 min	0.9 ± 0.6 (7)	3.8 ± 1.3 (7)*
	10 mg/kg	30 min	1.8 ± 1.0 (7)	4.7 ± 1.1 (7)*
Fenfluramine	10 mg/kg	30 min	1.4 ± 0.9 (6)	5.2 ± 1.8 (6)*

'Wet-dog' shakes were counted during a 1 min interval at the times indicated, and are expressed as mean ± s.e.mean. Figures in parentheses indicate number of animals in each group. Significance of differences between control and neuroleptic group: $*P < 0.05$; $**P < 0.01$ (Student's t test).

Results

Behavioural effects of 5-hydroxytryptophan, quipazine, fenfluramine and femoxetine

5-Hydroxytryptophan (50, 100, 150 mg/kg) In carbidopa-pretreated rats, 5-HTP produced its maximum behavioural response for all doses at 90 min in both the neuroleptic-treated rats and controls (see Figure 1 for 100 mg/kg dose). At 50 mg/kg 5-HTP, mild sniffing and occasional head bobbing (2/6 rats) was seen at 90 and 120 min in control animals: neuroleptic-treated rats exhibited more intense stereotyped sniffing and head bobbing (5/6 rats) at these times ($P > 0.05$). At 100 mg/kg 5-HTP, 3 out of 7 control animals showed stereotyped sniffing, head bobbing and backward walking at these times, while 7 out of 8 experimental animals scored these behaviours ($P < 0.05$). At the highest dose (150 mg/kg 5-HTP) the neuroleptic-treated rats all exhibited intense stereotyped sniffing, head bobbing and weaving, forepaw treading and munching movements: the effect in control rats was much less, with only half the animals showing head movements, two animals forepaw treading, and no rats munching ($P < 0.01$, $n = 6$ for both groups). The dose-effect of 5-HTP on these summed behaviours is shown in Figure 2. The number of 'wet-dog' shakes induced by all doses of 5-HTP was significantly increased in the neuroleptic rats when compared to control animals (Table 1).

Quipazine (1, 5, 10, 20 mg/kg) The effects of quipazine became maximal at 30 min in both neuroleptic and control animals (Figure 1). A 1 mg/kg dose in control animals produced little behavioural response, 2 out of 6 animals showing the occasional 'wet-dog' shake: however, in neuroleptic rats, this dose evoked mild sniffing and munching in all animals ($n = 6$) and body tremor was observed in half of these ($P < 0.01$) (Figure 2). Doses of 5, 10 and 20 mg/kg quipazine intensified these behaviours, the greater responses again being observed in the drug-treated animals. In the neuroleptic-treated rats 5-HT behavioural syndromes such as head weaving, body writhing and backward walking were seen at these doses, but these activities were only occasionally noted in the control animals. The dose-related behavioural effects of quipazine are shown in Figure 2. 'Wet-dog' shake behaviour after 5 and 10 mg/kg quipazine was significantly increased in the chronic neuroleptic-treated rats compared to the controls (Table 1).

Fenfluramine (5, 10, 20 mg/kg) The peak behavioural effect of fenfluramine appeared after 30 min (Figure 1). At 5 mg/kg both groups of animals appeared quiet, showing occasional sniffing and munching behaviours, although 3 out of 6 neuroleptic rats exhibited more intense sniffing at 30 min together with occasional head bobbing. The 10 mg/kg dose produced 'wet-dog' shakes and head bobbing in both groups although munching and tremor were only observed in the neuroleptic group ($P < 0.01$). The high dose of 20 mg/kg fenfluramine suppressed some of these behaviours; however, backward walking and a Straub tail effect became very prominent, and neuroleptic animals scored higher than control rats ($P < 0.05$) (Figure 2). 'Wet-dog' shake behaviour was enhanced in the neuroleptic group at 10 mg/kg fenfluramine (Table 1).

Femoxetine (2.5, 5, 10 mg/kg) Administration of femoxetine to either control or drug-treated rats produced little in the way of motor behavioural responses. Animals usually appeared sedated, but at the highest dose, body writhing was observed in half of the animals from each group. However, no difference between control and neuroleptic groups could be detected ($n = 6$).

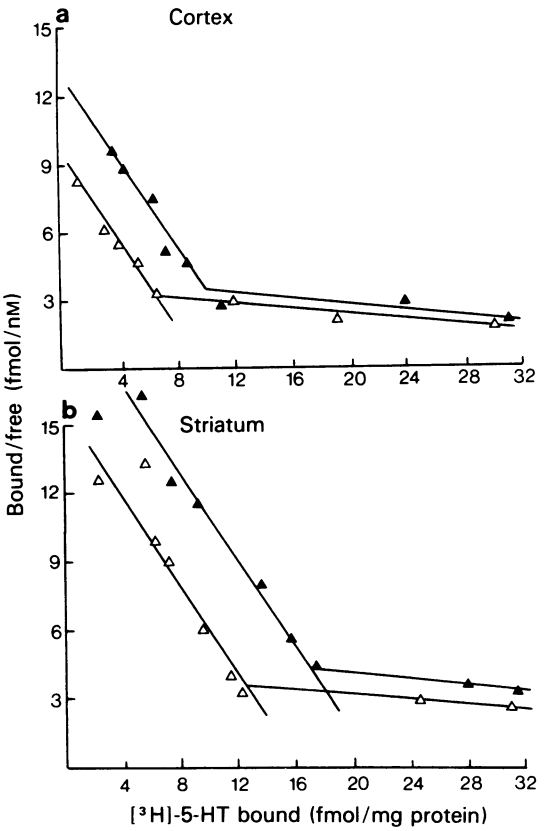

Figure 3 Scatchard analysis of specific [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) binding to membrane fractions prepared from cortex and striatum of control (△) or neuroleptic-treated rats (▲). Membranes were incubated with 0.5 to 25 nM [^{3}H]-5-HT, specific binding being defined as that displaced by the presence of 10 μM 5-HT. Each point is the mean of triplicate determinations, assayed on three separate occasions for striatum (b) and twice for cortex (a).

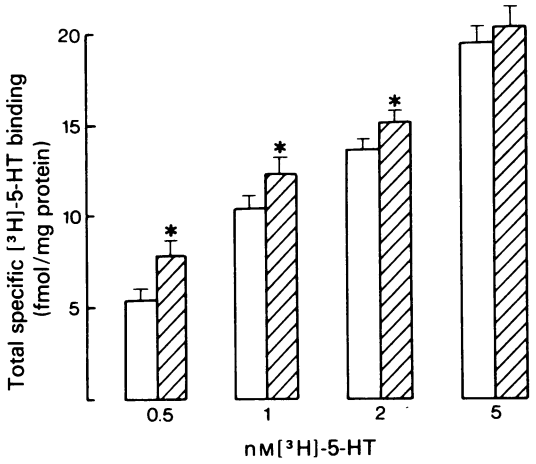

Figure 4 Total specific binding of [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) to membrane fractions prepared from substantia nigra of control (open columns) or neuroleptic-treated rats (hatched columns). Membranes were incubated with 0.5, 1, 2, 5 nM [^{3}H]-5-HT, specific binding being defined as that displaced by the presence of 10 μM 5-HT. Each point is the mean of quadruplicate determinations assayed on two separate occasions. Statistical comparison between two groups: $^*P < 0.05$ (Student's t test).

Radiolabelled 5-hydroxytryptamine binding

[^{3}H]-5-HT bound with high affinity to membrane fractions prepared from striatum and cortex (Figure 3) and substantia nigra (Figure 4). From Scatchard analysis two different populations of binding sites were observed in cortex and striatum. The K_d values were: cortex, 1.13 nM for high affinity binding and 25.6 nM for low-affinity binding; striatum, 1.28 nM for high-affinity binding and 30.1 nM for low-affinity binding.

Total specific high-affinity binding of 5-HT was significantly increased in membrane fractions of both cortex and striatum prepared from neuroleptic treated animals. Scatchard analysis of the data indicated that this was due to an increase in receptor numbers in both regions, the B values in cortex increasing from 11.3 ± 1.7 fmol per mg protein (control) to 16.2 ± 1.1 fmol per mg protein (neuroleptic) ($P < 0.05$). Similarly in striatum B, values increased from 20.3 ± 1.6 (control) to 27.9 ± 2.1 fmol/mg protein (neuroleptic ($P < 0.05$). The dissociation constants for [^{3}H]-5-HT did not differ significantly in the two experimental groups in either cortex (control K_d 1.13 nM; neuroleptic, 1.41 nM) or striatum (control K_d 1.28 nM; neuroleptic 1.25 nM) (Figure 3).

Total specific binding of [^{3}H]-5-HT was also significantly increased in the substantia nigra of neuroleptic-treated rats at concentrations of 0.5, 1 and 2 nM [^{3}H]-5-HT ($P < 0.05$) (figure 4).

High affinity 5-hydroxytryptamine uptake

No significant difference in high affinity uptake data was shown in striatal slices prepared from either control or neuroleptic rats. Lineweaver-Burk plots showed similar K_m values for the two groups (control, 3.2 μM; neuroleptic, 1.4 μM, calculated from the mean of quadruplicate determinations of 6 [^{3}H]-5-HT concentrations).

Discussion

Previous work has shown that chronic administration of trifluoperazine to rats increases cerebral dopamine receptor sensitivity. This has been well documented as an enhanced behavioural response to the dopamine agonist, apomorphine (Clow *et al.*, 1979a) and increased receptor binding of [^{3}H]-spiroperidol (Clow *et al.*, 1979b; 1980). Indeed in our own colony, chronically neuroleptic-treated rats displayed enhanced stereotyped responses to apomorphine (e.g. a 1 mg/kg dose of apomorphine, subcutaneously, induced stereotyped gnawing and licking behaviour in the neuroleptic-treated animals, while only continuous sniffing was observed in control rats) and increased specific binding of the dopamine agonist [^{3}H]-ADTN was observed in striatal membrane preparations (B value for control animals, 166 fmol/mg protein; B value for neuroleptic-treated rats, 295 fmol/mg protein). Thus it would seem that rats used in this study had enhanced dopamine receptor sensitivity after receiving trifluoperazine chronically for 4 to 6 months.

However, in addition to an enhanced dopamine component, these rats also exhibited potentiated behavioural responses believed to reflect central 5-HT function (Grahame-Smith, 1971; Trulson & Jacobs, 1976). Thus treatment of animals with 5-HTP, the direct precursor of 5-HT, produced head bobbing, body writhing, 'wet-dog' shakes and other 5-HT dependent behaviours which were significantly more pronounced in the rats receiving the neuroleptic. Similarly quipazine, a putative agonist at 5-HT receptors (Hong, Sancilio, Vargas & Pardo, 1969; Fuller, Snoddy, Perry, Roush, Molloy, Bymaster & Wong, 1974) and fenfluramine, a drug believed to release 5-HT in lower concentrations (Duhault & Verdavainne, 1967; Kannengiesser, Hunt & Raynaud, 1976) also produced significantly enhanced behavioural effects in the neuroleptic-treated animals when compared to control rats. Whilst it is likely that many of the behaviours observed do not solely reflect 5-HT function but will also involve other neurotransmitter systems such as the catecholamines (e.g. Curzon *et al.*, 1979; see below), these drugs also enhanced 'wet-dog' shake behaviour in neuroleptic-treated animals, a model which appears to depend mainly on 5-HT mechanisms (Bédard & Pycock, 1977).

In support of the enhanced behavioural effects to systemically administered 5-HT drugs, an increased specific binding of [^{3}H]-5-HT was observed in membrane fractions of striatum, cortex and substantia nigra taken from neuroleptic-treated rats. Scatchard analysis indicated an increased number of binding sites (B value) for striatum and cortex, the apparent affinity (K_d) remaining unchanged. Due to lack of tissue, a full Scatchard analysis could not be performed on tissue from the substantia nigra.

Thus, in addition to increased dopamine function we have demonstrated an enhanced 5-HT component following chronic neuroleptic administration. Although we believe that the behaviours quantified in this study basically reflect central 5-HT function, the enhanced dopaminergic component may have contributed to some of the motor responses observed. For example, stereotyped sniffing is usually associated with stimulation of striatal dopamine receptors and it is known that drugs such as 5-HTP and fenfluramine can displace dopamine from presynaptic terminals (Curzon *et al.* 1979). Therefore, a certain degree of caution should be exercised when interpreting some behavioural patterns. In this respect the 'munching' component observed mainly in the neuroleptic-treated rats is interesting, and certainly the relative roles of 5-HT and dopamine in this behaviour should be further investigated, especially if it bears any relation to the oro-facial dyskinesias observed in many humans receiving neuroleptic therapy.

The development of 5-HT receptor supersensitivity is possibly the direct result of drug administration as neuroleptic drugs are known to have affinity for 5-HT as well as dopamine receptors (Creese & Snyder, 1978; Leysen *et al.*, 1978). That receptors other than dopamine develop apparent supersensitivity after chronic neuroleptic treatment is not without precedent, as Gale (1980) reported increased γ-aminobutyric acid (GABA) binding in substantia nigra in such situations. Whether this is a direct effect of the neuroleptic drugs (haloperidol, chlorpromazine) or an indirect action of blocking striatal efferent pathways to the substantia nigra deserves further investigation. However, studies on acetylcholine, histamine and noradrenaline receptor binding showed no changes in rats receiving trifluoperazine for 6 months compared with controls (Clow, Glommeren, Jenner, Leysen, Marsden & Theodorou, 1980), although these authors did not attempt to investigate [^{3}H]-5-HT binding directly.

The apparent lack of effect of chronic neuroleptic therapy on high affinity 5-HT uptake systems or the behavioural effects of femoxetine does not necessarily preclude changes in presynaptic 5-HT terminal systems, although the more compelling 5-HT receptor binding data would predominantly point to enhanced postsynaptic mechanisms. Indeed, in this same group of animals we were unable to detect any changes in high-affinity dopamine uptake (Long & Pycock, unpublished observations) although it is well known that neuroleptic therapy enhances striatal dopamine metabolism (Clow *et al.*, 1980). Similarly it has been reported that neuroleptics enhance central 5-HT metabolism (Grabowska, 1976; Von Stralendorff, Ackenheil & Zimmerman, 1976).

Enhanced behavioural and biochemical indices of 5-HT function after chronic neuroleptic treatment

may have clinical relevance since neuroleptics are administered chronically to control psychiatric states. Whilst schizophrenia has been associated most closely with overactive dopamine systems, the involvement of 5-HT mechanisms has also been proposed (Wooley & Shaw, 1954; Saavedra & Axelrod, 1973; Chouinard, Annable, Young & Sourkes, 1978). Furthermore, a major side-effect of chronic neuroleptic therapy is the occurrence of tardive dyskinesias which may be related to the development of supersensitive dopamine receptors (Marsden, Tarsy & Baldessarini, 1975). However, 5-HT agonists or drugs which elevate synaptic 5-HT concentration are known to produce abnormal motor behaviour (Curzon *et al.*, 1979). Therefore, in view of the fact that enhanced 5-HT receptor function apparently occurs after neuroleptic treatment it should be considered that tardive dyskinesias may in part be related to the formation of supersensitive 5-HT receptors.

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