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REPEATED CHLORPROMAZINE ADMINISTRATION INCREASES A BEHAVIOURAL RESPONSE OF RATS TO 5-HYDROXYTRYPTAMINE RECEPTOR STIMULATION

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1 The hyperactivity syndrome produced in rats by administration of tranylcypromine (20 mg/kg i.p.) followed 30 min later by L-tryptophan (50 mg/kg i.p.) is generally considered to be due to increased 5-hydroxytryptamine (5-HT) functional activity. It is inhibited by chlorpromazine (30 mg/kg i.p.) injected 60 min before the tranylcypromine. However, chlorpromazine injection for 4 days either at a dose of 30 mg/kg once daily or 5 mg/kg twice daily results in an enhanced hyperactivity response to tranylcypromine and L-tryptophan administration 24 h after the final dose of chlorpromazine.

2 One injection of chlorpromazine (30 mg/kg) did not produce enhancement 24 h later and the inhibition of the tranylcypromine/L-tryptophan hyperactivity observed after acute chlorpromazine injection was seen if the rats were given tranylcypromine and L-tryptophan 1 h after the fourth chlorpromazine (30 mg/kg) dose.

3 Chlorpromazine (30 mg/kg) once daily or 5 mg/kg twice daily for 4 days resulted in rats displaying enhanced behavioural responses to the suggested 5-HT agonist 5-methoxy *N,N*-dimethyltryptamine (2 mg/kg) on day 5.

4 Chlorpromazine (30 mg/kg) once daily for 4 days produces a slight increase in brain 5-hydroxytryptamine (5-HT) concentration on day 5, but no difference in the rate of brain 5-HT synthesis or the rate of 5-HT accumulation after tranylcypromine and L-tryptophan administration.

5 There is some evidence that chlorpromazine blocks 5-HT receptors. It has also been observed that several other neuroleptic drugs do not produce enhanced 5-HT responses after repeated administration. It is suggested therefore that the enhanced behavioural response to 5-HT receptor stimulation following repeated chlorpromazine administration may be because this drug blocks 5-HT receptors.

Introduction

Administration to rats of tranylcypromine and tryptophan increases the synthesis of brain 5-hydroxytryptamine (5-HT) and produces a hyperactivity syndrome (Grahame-Smith, 1971a) which has been used to investigate the way that various drugs alter the functional activity of 5-HT in rat brain (Green & Grahame-Smith, 1976). Acute chlorpromazine administration inhibits this behavioural response (Grahame-Smith, 1971b; Heal, Green, Boullin & Grahame-Smith, 1976) as does pretreatment with several other neuroleptic drugs (Heal *et al.*, 1976). Presumably this is because they block the dopaminergic system involved in this behavioural response to 5-HT receptor stimulation (Green & Grahame-Smith, 1974).

It was further demonstrated that repeated administration of several neuroleptic drugs enhanced the dopamine-induced locomotor response produced by injection of tranylcypromine and L-DOPA but that only chlorpromazine treatment enhanced the 5-HT-induced hyperactivity (Heal *et al.*, 1976). There is some evidence that chlorpromazine blocks 5-HT post-synaptic receptors (see Discussion section). It therefore seemed possible that the increased response was the result of receptor blockade in an analogous manner to the increased behavioural sensitivity of dopaminergic systems observed after repeated neuroleptic administration (Gianutsos, Drawbaugh, Hynes & Lal, 1974; Moore & Thornburg, 1975; Heal *et al.*, 1976). However, since it has been reported that chlorpromazine can alter the availability of tryptophan to the brain (Bender, 1976), the possibility existed that the increased response was the result of enhanced 5-HT synthesis and therefore a presynaptic change.

The reason for the enhanced 5-HT behavioural

response has now been investigated and results suggest that it is a post-synaptic change and may reflect increased receptor sensitivity.

Methods

Procedures for the experiments were as follows: chlorpromazine was either injected once daily (at a dose of 30 mg/kg) between 14 h 00 min–15 h 00 min or twice daily (10 h 00 min and 17 h 00 min) at a dose of 5 mg/kg. Control rats were injected with 0.9% w/v NaCl solution (saline) at the same time. Rats were given chlorpromazine on the schedules for 4 days. On day 5, 24 h after the final dose all groups were given tranylcypromine (20 mg/kg), and L-tryptophan (50 mg/kg) or 5-methoxy *N,N*-dimethyltryptamine (2 mg/kg) after a further 30 minutes.

Activity was measured in groups of 3 animals on LKB Animex activity meters (sensitivity and tuning: 30 μA) as described previously (Grahame-Smith, 1971a; Green & Grahame-Smith, 1974). Results were collected as movements/min and graphs show the mean of every 5 min period. Total movements over the 60 min period after either L-tryptophan or 5-methoxy *N,N*-dimethyltryptamine were measured and the results are given in the legend to each figure as the mean of the total movements in 60 min ± the s.e. mean (3 experiments). Differences between groups were calculated using Student's *t* test.

Brain tryptophan was measured by the method of Denckla & Dewey (1967) and brain 5-HT by the method of Curzon & Green (1970).

Results

Effect of repeated chlorpromazine administration on the hyperactivity following tranylcypromine and L-tryptophan

Rats were injected with saline or chlorpromazine (30 mg/kg) once daily for 4 days. On day five, 24 h after the last injection both groups were given tranylcypromine (20 mg/kg) and L-tryptophan (50 mg/kg) after a further 30 minutes. The enhanced activity response of rats to tranylcypromine and L-tryptophan following repeated chlorpromazine treatment was confirmed (Figure 1). Enhancement was also seen if the rats had been injected with chlorpromazine (5 mg/kg) twice daily (10 h 00 min and 17 h 00 min) for 4 days and challenged with tranylcypromine and L-tryptophan on day 5 as described above (Figure 1). This dose does not produce the severe sedation seen after the higher chlorpromazine dose.

No enhancement was seen if the animals were challenged with tranylcypromine and tryptophan 24 h after a single dose of chlorpromazine (30 mg/kg).

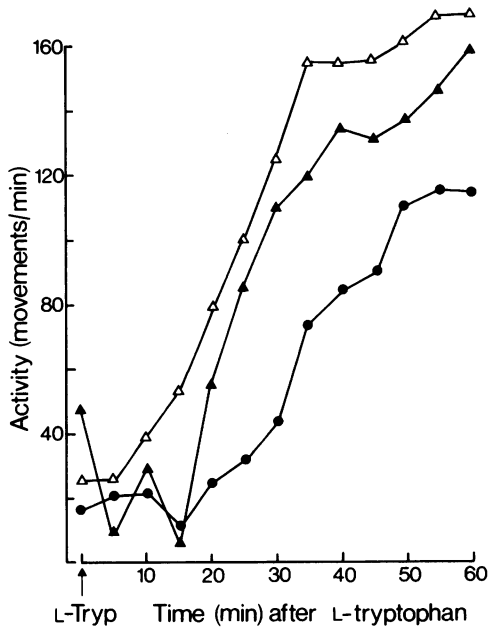

Figure 1 Effect of repeated chlorpromazine administration on the hyperactivity response of rats to tranylcypromine and L-tryptophan. Rats were injected daily for 4 days with saline (●) or chlorpromazine 30 mg/kg (△) or twice daily with chlorpromazine 5 mg/kg (▲). Twenty-four hours after the final injection all groups were given tranylcypromine (20 mg/kg) with L-tryptophan (L-Tryp, 50 mg/kg) after a further 30 minutes. Graph shows the typical hyperactivity following L-tryptophan. Activity was measured as movements/min on Animex meters as described in the Methods section. Total movements during 60 min following L-tryptophan, control: 2960 ± 55; chlorpromazine (30 mg/kg) pretreated: 6593 ± 291; chlorpromazine (5 mg/kg × 2) pretreated: 4000 ± 357. All results are the mean ± s.e. mean of 3 observations and chlorpromazine-treated groups are significantly different from controls at a level of significance of $P < 0.01$ and $P < 0.025$ respectively.

Furthermore, when this dose was given daily for 4 days with tranylcypromine and L-tryptophan given 1 h after the final dose, the normal inhibitory action of chlorpromazine on the tranylcypromine/L-tryptophan hyperactivity was still observed.

Effect of repeated chlorpromazine administration on brain tryptophan and 5-hydroxytryptamine concentrations and the rate of 5-hydroxytryptamine synthesis

An investigation was next made into the effect of the higher chlorpromazine dose on 5-HT biosynthetic mechanisms.

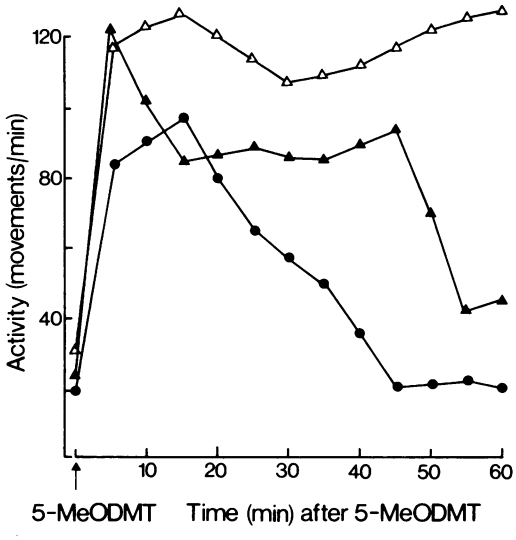

Figure 2 Effect of repeated chlorpromazine administration on the hyperactivity response of rats to tranylcypromine and 5-methoxy *N,N*-dimethyltryptamine (5-MeODMT). Rats were injected daily for 4 days with saline (●) or chlorpromazine 30 mg/kg (△) or twice daily with chlorpromazine 5 mg/kg (▲). Twenty-four hours after the final injection all groups were given tranylcypromine (20 mg/kg) with 5-MeODMT (2 mg/kg) after a further 30 minutes. Graph shows the typical hyperactivity following 5-MeODMT. Activity was measured as in Figure 1. Total movements during 60 min following 5-MeODMT, control: 3700 ± 509; chlorpromazine (30 mg/kg) pretreated: 8530 ± 452; chlorpromazine (5 mg/kg × 2) pretreated: 5710 ± 368. All results expressed as mean ± s.e. mean of 3 observations. Both chlorpromazine-treated results significantly different from control $P < 0.01$.

Rats were injected with saline or chlorpromazine (30 mg/kg) daily for 4 days. On the fifth day 24 h after the last injection they were killed and brain tryptophan and 5-HT measured. Further groups were injected with saline or chlorpromazine using the same protocol but 24 h after the final injection they were given tranylcypromine (20 mg/kg) and killed 60 min later and brain 5-HT measured. The rate of 5-HT synthesis following monoamine oxidase inhibition was then calculated by the method of Neff & Tozer (1968).

No differences were observed in the brain concentration of tryptophan between the control and chlorpromazine-treated group although the steady state concentration of 5-HT following repeated chlorpromazine administration showed a slight increase (Table 1). The rate of 5-HT synthesis in both groups was the same (Table 1). The rise of brain 5-HT after chlorpromazine has been observed by Bender (1976) but the changes reported here are much smaller.

These results suggested that the enhanced responses seen after repeated chlorpromazine treatment were not due to a change in 5-HT synthesis and this was confirmed by the observation that 1 h after L-tryptophan, when the chlorpromazine-treated animals were displaying considerably greater activity (see Figure 1) the brain 5-HT concentrations in both groups were the same (Table 1).

Effect of repeated chlorpromazine administration on the behavioural response to tranylcypromine and 5-methoxy N,N-*dimethyltryptamine*

Since the foregoing experiments showed that repeated chlorpromazine administration did not alter 5-HT biosynthetic mechanisms it seemed probable that post-synaptic changes were responsible for the enhanced 5-HT response following chlorpromazine. This was investigated by use of the suggested 5-HT agonist 5-methoxy *N,N*-dimethyltryptamine (5-MeODMT) which produces a qualitatively similar hyperactivity response to that seen after tranylcypromine and tryptophan, but with a different time course (Grahame-Smith, 1971b).

Rats were injected daily with saline or chlorpromazine as described above. Twenty-four hours after the final injection both groups were given tranylcypromine (20 mg/kg) followed 30 min later by 5-MeODMT (2 mg/kg). Chlorpromazine-treated animals showed a considerably enhanced response to 5-MeODMT (Figure 2) indicating altered post-synaptic responses following chlorpromazine.

Enhancement was also seen after the lower dose of chlorpromazine (5 mg/kg, twice daily, see above) (Figure 2).

Discussion

The fact that repeated chlorpromazine administration does not alter the rate of 5-HT synthesis but does produce an enhanced response to a 5-HT agonist strongly indicates that repeated chlorpromazine administration produces enhanced 5-HT responses as the result of a post-synaptic change. This does not appear to be due to the dopamine receptor blocking action of this drug as several other neuroleptics examined (haloperidol, spiroperidol and α-flupenthixol) do not have this action (Heal *et al.*, 1976). It seems probable that the enhancement is due to blockade of 5-HT receptors by chlorpromazine. Bradley, Wolstencroft, Hösli & Avanzino (1966) using microiontophoretic techniques observed blockade of some 5-HT neurones by chlorpromazine and recently Von Hungen, Roberts & Hill (1975) demonstrated that chlorpromazine, but not

Table 1 Effect of chlorpromazine on brain tryptophan and 5-hydroxytryptamine (5-HT) biosynthetic mechanisms

Injected	*Pretreatment*	*No. of observations*	*Brain concentrations in μg/g brain tissue (wet wt.)* 5-Hydroxytryptamine	Tryptophan
—	Saline	10	0.37 ± 0.01	2.98 ± 0.32
—	Chlorpromazine	10	0.43 ± 0.01*	2.79 ± 0.17
Tranylcypromine + L-Tryptophan	Saline	12	0.91 ± 0.04	18.40 ± 1.51
	Chlorpromazine	12	0.95 ± 0.03	18.66 ± 1.13
				Rate of 5-HT synthesis ($\mu g\ g^{-1}\ h^{-1}$)
Tranylcypromine	Saline	9	0.56 ± 0.01	0.19
Tranylcypromine	Chlorpromazine	8	0.60 ± 0.01	0.17

Results show brain tryptophan and 5-HT concentrations 24 h after 4 daily injections of chlorpromazine (30 mg/kg). The 5-HT accumulation in similarly treated rats given tranylcypromine (20 mg/kg) followed 30 min later by L-tryptophan (50 mg/kg) is also shown 60 min after the tryptophan administration. Finally the rate of 5-HT synthesis in a group of saline and chlorpromazine (30 mg/kg for 4 days)-treated rats on the 5th day has been measured. Rats were given tranylcypromine (20 mg/kg) and the 5-HT accumulation measured 60 min later. Subtraction of the mean control values from the value obtained 60 min after tranylcypromine gives the rate of synthesis in $\mu g\ g^{-1}\ h^{-1}$ (Neff & Tozer, 1968). Results show mean ± s.e. mean. The number of observations is noted.

* Different from saline-treated control, $P < 0.01$.

haloperidol, inhibited 5-HT-sensitive adenylate cyclase in the neonate rat, suggesting an action of chlorpromazine on the post-synaptic 5-HT receptor not shared by haloperidol.

While enhanced responses to dopamine receptor stimulation following repeated administration of drugs blocking the receptor now seem well established, the idea that 5-HT receptors can also exhibit this change has only recently been indicated. Klawans, D'Amico & Patel (1975) showed increased behavioural sensitivity to 5-hydroxytryptophan after chronic methysergide administration and Trulson, Eubanks & Jacobs (1976) have now demonstrated enhanced 5-HT post-synaptic responses following destruction of central 5-HT neurones with 5,7 dihydroxytryptamine. This was demonstrated by a shift in the dose-response curve of the behavioural responses to the 5-HT agonist 5-methoxy *N,N*-dimethyltryptamine. It should perhaps be noted however that Trulson *et al.* (1976) and I (unpublished observations) have been unable to demonstrate enhanced responses to 5-MeODMT following administration of *p*-chlorophenylalanine, the tryptophan hydroxylase inhibitor. This is at variance with the analogous situation in the dopaminergic system where treatment with the tyrosine hydroxylase inhibitor α-methyl *p*-tyrosine results in enhanced dopaminergic responses (Tarsy & Baldessarini, 1973; Dominic & Moore, 1969).

One interpretation of these findings would be that repeated chlorpromazine increases 5-HT receptor sensitivity. This is only observed however when the concentration of chlorpromazine in the brain has decreased since it was not apparent 1 h after the final administered dose, but was present 24 h later.

Whether the enhanced response is due to an alteration at the receptor itself or an alteration in some other neuronal system connected with the post-synaptic 5-HT receptor and which is seen as an increase in the 5-HT response, cannot be stated. However, the fact that haloperidol and several other neuroleptics do not produce this enhanced response argues strongly against the action of chlorpromazine on 5-HT responses being via its action on brain dopaminergic systems.

Since it has been demonstrated previously that the 5-HT hyperactivity syndrome is mediated via a post-synaptic dopaminergic system (Green & Grahame-Smith, 1974) the question arises as to why repeated administration of other neuroleptics, which demonstrably increase dopaminergic sensitivity (Heal *et al.*, 1976) does not result in increased 5-HT responses. No definitive answer can yet be given to this but it seems reasonable to suppose that transmitter interactions are modulatory and some alteration in the sensitivity of a dopaminergic system need not result in changes in 5-HT function.

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