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Behavioural hyperactivity in rats treated with selective monoamine oxidase inhibitors and LM 5008, a selective 5-hydroxytryptamine uptake blocker

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1 The administration of 4-[2-(3-indolyl)ethyl]piperidine (LM 5008), a selective 5-hydroxytryptamine (5-HT) uptake blocker to rats pretreated with tranylcypromine (Tcp) resulted in a behavioural syndrome of locomotor hyperactivity which is indistinguishable from that following combined treatment with Tcp and L-tryptophan.

2 A similar behavioural response was elicited by the administration of LM 5008 to rats pretreated with 5-hydroxytryptophan.

3 The response to LM 5008 after monoamine oxidase (MAO) inhibition was abolished by pretreatment with *p*-chlorophenylalanine, indicating the involvement of 5-HT in producing the hyperactivity syndrome.

4 The administration of imipramine and chlorimipramine in combination with Tcp also resulted in hyperactivity, but these drugs were much less potent than LM 5008 in producing the syndrome.

5 In contrast to L-tryptophan, which can produce hyperactivity only after the inhibition of both type A and type B MAO, LM 5008 can elicit the syndrome after selective inhibition of MAO type A only but not after inhibition of MAO type B.

6 The behavioural results indicate that when MAO type A is inhibited, LM 5008 treatment elicits hyperactivity by preventing the availability of 5-HT to be metabolized by MAO-B component.

Introduction

When rats are treated with an irreversible monoamine oxidase (MAO) inhibitor and L-tryptophan they display a stereotyped hyperactivity syndrome (Hess & Doepfner, 1961; Horita & Carino, 1970; Grahame-Smith, 1971; Modigh & Svensson, 1972). This syndrome is believed to be mediated by 5-hydroxytryptamine (5-HT), since inhibition of 5-HT synthesis by prior treatment with *p*-chlorophenylalanine (PCPA) prevents its production (Horita & Carino, 1970; Grahame-Smith, 1971; Modigh and Svenssen, 1972). Hyperactivity is not closely correlated with the cerebral content of 5-HT (Hess & Doepfner, 1961) but is related to the rate of 5-HT accumulation. Grahame-Smith (1971) has suggested that when the nerve terminal is unable to store the excess of 5-HT, which accumulates as a result of precursor loading combined with decreased metabolic degradation, the amine 'spills over' into the synaptic cleft and causes the hyperactivity syndrome. If this is so, then the inhibition of 5-HT reuptake in association with increased neuronal 5-HT synthesis would also provide extraneuronal excess of 5-HT and should result in hyperactivity. Potentiation of behavioural response to L-tryptophan by the 5-HT uptake inhibitors, chlorimipramine and fluoxetine, in rats pretreated with MAO inhibitors has been reported by Fuller, Perry & Molloy (1974). Holman, Seagraves, Elliot & Barchas (1976) have shown that the combined treatment with the non-selective MAO inhibitor tranylcypromine (Tcp) and either Lilly 110140 or 5-hydroxytryptoline (which selectively inhibit 5-HT uptake) resulted in a behavioural hyperactivity similar to that seen after Tcp and L-tryptophan administration. Green & Youdim (1975) and Squires & Lassen (1975) attempted to define the role of MAO-A and MAO-B in eliciting behavioural excitation in rats loaded with L-tryptophan. Both groups found that L-tryptophan can produce hyperactivity only when both enzyme forms are inhibited by at least 86%. Green & Youdim (1975) further showed that when MAO-A is selectively inhibited *in vivo*, brain 5-HT

rises. They concluded that when MAO-A is selectively inhibited, 5-HT can behave as a substrate for MAO type B.

Recently, it has been reported that LM 5008 (4-[2-(3-indolyl) ethyl] piperidine) is a potent selective inhibitor of 5-HT uptake in brain synaptosomes and platelets (Le Fur & Uzan, 1977). We have, therefore, studied the effect of LM 5008 on the behaviour of rats pretreated with either Tcp, 5-hydroxytryptophan (5-HTP) or the selective MAO inhibitors, clorgyline and deprenyl.

Methods

Behavioural experiments

Sprague-Dawley male rats ranging in weight from 150 to 250 g were used in all experiments. Rats were housed in groups of 5 with food and water *ad libitum*, and maintained on a 12 h–12 h light-dark cycle. One hour before testing, individual rats were placed in plastic cages that were put on LKB Animax activity meters (sensitivity and tuning 30 μA). Activity was monitored every 10 min, starting with the injection of the first drug, MAO inhibitor or 5-HTP. Thirty min later the animal received the 5-HT uptake blocker being studied or saline for control. The animal was not disturbed again until the end of the session (120 min).

Biochemical procedures

All biochemical determinations were performed at the end of behavioural studies. MAO activity was measured in whole brain homogenates with [^{14}C]-5-HT as a substrate for MAO type A and [^{14}C]-phenylethylamine as substrate for MAO type B using the procedure described by Tipton & Youdim (1976). 5-HT and 5-hydroxyindole acetic acid (5-HIAA) in the brain were determined according to the method developed by Vogt & Wilson (1972). Protein was estimated by the procedure of Lowry, Rosebrough, Farr & Randall (1951).

Materials

Tranylcypromine was generously donated by Smith, Kline and French, clorgyline was donated by May & Baker. Deprenyl was a gift from Professor J. Knoll, Budapest, Hungary. LM 5008 was generously given by Pharmindustrie (France). Imipramine and chlorimipramine were a gift from Ciba Geigy, Basle; PCPA and 5-HTP were purchased from Sigma.

All drugs except LM 5008 and PCPA were dissolved in 0.9% w/v NaCl (saline) and injected intraperitoneally. LM 5008 was dissolved in 0.1 N HCl to make 29% solution. It was then titrated to pH 6 with 0.1 N NaOH and diluted to 1% solution with saline. *p*-Chlorophenylalanine (PCPA) was administered intraperitoneally as a suspension in 1% Tween in saline 72 h before the start of a test session.

Results

Behavioural changes following the combined treatment with tranylcypromine and either 5-hydroxytryptophan or LM 5008

The administration of either Tcp (5 mg kg^{-1}, i.p.) or LM 5008 (20 mg kg^{-1}) had no effect on the behavioural response of the rat. However, when LM 5008 (20 mg kg^{-1}, i.p.) was injected 30 min after Tcp (5 mg kg^{-1}, i.p.) a profound effect on behaviour was observed. This drug combination elicited stereotyped behaviour characterized by forepaws padding, head weaving and circling, which were recorded as a whole as hyperactivity using the activity meters. The hyperactivity started 30–40 min after LM 5008 administration and lasted for several hours. Depletion of brain 5-HT by pretreatment with *p*-chlorophenylalanine (PCPA) (250 mg kg^{-1}, i.p. 72 h

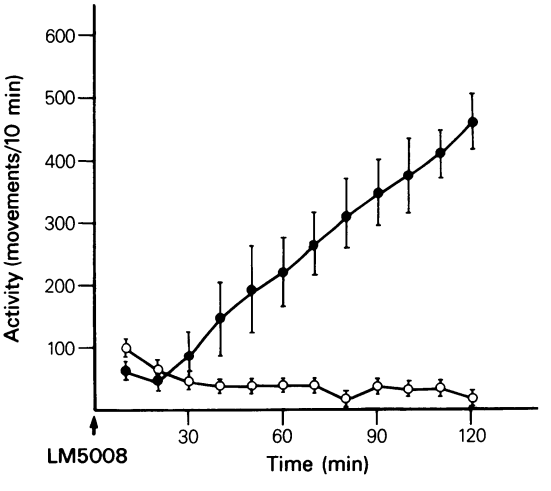

Figure 1 Effect of pretreatment with *p*-chlorophenylalanine (PCPA) on the hyperactivity syndrome elicited by the administration of tranylcypromine (Tcp) and LM 5008. Rats were given PCPA (250 mg kg^{-1} i.p., ○), 72 h later they were given Tcp (5 mg kg^{-1} i.p.) followed 30 min later by LM 5008 (20 mg kg^{-1} i.p.) administration. Activity of individual rats was then recorded for the next 2 hours. Control animals (●) were injected with saline in place of PCPA. Each point represents mean movements during 10 min periods; s.e.mean shown by vertical lines. $n = 5-7$ animals for each treatment.

before Tcp injection) abolished the hyperactivity syndrome to Tcp and LM 5008 (Figure 1).

No behavioural stimulation was noticed after the injection of 5-hydroxytryptophan (5-HTP), (10–100 mg kg^{-1}, i.p.). However, LM 5008 (20 mg kg^{-1}, i.p.) given 30 min after 5-HTP (50 mg kg^{-1}, i.p.) administration resulted in a similar behavioural syndrome to that observed after the combined treatment with Tcp and LM 5008. In this experiment the hyperactivity syndrome appeared much sooner, viz. 10 min after LM 5008 injection, and lasted for 60–80 min (Figure 2).

Hyperactivity induced by tranylcypromine and tricyclic antidepressant treatment

The comparison between LM 5008 and the tricyclic uptake blockers, imipramine and chlorimipramine, was carried out in rats that were pretreated with Tcp (5 mg kg^{-1}, i.p.). All 5-HT uptake blockers were injected at a dose of 20 mg kg^{-1}, i.p. 30 min after administration of the MAO inhibitor. Results are summarized in Figure 3, and it can be seen that LM 5008 was more potent in eliciting the behavioural hyperactivity than the tricyclic uptake blockers. Chlorimipramine (at 20 mg kg^{-1}) did not cause behavioural changes in rats pretreated with Tcp. However, increasing the dose of chlorimipramine to 50 mg kg^{-1} (i.p.) resulted in the appearance of the hyperactivity syndrome.

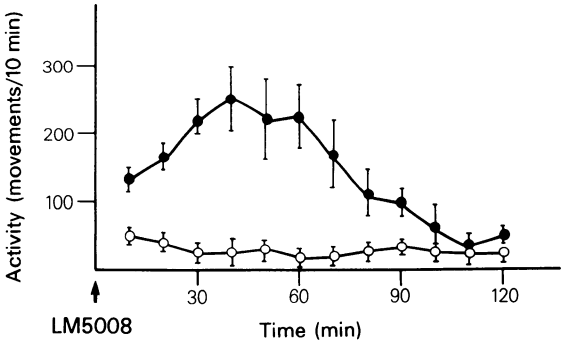

Figure 2 The effect of LM 5008 on the behaviour of rats pretreated with 5-hydroxytryptophan (5-HTP). Rats were given 5-HTP (100 mg kg^{-1}, i.p.) followed 30 min later with either LM 5008 (20 mg kg^{-1}, i.p.) or vehicle solution: 5-HTP alone (○); 5-HTP plus LM 5008 (●). Activity of individual rats was recorded for 2 h after LM 5008 administration. Each point represents mean movement over 10 min periods; s.e.mean shown by vertical lines. $n = 5-7$ animals in each group.

Behavioural responses following selective monoamine oxidase inhibitors and LM 5008

Green & Youdim (1975) and Youdim (unpublished data) showed that when MAO-A or -B are selectively inhibited by clorgyline or deprenyl respectively and the rats receive either L-tryptophan or L-5-HTP, no hyperactivity syndrome is elicited. In contrast to the above result, we found LM 5008 induced hyperactivity in rats previously treated with clorgyline (10 mg kg^{-1}) but not in those pretreated with deprenyl (10 mg kg^{-1}) (Figure 4). The clorgyline-LM 5008 behavioural syndrome was identical to that described for the combination of Tcp with LM 5008.

Biochemical changes following selective monoamine oxidase inhibitors and LM 5008

The data pertaining to biochemical changes following the various treatments are shown in Table 1. All determinations were made 120 min after saline or MAO inhibitor administration. Clorgyline treatment caused a near complete selective inhibition of MAO-A (5-HT) accompanied by an increase in 5-HT and a decrease in 5-HIAA levels in the brain. In contrast, deprenyl (10 mg kg^{-1}) selectively inhibited MAO-B

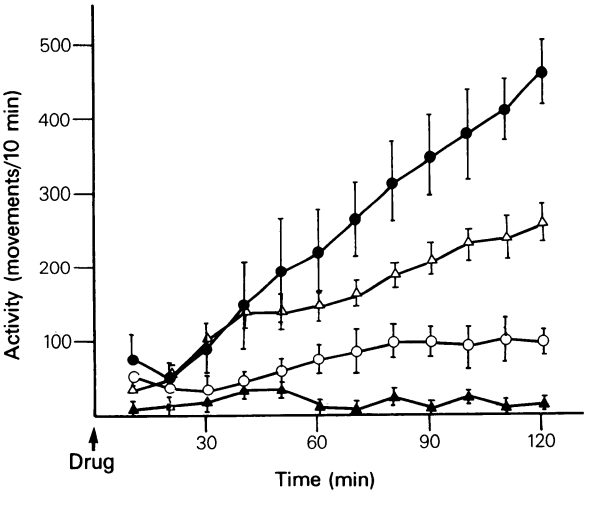

Figure 3 Comparison between tricyclic uptake blockers and LM 5008 in their ability to produce the hyperactivity syndrome in rats. Rats were given tranylcypromine (Tcp) (5 mg kg^{-1}, i.p.); 30 min later they were given either imipramine (▲, 20 mg kg^{-1}), chlorimipramine (○, 20 mg kg^{-1} or △, 50 mg kg^{-1}) or LM 5008 (●, 20 mg kg^{-1}). All injections were i.p. Activity was then recorded for individual rats over the next 2 h. Each point represents mean movements over 10 min period; s.e.mean shown by vertical lines. $n = 5$ animals in each group.

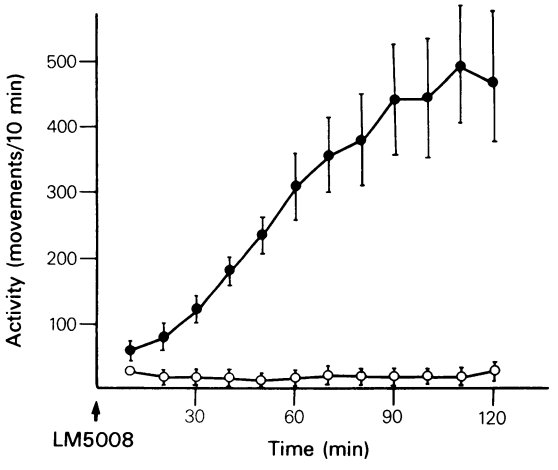

Figure 4 Appearance of the hyperactivity syndrome following LM 5008 administration to rats pretreated with either clorgyline or deprenyl. Rats were injected with clorgyline (●) or deprenyl (○) (10 mg kg^{-1}, i.p.) followed 30 min later by LM 5008 injection (20 mg kg^{-1}, i.p.). Activity of individual rats was then measured over the next 2 h. Each point represents mean movement over 10 min period; s.e.mean shown by vertical lines. $n = 6$ animals in each group.

(phenylethylamine) without causing a significant change in brain 5-HT or 5-HIAA.

LM 5008 (20 mg kg^{-1}) alone produced slight inhibition of MAO-A activity, a non-significant elevation in 5-HT and a significant decrease in 5-HIAA levels. The brain levels of 5-HT after combination of LM 5008 and the selective MAO inhibitors (clorgyline and deprenyl) were similar to those determined after treatment with the selective MAO inhibitors alone. Brain 5-HIAA concentration was more markedly decreased by the combination of clorgyline and LM 5008 than by each of these drugs given alone.

Discussion

When rats are treated with compounds which either increase synaptic 5-HT or directly stimulate postsynaptic 5-HT receptors a stereotyped behaviour is produced, one feature of which is a hyperactivity syndrome (for review see Jacobs, 1976; Green & Grahame-Smith, 1976). The administration of non-selective MAO inhibitors which irreversibly inhibit both forms of the enzyme or treatment with the 5-HT precursors (5-HTP or L-tryptophan), increases the content of 5-HT in the brain (Udenfriend, Weissbach & Bogdanski, 1957; Grahame-Smith, 1971), but such treatment alone is not sufficient to produce the hyperactivity syndrome.

Green & Youdim (1975) showed that the selective inhibition of MAO-A or MAO-B, with clorgyline or deprenyl respectively, followed by L-tryptophan administration did not result in a hyperactivity syndrome. In contrast, hyperactivity did result after inhibition of both MAO-A and -B (either with the combination of selective inhibitors or with Tcp) together with L-tryptophan administration. In the latter case, the brain 5-HT concentrations are much higher than those seen with the selective inhibitors of MAO. Inhibition of MAO-B alone has very little effect on brain 5-HT (Table 1 and see Green & Youdim, 1975). These results provided evidence to indicate that when type A MAO is selectively inhibited, the 5-HT synthesized accumulates to an extent that would satisfy the much higher *Km* of this sub-

Table 1 5-Hydroxytryptamine (5-HT) and 5-hydroxyindole acetic acid (5-HIAA) levels and monoamine oxidase (MAO) activity in the brain of rats after MAO inhibitor and LM 5008 treatment

Treatment	*5-HT* (ng g^{-1})	*5-HIAA* (ng g^{-1})	*MAO activity (% of control ±)* *5-HT substrate*	*PEA substrate*
Control	322 ± 31	298 ± 40	100	100
LM 5008 (20 mg kg^{-1})	399 ± 57	146 ± 18*	78 ± 12	112 ± 6
Clorgyline (10 mg kg^{-1})	641 ± 89*	118 ± 23*	5 ± 1†	81 ± 15
Deprenyl (10 mg kg^{-1})	382 ± 31	252 ± 32	80 ± 13	15 ± 5†
Clorgyline followed 30 min later by LM 5008	589 ± 95*	63 ± 13**†	10 ± 4†	76 ± 3*
Deprenyl followed 30 min later by LM 5008	404 ± 97	159 ± 26*	88 ± 9	11 ± 2†

Brain 5-HT, 5-HIAA and MAO-A (5-HT) and MAO-B (PEA) activities were determined 120 min after drug treatments. The results are expressed ± s.e.mean ($n = 6-12$ rats).
*$P < 0.01$ significantly different from control values; **$P < 0.01$ significantly different from clorgyline- or LM 5008-treated animals; †$P < 0.001$ significantly different from control value.

strate for MAO-B (Green & Youdim, 1975; Fowler & Tipton, 1982), and it could be deaminated by this enzyme at a slower rate. Thus the rate of 5-HT accumulation in the synaptic cleft never reaches that seen after the combination of selective MAO-A and -B inhibitors or a non-selective inhibitor (Green & Youdim, 1975). Recently Fowler & Tipton (1982) have confirmed by *in vitro* studies that 5-HT can be a substrate for MAO-B, albeit at high 5-HT concentration. The present study has demonstrated that selective inhibition of MAO-A but not of -B in combination with a selective 5-HT uptake blocker, LM 5008, results in the hyperactivity syndrome. This is consistent with a predominantly intraneuronal location of MAO-A (Neff & Fuentes, 1976). Furthermore, the results indicate that the 5-HT which accumulates at the synaptic cleft after the inhibition of MAO-A and the amine uptake process is not available for deamination by MAO-B. It can be speculated that LM 5008 inhibits the access of synaptic 5-HT either to a small B component situated intraneuronally or to the bulk of MAO-B enzyme, which is believed to be located extraneuronally (Neff & Fuentes, 1976). In this context, evidence for a 5-HT uptake system located in glial cells sensitive to inhibition by amine uptake blockers has been reported by Whitaker & Warsh (1983).

Acute administration of 5-HT uptake blockers such as LM 5008 or the tricyclic uptake blockers alone (imipramine and/or chlorimipramine) fails to change motor activity (unpublished observation). However, in our experiments the combination of increased 5-HT content in the brain by either Tcp or 5-HTP treatment with LM 5008 or tricyclic antidepressants produced the behavioural syndrome of hyperactivity. Thus our results are in agreement with those reported by Holman *et al.* (1976) and Ortman, Waldmeier, Radeke, Felner & Delini-Stula (1981) and support the hypothesis of Grahame-Smith (1971) that the hyperactivity syndrome is related to the accumulation of extraneuronal 5-HT. The involvement of 5-HT in the hyperactivity syndrome is shown in the studies where the hyperactivity was blocked by pretreatment with PCPA, which inhibits tryptophan hydroxylase, thus reducing available 5-HT.

Since a major neurochemical mechanism of action of tricyclic antidepressants has been ascribed to an inhibition of 5-HT uptake (Carlsson, Fuxe & Ungerstedt, 1968; Segawa, Normura, Tanaka & Murakami, 1977), we have studied the effect of imipramine and chlorimipramine in producing the hyperactivity syndrome in rats pretreated with Tcp. *In vitro* LM 5008 is as potent as imipramine and chlorimipramine in inhibiting [^{3}H]-5-HT uptake into synaptosomes (Le Fur & Uzan, 1977). However, *in vivo* LM 5008 was far more potent than the tricyclic uptake blockers (Le Fur, Mitrani & Uzan, 1977). Our behavioural studies confirm the *in vivo* data of Le Fur *et al.* (1977). The differences in the potencies between LM 5008 and the tricyclic uptake blockers in eliciting the behavioural syndrome of hyperactivity cannot be attributed to the 5-HT receptor blocking property of the latter (Ogren, Fuxe, Agnati, Gustafsson, Jonsson & Holm, 1979), since increasing the dose of chlorimipramine resulted in the appearance of the hyperactivity syndrome in rats previously treated with Tcp. The different potencies of these drugs may reflect differences in absorption, distribution or efficacy. They may also reflect differences in metabolism, since *in vivo* N-demethylation of the tricyclic uptake blockers to their respective secondary amines which are more potent as noradrenaline uptake blockers and less potent as 5-HT uptake blockers could occur. If selective 5-HT uptake inhibition plays a major role in the antidepressant activity then a drug like LM 5008 may possess an advantage over the tricyclic antidepressants by being more potent and more specific in its effects on the 5-hydroxytryptaminergic system.

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