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Relationship between extracellular 5-hydroxytryptamine and behaviour following monoamine oxidase inhibition and L-tryptophan

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1 The present study investigates the effects of selective and a non-selective monoamine oxidase (MAO) inhibitors combined with L-tryptophan on MAO-A and -B activity, hypothalamic extracellular 5-hydroxytryptamine (5-HT) *in vivo* and the occurrence of the 5-HT behavioural syndrome.

2 Selective inhibition of intraneuronal MAO-A with MDL 72394 (0.5 mg kg^{-1}, i.p.) had no effect on extracellular 5-HT and following administration of L-tryptophan (50 mg kg^{-1}, i.p.) the 5-HT behavioural syndrome was not induced.

3 Selective inhibition of MAO-A at all sites with clorgyline (5 mg kg^{-1}, i.p.) increased extracellular 5-HT but did not induce the 5-HT behavioural syndrome when combined with L-tryptophan administration.

4 Selective inhibition of MAO-B with selegiline (10 mg kg^{-1}, i.p.) had no effect on extracellular 5-HT and the 5-HT behavioural syndrome was not observed after L-tryptophan administration.

5 Inhibition of MAO-A and -B with a higher and therefore non-selective, dose of MDL 72394 (2 mg kg^{-1}) markedly increased extracellular 5-HT but failed to induce the 5-HT behavioural syndrome after L-tryptophan administration.

6 Inhibition of MAO-A and -B at all sites in the brain (tranylcypromine 20 mg kg^{-1}, i.p. or clorgyline 5 mg kg^{-1} plus selegiline 10 mg kg^{-1}) increased extracellular 5-HT and induced the behavioural syndrome on administration of L-tryptophan.

7 The results demonstrate that inhibition of MAO-A and -B both within amine neurones and elsewhere in the brain is essential for the development of the 5-HT behavioural syndrome. Whilst the syndrome is associated with increased extracellular 5-HT this does not appear necessarily to result in the syndrome and may indicate that increased extracellular 5-HT is not solely involved in the induction of the '5-HT behavioural syndrome'.

Introduction

Rats treated with a monoamine oxidase (MAO) inhibitor and L-tryptophan exhibit a characteristic behavioural syndrome of reciprocal forepaw treading, lateral head weaving, hindlimb abduction, Straub tail, hyperpyrexia and hyperactivity (Hess & Doepfner, 1961; Grahame-Smith, 1971). It was thought that L-tryptophan caused an accumulation of 5-hydroxytryptamine (5-HT) in the brain and that MAO inhibition allowed the 5-HT to 'spill over' into the synapse (Grahame-Smith, 1971). This hypothesis was supported, in part by the ability of certain 5-HT receptor antagonists to abolish some of the components of the behavioural syndrome and of 5-HT uptake inhibitors to potentiate the syndrome (Deakin & Green, 1978). Consequently, this model has been used as a functional test of 5-HT receptor activity (Green & Grahame-Smith, 1976).

MAO exists as two forms termed MAO-A and MAO-B (Johnston, 1968; Youdim *et al.*, 1969) of which 5-HT is preferentially deaminated by MAO-A *in vitro* (Johnston, 1968). It was found that inhibition of MAO-A alone plus L-tryptophan treatment was not sufficient to evoke the syndrome (Green & Youdim, 1975; Squires & Lassen, 1975; Archer *et al.*, 1985). In

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addition, selective MAO-B inhibition and L-tryptophan treatment also failed to produce the syndrome while a combination of an MAO-A and MAO-B inhibitor plus L-tryptophan did (Green & Youdim, 1975).

A number of other neurotransmitters may be involved in the syndrome. For instance, catecholamines have been suggested to have a role (Deakin & Green, 1978); reserpinised rats fail to exhibit some behavioural effects of 5-HT receptor stimulation (Tricklebank *et al.*, 1984) and dopamine antagonists attenuate the syndrome (Heal *et al.*, 1976). Squires & Lassen (1975) suggested that the syndrome may be dependent on N-substituted derivatives of 5-HT which are partly deaminated by MAO-B. Tryptamine may play a part in eliciting the behavioural syndrome since treatment with an MAO inhibitor and L-tryptophan increases brain levels of tryptamine more than 5-HT (Marsden & Curzon, 1978). Furthermore, inhibitors of the enzyme aromatic L-amino acid decarboxylase, that have little effect on brain 5-HT, decrease brain tryptamine and prevent the behavioural syndrome (Marsden & Curzon, 1979).

Nevertheless, 5-HT is considered to play an important part in the behavioural syndrome especially since *in vivo* 5-HT may also be deaminated by MAO-B if MAO-A has been inhibited (Fowler & Tipton, 1982). Recently Archer *et al.* (1985) reported that amiflamine and L-tryptophan give rise to increased total activity, locomotion and rearing behaviour even though amiflamine is a selective inhibitor for MAO-A.

Although there have been a number of measurements of brain 5-HT, no studies have been made of the effects of MAO inhibitors and L-tryptophan on the release of 5-HT *in vivo*. Intracranial dialysis offers a method of measuring extracellular levels of 5-HT in freely behaving rats which can be used as an index of 5-HT release from neurones (Ungerstedt, 1984; Sharp *et al.*, 1985; Brazell *et al.*, 1985). Therefore we have used this technique to measure extracellular levels of 5-HT in freely moving rats and determined whether the occurrence of the 5-HT behavioural syndrome correlated with an increase in 5-HT release following the administration of L-tryptophan and an MAO inhibitor. In order to study the roles of different pools of MAO, selective inhibitors were used; the MAO-A inhibitor clorgyline (Johnston, 1968; Yang & Neff, 1973), the MAO-B inhibitor selegiline (Yang & Neff, 1973), the MAO-A inhibitor (*E*)-β-fluoromethylene-*meta*-tyrosine (MDL 72394) at a neuronally selective dose (Palfreyman *et al.*, 1985) as well as the non-selective MAO inhibitor tranylcypromine.

Part of this work has been previously published in abstract form (Marsden *et al.*, 1987; Sleight *et al.*, 1987).

Methods

Male Wistar rats weighing 200–300 g were used in all experiments. They were housed in groups of 5 under a 12 h light-dark cycle and given free access to food and water.

Behavioural methods

Behaviour and locomotor activity was measured in groups of 5 rats with the rats in individual observation chambers. Thirty minutes after tranylcypromine (20 mg kg^{-1}, i.p.) and 2 h after saline, MDL 72394 (0.5 or 2 mg kg^{-1}, i.p.), clorgyline (5 mg kg^{-1}, i.p.), selegiline (10 mg kg^{-1}, i.p.), or clorgyline (5 mg kg^{-1}, i.p.) plus selegiline (10 mg kg^{-1}, i.p.), L-trytophan (50 mg kg^{-1}, i.p.) was given. The components of the behavioural syndrome produced by MAO inhibition plus L-tryptophan were scored on a rating scale where 0 = absent, 1 = occasional, 2 = frequent and 3 = constant, 10, 20, 30, 40, 50 and 60 min after the L-tryptophan injection. The components scored were reciprocal forepaw treading, lateral head weaving, Straub tail and hindlimb abduction. All behavioural studies were performed between 3 and 6 h after the start of the light phase of the light-dark cycle.

Measurement of indole extracellular levels by intracranial dialysis

Dialysis probes were manufactured according to the method of Brazell *et al.* (1985). Dialysis tubing was positioned in the lumen of two stainless steel cannulae (23 g) so as to leave 2 mm of dialysis tubing exposed and secured with epoxy resin. A small length of nylon thread was inserted into the dialysis tubing and the probe was perfused with artificial CSF (composition (nM): NaCl 125, $NaHCO_3$ 27, KCl 2.5, $NaH_2PO_4 2H_2O$ 0.5. $NaHO_4$ 1.2, $NaSO_4$ 0.5, $MgCl_2 6H_2O$ 1, $CaCl_2$ $2H_2O$ 1, glucose 5, pH 7.4) at a rate of 1 μl min^{-1}. The probe was then made into a loop with a 180° angle and implanted into the rat ventromedial hypothalamus (rostral-caudal + 0.2 mm from bregma, sagittal ± 0.8 mm from bregma, vertical −8.5 from the dura) (Pellegrino *et al.*, 1981) under 2–3% halothane/O_2/N_2O anaesthesia. The outlet tube from the dialysis loop ran to a harness on the back of the rat. The animal was allowed 2–3 h to recover from the anaesthesia and then 20 min dialysis samples were collected into an Eppendorf tube incorporated into the harness. The indoles in the dialysate were immediately assayed by high performance liquid chromatography with electrochemical detection (h.p.l.c.–e.c.d.)

H.p.l.c. assay of 5-HT and 5-HIAA

5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were

separated by ion-pair, reverse phase chromatography. Separation took place on a column (length 100 mm internal diameter 2 mm) packed with 3 μm Hypersil. Mobile phase, consisting of 0.15 M NaH_2PO_4, $2H_2O$, 0.1 mM EDTA, 0.5 mM sodium octydyl sulphonic acid, 15% methanol was pumped through the column at a rate of 0.4 ml min^{-1}. The amines eluted from the column were measured with a glassy carbon working electrode maintained at a potential of +0.65 V.

Basal indole levels were measured for 1 h after which time rats were given either tranylcypromine (20 mg kg^{-1} i.p.), MDL 72394 (0.5 or 2 mg kg^{-1}, i.p.) clorgyline (5 mg kg^{-1}, i.p.), selegiline (10 mg kg^{-1}, i.p.) or clorgyline (5 mg kg^{-1}, i.p.) plus selegiline (10 mg kg^{-1}, i.p.). Thirty minutes after tranylcypromine treatment and 2 h after all the other treatments, L–tryptophan (50 mg kg^{-1}, i.p.) was given and samples were then collected for a further 80 min. A separate group of rats was given 0.9% saline as control.

Determination of monoamine oxidase activity

Groups of 5 rats were given either saline, tranylcypromine (20 mg kg^{-1}, i.p.) MDL 72394 (0.5 or 2 mg kg^{-1}, i.p.), clorgyline (5 mg kg^{-1}, i.p.), selegiline (10 mg kg^{-1}, i.p.), or cloryline (5 mg kg^{-1}, i.p.) plus selegiline (10 mg kg^{-1}, i.p.). Thirty minutes after tranylcypromine and 2 h after other treatments the rats were killed by decapitation and their hypothalami removed, weighed and frozen in liquid nitrogen. Each hypothalamus was sonicated in 600 μl phosphate buffer (pH 7.4) and centrifuged at 13,500 *g* for 20 min. MAO activity was measured in the supernatant according to the method of Zreika *et al.* (1984) with [^{14}C]-5-HT (Amersham) and [^{14}C]-phenylethylamine (PEA) (New England Nuclear) used as substrates for MAO-A and MAO-B respectively.

Drugs

Phenylethylamine (PEA), 5-hydroxytryptamine (5-HT), 5-hydroxyindole acetic acid (5-HIAA) and L-tryptophan (L-TP) were purchased from Sigma U.K. The sources of the MAO inhibitors were MDL 72394 (Merrell-Dow Research Institute, Strasbourg), tranylcypromine (Smith, Kline and French), clorgyline (May and Baker) and selegiline (donated by Dr P. Jenner).

Statistical analysis

Dialysis results were analysed by the unpaired Student's *t* test. The mean value of the first three dialysis 5-HT values was taken as the control value and all values were then expressed as a percentage of the controls. In the figures the data are shown as percentage of the pre-injection values with mean pre-injection values ± s.e.mean stated in the legend in fmol 20 μl^{-1} dialysis perfusate. Student's *t* test values were determined on the original values (fmol 20 μl^{-1} dialysis perfusate) and not the percentage values. The behavioural data were analysed by the Mann-Whitney U-test.

Results

Tranylcypromine plus L-tryptophan

L-Tryptophan (50 mg kg^{-1}, i.p.) alone did not produce the behavioural syndrome and had no effect on the basal extracellular 5-HT and 5-HIAA levels determined by intracerebral dialysis compared to saline injected controls. When L-tryptophan (50 mg kg^{-1}) was given 30 min after the administration of the non-selective MAO inhibitor tranylcypromine (20 mg kg^{-1}, i.p.) the characteristic behavioural syndrome developed and this was associated with a rise in hypothalamic extracellular 5-HT (Figure 1) and a decrease in 5-HIAA (results not shown). Tranylcypromine alone also significantly increased hypothalamic extracellular 5-HT but did not induce the behavioural response (Figure 1). At the dose of tranylcypromine used (20 mg kg^{-1}, i.p.) both MAO-A and -B were fully inhibited (Table 1).

MDL 72394 plus L-tryptophan

MDL 72394 at a dose (2 mg kg^{-1}), which significantly reduced both MAO-A and -B activity but B to a lesser extent than that observed after tranylcypromine (Table 1), significantly and markedly increased hypothalamic extracellular 5-HT but failed to induce the '5-HT behavioural response' (Figure 2a). The addition of L-tryptophan 2 h after MDL 72394 was associated with a further rise in extracellular 5-HT (Figure 2a) but we did not observe the behavioural syndrome. Administration of MDL 72394 at a lower dose (0.5 mg kg^{-1}, i.p.) only reduced MAO-A activity (Table 1) and neither extracellular 5-HT (Figure 2b) nor behaviour changed.

Clorgyline plus L-tryptophan

Clorgyline (5 mg kg^{-1}, i.p.) significantly reduced MAO-A activity but had no effect on MAO-B (Table 1). When L–tryptophan (50 mg kg^{-1}, i.p.) was given 2 h after clorgyline there was a small but significant increase in hypothalamic extracellular 5-HT but again the behavioural syndrome was not observed (Figure 3a).

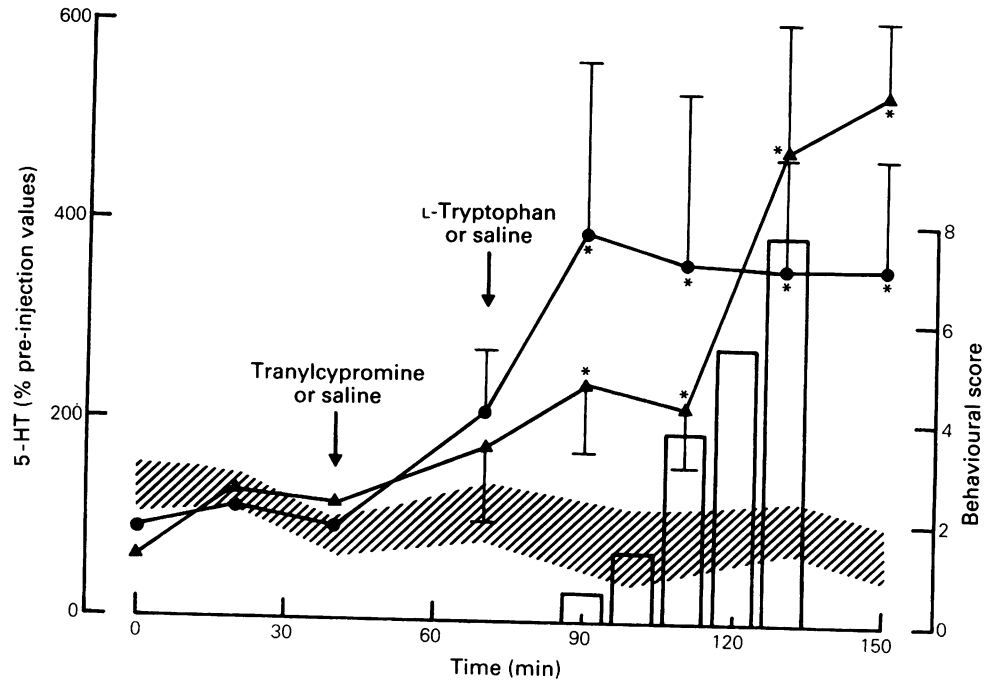

Figure 1 Effect of 0.9% saline plus 0.9% saline (hatched area, $n = 5$), tranylcypromine (20 mg kg^{-1}, i.p.) plus 0.9% saline (▲, $n = 5$) or tranylcypromine plus L-tryptophan (50 mg kg^{-1}, i.p.) (●, $n = 5$) on hypothalamic extracellular 5-hydroxytryptamine (5-HT). The 5-HT level is expressed as a percentage of the mean of the three pre-injection values ± s.e.mean. The mean pre-injection concentration of 5-HT was 38 ± 10 fmol 20 µl^{-1} in the dialysis perfusate in saline-treated animals, 21 ± 10 fmol 20 µl^{-1} in animals treated with tranylcypromine plus saline and 38 ± 12 fmol 20 µl^{-1} in animals treated with tranylcypromine and L-tryptophan. Tranylcypromine or saline was administered 30 min before L-tryptophan or saline. The open histogram columns represent the behavioural score (see methods for details) apparent after administration of L-tryptophan to tranylcypromine pretreated rats. The behavioural syndrome was not observed in control or tranylcypromine plus saline-injected rats.
*$P < 0.05$ compared to saline ± saline control animals.

Table 1 Effects of tranylcypromine (20 mg kg^{-1}), MDL 72394 (0.5 and 2 mg kg^{-1}), clorgyline (5 mg kg^{-1}), selegiline (10 mg kg^{-1}) and clorgyline plus selegiline followed by L-tryptophan (50 mg kg^{-1}) on monoamine oxidase (MAO)-A and -B activity in hypothalamic extracts

Drug	*MAO-A*	*MAO-B*
Control (0.9% saline)	63.3 ± 6.9	93.9 ± 9.39
Tranylcypromine	0 ± 0*	0 ± 0*
MDL 72394 (0.5 mg kg^{-1})	19.3 ± 11.3*	82.4 ± 12.2
MDL 72394 (2 mg kg^{-1})	0.31 ± 0.31*	16.9 ± 16.9*
Clorgyline	0.31 ± 0.6*	62.9 ± 28.2
Selegiline	48.1 ± 13.9	10.3 ± 6.6*
Clorgyline + selegiline	6.3 ± 4.4*	0 ± 0*

Tranylcypromine was given 30 min before L-tryptophan and the other inhibitors 2 h before L-tryptophan; 2 h later the rats were killed and MAO activity measured in the hypothalamus. Results are expressed as either nmol 5-HT oxidised mg^{-1} wet wt h^{-1} (MAO-A) or nmol phenylethylamine oxidised mg^{-1} wet wt h^{-1}.
*$P < 0.001$ compared to saline-injected controls.

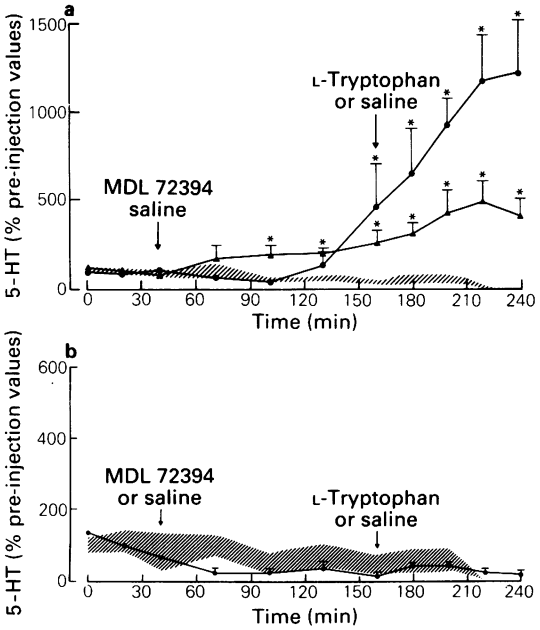

Figure 2 (a) Effects of MDL 72394 (2 mg kg^{-1}, i.p.) followed 2 h later by either 0.9% saline (▲, $n = 5$) or L-tryptophan (●, $n = 5$) on hypothalamic extracellular 5-hydroxytryptamine (5-HT) compared with rats given 0.9% saline on both occasions (hatched area, $n = 5$). The 5-HT levels are expressed as percentages of the mean of the three pre-injection control values ± s.e.mean. The mean pre-injection concentration of 5-HT was 20 ± 5 fmol 20 µl^{-1} in the dialysis perfusate in animals treated with MDL 72394 plus saline and 12 ± 4 fmol 20 µl^{-1} in animals treated with MDL 72394 plus L-tryptophan. *$P < 0.05$ compared to saline-injected controls. (b) Effects of MDL 72394 (0.5 mg kg^{-1}, i.p.) followed by L-tryptophan 50 mg kg^{-1}, i.p.) 2 h later (●, $n = 5$) on hypothalamic extracellular 5-HT levels compared with the effects of 0.9% saline (hatched area, $n = 5$). The data were analysed as in (a). The mean pre-injection concentration of 5-HT in the dialysis perfusate in animals treated with MDL 72394 + L-tryptophan was 44 ± 11 fmol 20 µl^{-1}. None of the treatment schedules in either (a) or (b) resulted in the appearance of any of the components of the behavioural score.

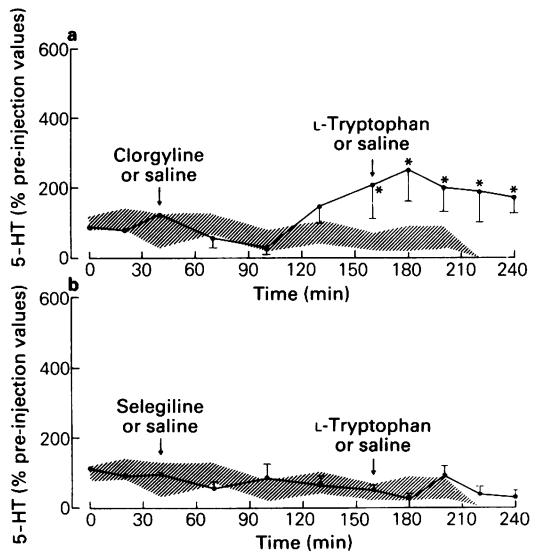

Figure 3 (a) Effect of clorgyline (5 mg kg^{-1}, i.p.) followed by L-tryptophan (50 mg kg^{-1}, i.p.) 2 h later (●, $n = 5$) on hypothalamic extracellular 5-hydroxytryptamine (5-HT) levels compared with the effects of 0.9% saline (hatched area, $n = 5$). The data were analysed as in Figure 2a. The mean pre-injection concentration of 5-HT in the dialysis perfusate in animals treated with clorgyline plus L-tryptophan was 28 ± 5 fmol 20 µl^{-1}. *$P < 0.05$ compared to saline-injected controls. (b) Effect of selegiline (10 mg kg^{-1}, i.p.) followed by L-tryptophan 2 h later (●, $n = 5$) on hypothalamic extracellular 5-HT levels compared with the effects of 0.9% saline (hatched area, $n = 5$). The data were analysed as in Figure 2a. The mean pre-injection concentration of 5-HT in the dialysis perfusate in animals treated with selegiline and L-tryptophan was 41 ± 7 fmol 20 µl^{-1}. None of the treatments scheduled in either (a) or (b) resulted in the appearance of any of the components of the behavioural score.

Selegiline plus L-tryptophan

Selegiline (10 mg kg^{-1}, i.p.) selectively inhibited MAO-B (Table 1) but addition of L-tryptophan (50 mg kg^{-1}, i.p.) 2 h later had no effect on extracellular 5-HT and failed to induce the behavioural syndrome (Figure 3b).

Selegiline plus clorgyline and L-tryptophan

Selegiline (10 mg kg^{-1}) plus clorgyline (5 mg kg^{-1}) significantly inhibited both MAO-A and -B activity (Table 1) and there was a significant increase in hypothalamic extracellular 5-HT prior to the administration of L-tryptophan 2 h after the MAO inhibitors (Figure 4); this was maintained and enhanced after L-tryptophan and the behavioural syndrome appeared within 20 min of L-tryptophan administration.

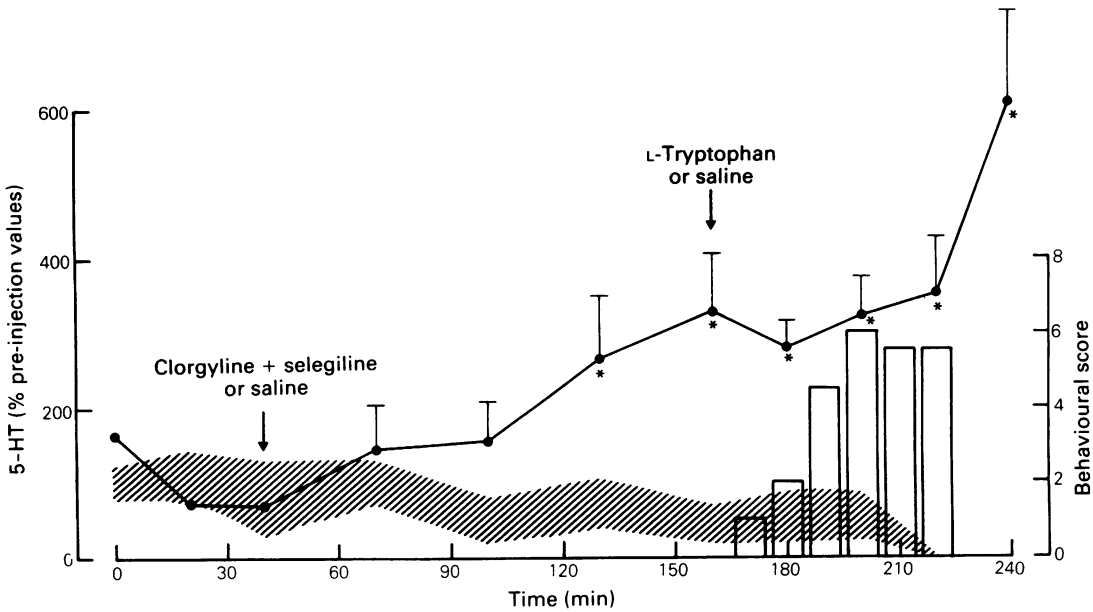

Figure 4 Effect of clorgyline (5 mg kg^{-1}, i.p.) plus selegiline (10 mg kg^{-1}, i.p.) followed 2 h later by L-tryptophan (50 mg kg^{-1}, i.p.) (●, $n = 5$) on hypothalamic extracellular 5-hydroxytryptamine (5-HT) levels compared with the effects of 0.9% saline (hatched area, $n = 5$). The data were analysed as in Figure 2a. The mean pre-injection concentration of 5-HT in the dialysis perfusate in animals treated with clorgyline plus selegiline plus L-tryptophan was 37 ± 7 fmol 20 µl^{-1}. *$P < 0.05$ compared to saline-injected controls. The open histogram columns represent the behavioural score observed after administration of clorgyline and selegiline followed by L-tryptophan.

Discussion

Earlier work has indicated that 5-HT is involved in the behavioural response following the administration of an MAO inhibitor and L-tryptophan (Hess & Doepfner, 1961; Grahame-Smith, 1971). Although brain levels of 5-HT increase following administration of an MAO inhibitor and L-tryptophan (Grahame-Smith, 1971) no previous studies have determined whether this behavioural syndrome is linked directly to an increase in 5-HT release *in vivo*. Similarly the roles of various pools of MAO in the control of extracellular 5-HT have not be studied. This paper, using the technique of intracranial dialysis to measure extracellular levels of 5-HT, (Ungerstedt, 1984; Sharp *et al.*, 1985; Brazell *et al.*, 1985) which, in turn, can be used as an index for the release of 5-HT from neurones, demonstrates that the 5-HT behavioural syndrome is associated with raised extracellular 5-HT but that raised extracellular 5-HT does not itself necessarily result in the syndrome. Administration of L-tryptophan alone had no effect on extracellular levels of either 5-HT or 5-HIAA, while complete inhibition of MAO-A and MAO-B by tranylcypromine, however, produced a large rise in extracellular 5-HT concentrations and a concurrent decrease in extracellular 5-HIAA but no behavioural syndrome. The subsequent administration of L-tryptophan to these animals induced the characteristic behavioural syndrome. Selective inhibition of MAO-A by clorgyline produced a slight increase in extracellular 5-HT though this was not as large as that seen in animals treated with tranylcypromine but rats pretreated with clorgyline did not display the behavioural syndrome when administered L-tryptophan. A neuronally selected dose of MDL 72394 (0.1 mg kg^{-1}) produced no change in extracellular 5-HT, however a large increase was seen following treatment with a non-selective dose of MDL 72394 (2 mg kg^{-1}) and L-tryptophan but the behavioural syndrome was not seen in either case. Selective inhibition of MAO-B by selegiline had no effect on extracellular 5-HT and the characteristic behavioural syndrome was not seen after administration of L-tryptophan.

MDL 72394 selectively inhibits the neuronal pool of MAO-A since inhibition of striatal MAO-A by MDL 72394 is attenuated by treatment with the neurotoxin,

6-hydroxydopamine (Palfreyman *et al.*, 1985). Treatment with a neuronally selective dose of MDL 72394 (0.5 mg kg^{-1}) did not alter extracellular 5-HT levels suggesting that the MAO-A contained in amine neurones does not play a major role in the regulation of extracellular 5-HT. Clorgyline inhibits all pools of MAO-A and following treatment with clorgyline we observed a small increase in extracellular 5-HT. These findings suggest that following its release, 5-HT is not necessarily metabolized by MAO-A in amine neurones but by MAO in non-amine containing cells; though at this stage we are unable to say what type of cells these are. The greatest increase in extracellular 5-HT was seen following inhibition of both MAO-A and MAO-B suggesting that although 5-HT is preferentially metabolized by MAO-A it can also be metabolized by MAO-B if MAO-A is inhibited. Consequently, both MAO-A and MAO-B are involved in the metabolism of released 5-HT, though the contribution of each type to the process may vary according to the conditions.

Previous work has suggested that the behavioural syndrome observed following the administration of MAO inhibitors and L-tryptophan can be linked to an increase in tissue levels of 5-HT. However, results presented here, suggest that the 5-HT behavioural syndrome cannot be explained purely on the basis of an increase in 5-HT release. Of all the experimental groups, the largest increase in extracellular 5-HT was seen following treatment with the non-selective dose of MDL 72394 (2 mg kg^{-1}) plus L-tryptophan; however, the behavioural syndrome was not observed in this group. The behavioural syndrome was only observed however, when extracellular 5-HT increased. Therefore the behavioural syndrome may be linked to an increase in 5-HT release, but may also be the result of a change in the extracellular levels of some other compound. This view is supported by the observation that the addition of L-tryptophan to rats pretreated with MDL 72394 (2 mg kg^{-1}) resulted in a further increase in extracellular 5-HT. This was not the case in rats pretreated with tranylcypromine and L-tryptophan, and the behavioural syndrome was only observed in rats receiving tranylcypromine plus L-tryptophan. In addition, there was no correlation between the magnitude of the rise in extracellular 5-HT and the intensity of the syndrome.

There is evidence, however, that the behavioural syndrome arises in the spinal cord and brain stem (Jacobs & Klemfuss, 1975). Since implantation of a dialysis probe into the rat spinal cord poses major technical problems, we have studied the effect of tranylcypromine and L-tryptophan on extracellular 5-HT levels in the frontal cortex. This treatment produced a rise in extracellular 5-HT of 431.7 ± 10.3% similar to that seen in the hypothalamus suggesting that following the various treatments with MAO inhibitors and L-tryptophan similar changes in 5-HT occur thoughout the brain.

The present results confirm those of Green & Youdim (1975) that complete inhibition of both MAO-A and MAO-B is required to produce the syndrome. Although MDL 72394 (2 mg kg^{-1}) fully inhibited MAO-A and inhibited MAO-B by 82%, this was not sufficient to produce the behavioural syndrome upon administration of L-tryptophan.

To summarise, our results suggest that although 5-HT may be associated with the behavioural syndrome it is not necessarily the only factor involved. Tricklebank *et al.* (1984) proposed that a catecholaminergic system may be involved. Inhibition of MAO-A and -B markedly increases brain tryptamine levels and tryptamine given to tranylcypromine pretreated rats produces the behavioural syndrome (Marsden & Curzon, 1978). Inhibition of peripheral decarboxylase activity has no effect on brain 5-HT but significantly reduces rat brain tryptamine levels, and locomotor and behavioural scores observed in rats given tranylcypromine and L-tryptophan. Increased brain tryptamine could increase the presynaptic release of 5-HT (Marsden & Curzon, 1979; Robinson & Marsden, 1984) and/or act directly on postsynaptic mechanisms (Luscombe *et al.*, 1982; Jones, 1982; Irons *et al.*, 1984).

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