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Increased total activity in the rat after L-tryptophan plus the monoamine oxidase-A inhibitor amiflamine but not after L-tryptophan plus clorgyline

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1 The effect of pretreatment with either saline or the monoamine oxidase-A inhibitors clorgyline and amiflamine upon the total activity, locomotion and rearing behaviour of the rat induced by various doses of the monoamine precursor L-tryptophan was studied by use of automated activity boxes.

2 Amiflamine (2.5 and 5.0 mg kg^{-1}, i.p.) increased in a dose-dependent manner total activity and to a lesser extent, locomotion when given 60 min before L-tryptophan (100 mg kg^{-1}, i.p.). The increased activity was seen after amiflamine plus either 25 or 75 mg kg^{-1} L-tryptophan. Rearing behaviour was not affected.

3 Analysis of 5-hydroxytryptamine (5-HT) and its deaminated metabolite 5-hydroxyindoleacetic acid (5-HIAA) by high performance liquid chromatography with electrochemical detection indicated that in both frontal cortex and hypothalamus, amiflamine (at both doses) increased 5-HT and reduced 5-HIAA concentrations. Combination of amiflamine with L-tryptophan (100 mg kg^{-1}, i.p.) resulted in a higher 5-HT concentration being found than after amiflamine alone. L-Tryptophan treatment alone did not change 5-HT concentrations but increased 5-HIAA concentrations.

4 Clorgyline, at a dose of either 1 or 5 mg kg^{-1} i.p. plus L-tryptophan (25 or 100 mg kg^{-1}, i.p.) did not increase total activity, locomotion or behaviour.

5 A number of possible explanations for the differences in the behavioural effects of clorgyline and amiflamine when given with L-tryptophan are discussed. It is concluded that in addition to monoamine oxidase-A inhibition, other pharmacological effects of the drugs, such as 5-HT release (amiflamine) and inhibition of tryptophan hydroxylation (clorgyline) may be of importance in determining the magnitude of the increase in activity when the compounds are given together with L-tryptophan.

Introduction

By use of both automated and observational techniques, it has been reported that pretreatment of rats with a monoamine oxidase inhibitor (MAOI) prior to the administration of L-tryptophan results in behavioural hyperactivity and expression of several components of the 5-hydroxytryptamine (5-HT) behavioural syndrome (Hess & Doepfner, 1961; Grahame-Smith, 1971; Jacobs, 1974; Green & Youdim, 1975; Marsden & Curzon, 1978). These behavioural responses have been assumed to reflect the increased functional activity at central 5-HT receptors (see e.g. Green & Grahame-Smith, 1976; Jacobs, 1976; Trulson & Jacobs, 1976).

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Monoamine oxidase, however exists as two catalytically active forms, termed MAO-A and MAO-B, where the activity of the A form is sensitive to inhibition by the acetylenic inhibitor clorgyline, and the activity of the B form sensitive to inhibition by (−)-deprenyl ((−)-selegiline) (Johnston, 1968; Knoll & Magyar, 1972). Initial studies performed *in vitro* demonstrated that 5-HT was a substrate for MAO-A alone (Johnston, 1968). However, more recent studies have demonstrated that whilst MAO-A predominated at low concentrations of 5-HT, a small (about 10%) deamination by MAO-B could be detected at high concentrations (Mitra & Guha, 1980; Fowler & Tipton, 1982). Such a conclusion had also been reached earlier by Green & Youdim (1975) on the

basis of behavioural studies. These authors showed that little or no potentiation of hyperactivity was found with L-tryptophan plus the MAO-A inhibitor clorgyline whereas L-tryptophan plus either the non selective MAO inhibitor tranylcypromine or both clorgyline plus the MAO-B selective inhibitor (−)-deprenyl gave considerable potentiation (Green & Youdim, 1975). However, both tranylcypromine and (−)-deprenyl (but not clorgyline) produce, in addition to their MAO inhibitory properties, an activation of dopaminergic neurones (Da Prada *et al.*, 1982), and dopamine agonists have been shown to produce hyperactivity in rats (see e.g. Beninger, 1983, for review).

The combination of an MAO inhibitor and L-tryptophan has been found to produce a greater percentage increase in brain tryptamine concentrations than in 5-HT concentrations (Marsden & Curzon, 1974; Weil-Malherbe, 1976; Warsh *et al.*, 1977). Subsequently, it was shown that the increased activity resulting from an MAO inhibitor plus L-tryptophan may have been due either to the elevated levels of tryptamine or due to a concomitant release of 5-HT (Marsden & Curzon, 1978; 1979). If this latter explanation is correct, then it would be predicted that a compound which has both MAO inhibitory and 5-HT releasing properties should potentiate activity when given with L-tryptophan to a greater extent than a compound which inhibits MAO but does not *per se* release 5-HT. Such a hypothesis has been tested by comparing the effects of two MAO-A selective inhibitors, namely clorgyline (which does not release 5-HT) (Johnston, 1968; Renyi, 1984) with amiflamine (which releases 5-HT) (Ögren *et al.*, 1981; Ask *et al.*, 1982; Renyi, 1984). An automated device consisting of boxes monitored by photocells has been used to register locomotion, rearing and general activity. In addition, the concentrations of 5-HT and its deaminated metabolite 5-hydroxyindoleacetic acid (5-HIAA) have been measured by high-performance liquid chromatography (h.p.l.c.) with electrochemical detection in the hypothalamus and frontal cortex after amiflamine and L-tryptophan administration.

Methods

Male Sprague-Dawley rats (Anticimex AB, Sollentuna, Sweden), body weight 350–400g were randomly allocated to the different treatment groups. They were housed in groups of three rats per cage under laboratory conditions with a 12 h on/12 h off lighting schedule in a thermostatically controlled room (21 ± 1°C).

Activity measurements

An automated device consisting of rat cages (40 × 25 × 15 cm) each placed within two series of infra-red beams (at different heights: one high and one

Table 1 The effect of L-tryptophan (100 mg kg^{-1}, i.p.) on locomotion and rearing counts from activity test boxes for rats pretreated with either saline or amiflamine (2.5 or 5.0 mg kg^{-1}, i.p.)

		Behavioural parameters measured over time intervals (min):				
Treatment	*Behaviour*	0–30	31–60	61–90	91–120	121–150
Saline + saline	Locomotion	203	32	54	20	8.1
	Rearing	1467	409	564	233	41
Saline + tryptophan (100)	Locomotion	225	40	53	24	23
	Rearing	1643	477	596	115	174
Amiflamine (2.5) + saline	Locomotion	147[a]	37	29	16	14
	Rearing	715[a]	296	395	164	201
Amiflamine (2.5) + tryptophan (100)	Locomotion	188[b]	50	62	58[c]	26
	Rearing	795[b]	323	139	204	68
Amiflamine (5.0) + saline	Locomotion	118[a]	36	38	25	29
	Rearing	269[a]	208	350	248	208
Amiflamine (5.0) + tryptophan (100)	Locomotion	167[b]	47	45	81[d]	61
	Rearing	430[b]	144	247	283	96

Rats were injected with amiflamine or saline immediately before they were placed in the test boxes. L-Tryptophan or saline was administered 60 min later. Values are expressed as means ($n = 8$). [a]$P < 0.01$ vs saline + saline group; [b]$P < 0.01$ vs saline + L-tryptophan group; [c]$P < 0.01$ vs amiflamine (2.5) + saline group; [d]$P < 0.01$ vs amiflamine (5.0) + saline group (Dunnett's test). Two-way ANOVA for locomotion, rearing, 5-HT and 5-HIAA (given in Table 2): $F(20,210) > 4.9$, $P < 0.01$.

low) was used (Rat-o-Matic, ADEA Elektronik AB, Uppsala, Sweden). The following parameters were measured:

Locomotion was registered by the low level grid of infra-red beams. Counts were registered only when the rats moved in a horizontal plane around the box.

Rearing was measured when the rats raised their front legs and/or rested on their haunches, with the upper part of the body breaking the high-level infra-red beams. Counts were registered during the period when at least one high level beam was broken, i.e. the number of counts were proportional to the time the rat spent rearing.

Activity was measured by a sensor (mounted on a lever with a counter-weight) with which the test box was in contact. The sensor registered all types of vibrations within the test box, such as those produced by locomotion, shaking (tremors) or grooming. Since locomotion contributes to activity counts, an increased activity (measured in this way) with little change in locomotion (measured in isolation as described above) may be assumed to reflect an increased incidence of tremors.

All three parameters were measured over 30 min periods. Thus, in the Tables and Figures, the total counts during the periods 0–30, 31–60, 61–90, 91–120, 121–150 and, where measured, 151–180 min are given.

Combination of amiflamine with L-tryptophan

Three experiments were performed. In the first experiment, groups ($n = 8$) of rats were treated either with saline or with amiflamine (2.5 or 5.0 mg kg^{-1}, i.p.) immediately before they were placed in the activity boxes. Sixty min later, either saline or L-tryptophan (100 mg kg^{-1}, i.p.) was injected and activity measurements continued over a further 90 min. Immediately after this observation period, the rats were removed from the activity boxes, killed by decapitation, and the brain regions used in the study (frontal cortex and hypothalamus) dissected out as described by Jonsson *et al.* (1982). The brain regions were then stored at − 70°C until used for assay of 5-HT and 5-HIAA concentrations as described below.

In the second experiment, groups ($n = 8$) of rats were given amiflamine (2.5 mg kg^{-1}, i.p.) immediately before they were placed in the activity boxes. Sixty min later, either saline or L-tryptophan (25, 75 and 100 mg kg^{-1}, i.p.) was injected and activity measurements continued over a further 120 min, after which time the animals were removed from the activity boxes and killed by decapitation.

In the third experiment, the groups and conditions were as for the second experiment, but with 5.0 mg kg^{-1} i.p. amiflamine instead of 2.5 mg kg^{-1}.

Combination of clorgyline with L-tryptophan

Groups ($n = 8$) of rats were injected with either saline or clorgyline (1.0 or 5.0 mg kg^{-1}, i.p.) immediately before they were placed in the activity boxes. Sixty min later, either saline or L-tryptophan (25 or 100 mg kg^{-1}, i.p.) was injected and activity measurements continued over a further 120 min, after which time the animals were removed from the activity boxes and killed by decapitation.

Measurement of 5-HT and 5-HIAA concentrations in the frontal cortex and hypothalamus

The dissected tissues from the first amiflamine experiment (see above) were homogenized by sonication in 0.5 ml of 0.1 M perchloric acid containing epinine as an internal standard. The concentrations of 5-HT and 5-HIAA in the homogenates were assayed by direct injection into an h.p.l.c. system with electrochemical

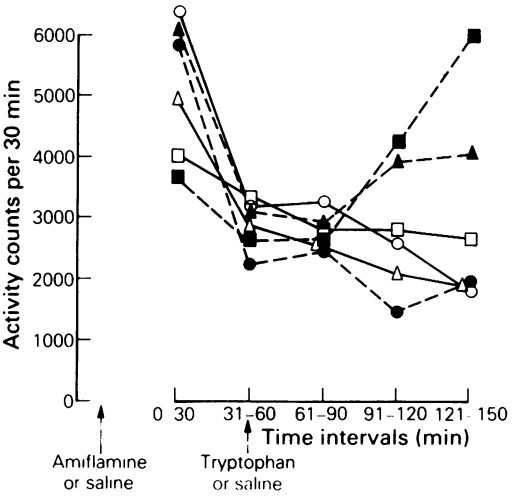

Figure 1 Total activity counts from the photocell activity test boxes at various intervals following amiflamine and/or L-tryptophan administration. Rats were injected with saline or amiflamine immediately before they were placed in the test boxes. Saline or L-tryptophan (100 mg kg^{-1} i.p.) was injected 60 min later. Values are mean activity counts for each treatment group. Treatment groups, each consisting of 8 rats, were: saline plus saline (○); amiflamine (2.5 mg kg^{-1} i.p.) plus saline (△); amiflamine (5.0 mg kg^{-1} i.p.) plus saline (□); saline plus L-tryptophan (●); amiflamine (2.5 mg kg^{-1} i.p.) plus L-tryptophan (▲); amiflamine (5.0 mg kg^{-1}, i.p.) plus L-tryptophan (■). Two-way analysis of variance for activity data: $F(20, 210) = 10.3$, $P < 0.01$. Significant differences: 91–120 min and 121–150 min data ▲ > △; ■ > □; ▲, ■ > ● (Dunnett's test).

detection essentially as described earlier (Magnusson *et al.*, 1980). The mobile phase of the chromatographic column (Nova Pak C18, Waters Associates) was citrate buffer, pH 4.0, containing 1.2 mM hexyl sulphate and 8% methanol. The detector potential was set at 600 mV vs an Ag/AgCl reference electrode. Tissue 5-HT and 5-HIAA concentrations are expressed as pmol mg^{-1} wet weight.

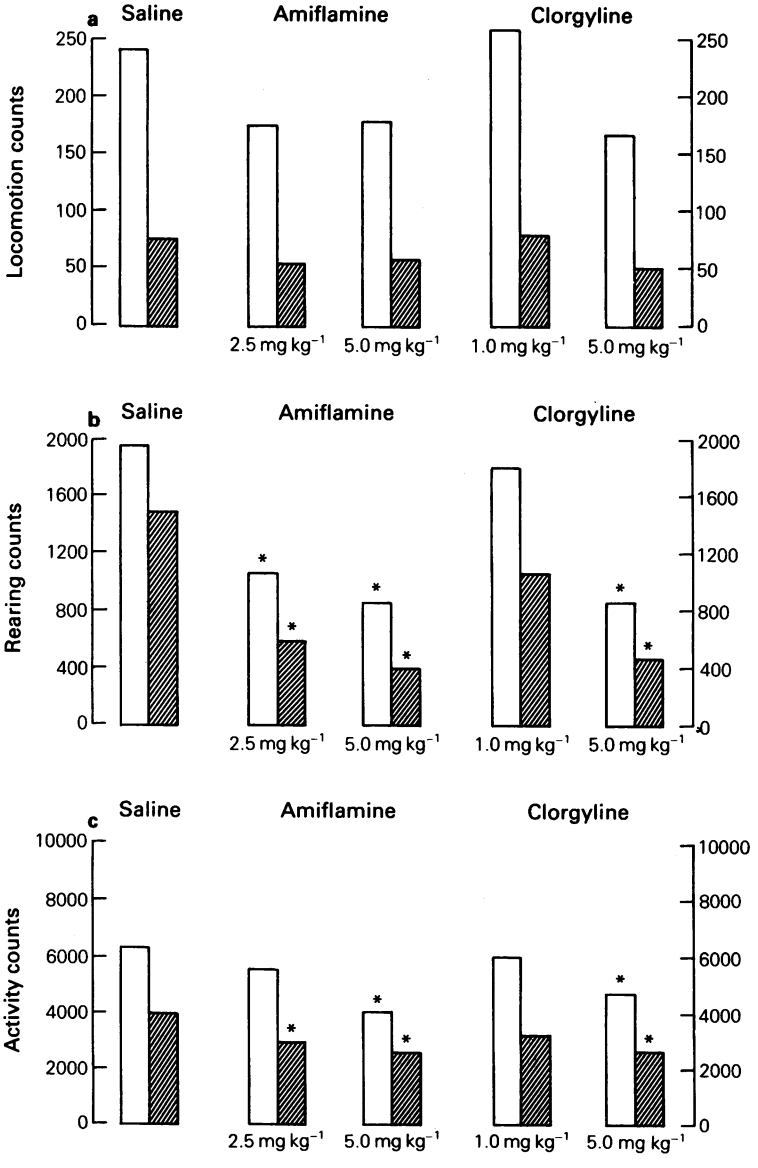

Figure 2 (a) Locomotion (b) rearing and (c) activity counts from the activity test boxes over the first 60 min following amiflamine and clorgyline administration. Rats were injected i.p. with either saline, amiflamine or clorgyline at the given doses and placed in the activity boxes. The columns represent the mean values for 0–30 (open columns) and 31–60 min (hatched columns) for all the animals tested ($n = 32$ for saline and amiflamine, $n = 24$ for clorgyline) in the experiments presented in Tables 1 and 2 and Figures 1, 3, 4 and 5, since the L-tryptophan was not administered until after the first 60 min. $^*P < 0.01$ with respect to the saline-treated rats. (Dunnett's test).

Table 2 The effect of L-tryptophan (100 mg kg^{-1}, i.p.) on 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations in the frontal cortex and hypothalamus of rats pretreated with either saline or amiflamine (2.5 or 5.0 mg kg^{-1}, i.p.)

Treatment	*5-HT and 5-HIAA concentrations* (pmol mg^{-1} wet weight)			
	Frontal cortex		*Hypothalamus*	
	5-HT	*5-HIAA*	*5-HT*	*5-HIAA*
Saline + saline	5.2 ± 0.40 (100%)	5.8 ± 0.37 (100%)	4.0 ± 0.12 (100%)	2.4 ± 0.16 (100%)
Saline + tryptophan (100)	6.5 ± 0.64 (124%)	11.8 ± 0.55[a] (205%)	4.5 ± 0.32 (114%)	4.0 ± 0.29[a] (164%)
Amiflamine (2.5) + saline	13.7 ± 0.74[a] (262%)	2.1 ± 0.26[a] (36%)	7.7 ± 0.33[a] (193%)	0.71 ± 0.11[a] (29%)
Amiflamine (2.5) + tryptophan (100)	16.2 ± 0.89[b,c] (309%)	3.8 ± 0.56[b] (66%)	9.4 ± 0.65[b,c] (238%)	1.1 ± 0.18[b] (46%)
Amiflamine (5.0) + saline	14.1 ± 0.52[a] (269%)	1.8 ± 0.30[a] (31%)	7.2 ± 0.31[a] (181%)	0.77 ± 0.07[a] (32%)
Amiflamine (5.0) + tryptophan (100)	17.8 ± 0.59[b,d] (340%)	2.6 ± 0.43[b] (45%)	10.2 ± 0.41[b,d] (256%)	0.72 ± 0.08[b] (30%)

The rats were treated as described in Table 1. The animals were killed within 20 min after removal from the test boxes (for the behavioural testing given in Table 1 and Figure 1) and frontal cortices and hypothalami removed as described in Methods. Values are expressed as means ± s.e.mean ($n = 8$). Figures in parentheses indicate the 5-HT or 5-HIAA concentrations as a percentage of the saline + saline group mean concentrations. [a]$P < 0.01$ vs saline + saline group; [b]$P < 0.01$ vs saline + L-tryptophan group; [c]$P < 0.01$ vs amiflamine (2.5) + saline group; [d]$P < 0.01$ vs amiflamine (5.0) + saline group (Dunnett's test).

Statistical analysis

The data in all cases were analysed for significance by use of an unsystematic two-way ANOVA (Snedecor & Cochran, 1967). Testing for significance between group pairs were performed with Dunnett's two-tailed *t* test (Kirk, 1968). Significance was taken in all cases at the level of $P < 0.01$. Thus, the statistical tests used were relatively conservative, but this was because of the considerable variability of the parameters (particularly rearing) from animal to animal. This variance explains why, in the Tables, there are considerable differences in some mean values but the differences do not reach significance.

Compounds used

Amiflamine (FLA 336(+), (S)-(+)-4-dimethylamino-2, α-dimethyl phenethylamine) tartrate and clorgyline hydrochloride were synthesized by Dr L. Florvall, Astra Läkemedel AB, Södertälje, Sweden. L-Tryptophan HCl was purchased from Fluka AG, Switzerland. Amiflamine and clorgyline were dissolved in 0.9% w/v NaCl solution (saline) at room temperature, L-tryptophan at 40°C. All other reagents were standard laboratory reagents of analytical grade wherever possible.

Results

Combination of amiflamine (2.5 or 5.0 mg kg^{-1}) with L-tryptophan (100 mg kg^{-1}, i.p.)

Administration to rats of L-tryptophan (100 mg kg^{-1} i.p.) had little or no effect on the locomotion, rearing or total activity parameters when compared with saline-treated controls (Table 1, Figure 1). Similarly, the two doses of amiflamine tested (2.5 and 5.0 mg kg^{-1}, i.p.) had little or no effect on locomotion, rearing or activity (Table 1, Figure 1), apart from during the initial acclimatisation period, where both doses of amiflamine decreased rearing over the first 30 min and decreased rearing and activity but not locomotion over the next 30 min (Figure 2).

Rats that received L-tryptophan (100 mg kg^{-1}, i.p.) in combination with amiflamine (2.5 or 5.0 mg kg^{-1}, i.p.) showed a considerably greater level of activity than rats treated with either L-tryptophan or amiflamine alone. The activity increase was most evident

60 and 90 min after the L-tryptophan injection and was greater for 5.0 mg kg^{-1} than for 2.5 mg kg^{-1} amiflamine (Figure 1). The amiflamine plus L-tryptophan combination also produced an increase in locomotion counts 60 min after the L-tryptophan injection, whereas no effects upon rearing were observed (Table 1).

The concentrations of 5-HT and its deaminated metabolite 5-HIAA in the hypothalamus and frontal cortex of the rats found 90 min after the L-tryptophan (and 150 min after amiflamine) administration are given in Table 2. L-Tryptophan alone increased 5-HIAA levels in both regions without effect upon the 5-HT levels, a finding consistent with the notion of an increased 5-HT turnover (with respect to the saline-treated rats) being found after L-tryptophan administration. Amiflamine alone produced a dose-dependent increase in 5-HT levels together with a concomitant decrease in 5-HIAA levels, consistent with inhibition of 5-HT deamination in these animals. The combination of amiflamine and L-tryptophan potentiated the increase in 5-HT produced by amiflamine alone in both tissues.

Combination of amiflamine (2.5 mg kg^{-1}, i.p.) with various doses of L-tryptophan

Rats that received the highest dose of L-tryptophan (100 mg kg^{-1}, i.p.) in combination with amiflamine (2.5 mg kg^{-1}, i.p.) showed significantly more activity than the rats that received amiflamine alone (Figure 3a), in agreement with the first experiment (see Figure 1). The effect was noted from 90 min after the L-tryptophan administration. The increase in locomotion by the amiflamine plus L-tryptophan (100 mg kg^{-1}) group was noted 60 min after the L-tryptophan administration (Figure 3b). No effects upon rearing behaviour were noted (Figure 3c). The combination of amiflamine with lower doses of L-tryptophan (75 and 25 mg kg^{-1}, i.p.) produced similar activity, locomotion and rearing characteristics to the rats given amiflamine alone (Figure 3a–c).

Combination of amiflamine (5.0 mg kg^{-1}, i.p.) with various doses of L-tryptophan

When the higher dose of amiflamine was used, all three L-tryptophan dose groups showed significantly more activity than the rats that received amiflamine alone. The activity increase was noted from 60 min after the L-tryptophan injection for the amiflamine plus tryptophan (100 mg kg^{-1}) group, and from 90 min for the other two groups (Figure 4a). A significant increase in locomotion was obtained for the amiflamine plus L-tryptophan (100 mg kg^{-1}, i.p.) group from 60 to

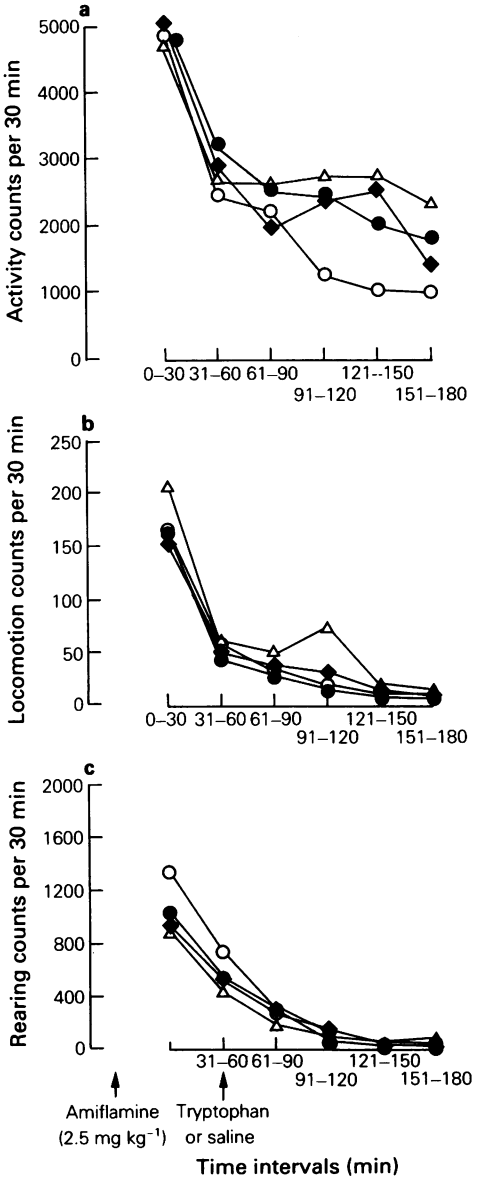

Figure 3 (a) Total activity (b) locomotion and (c) rearing counts from the activity test boxes at various intervals following amiflamine and L-tryptophan administration. Rats were injected with 2.5 mg kg^{-1} i.p. amiflamine immediately before they were placed in the test boxes and either saline (○) or 25 mg kg^{-1} (●), 75 mg kg^{-1} (◆) or 100 mg kg^{-1} (△) L-tryptophan injected 60 min later. Values are mean counts for each treatment group ($n = 8$). Two-way analysis of variance for activity data: $F(15, 162) = 1.87$, $P < 0.05$. Significant differences with respect to the amiflamine-saline group ($P < 0.01$, Dunnett's test): (a) △ > ○ at 121–150 min interval; (b) △ > ○ at 91–120 min interval.

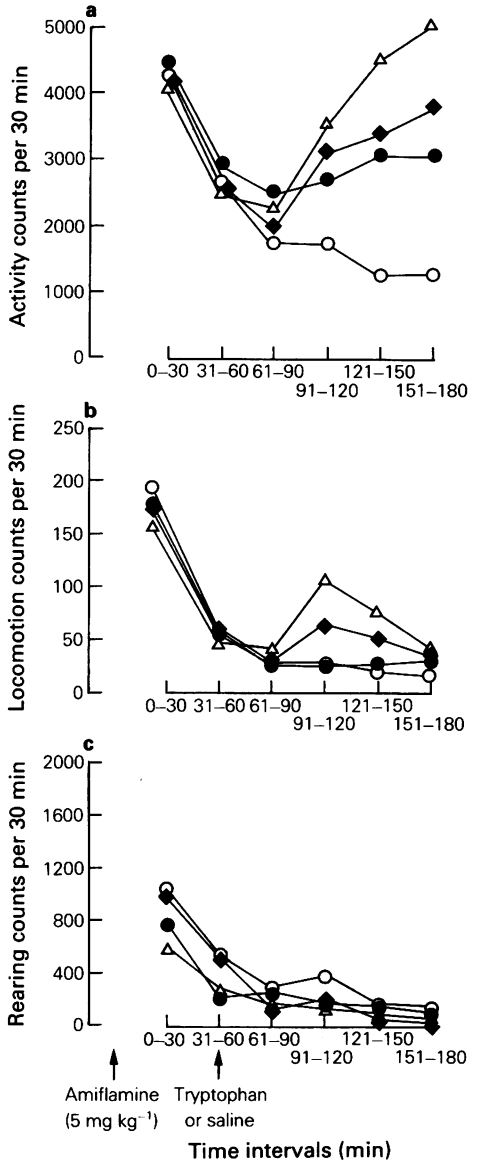

Figure 4 (a) Total activity, (b) locomotion and (c) rearing counts from the activity test boxes at various intervals following amiflamine and L-tryptophan administration. Rats were injected with 5 mg kg^{-1} i.p. amiflamine immediately before they were placed in the test boxes and either saline (○) or 25 mg kg^{-1} (●), 75 mg kg^{-1} (◆) or 100 mg kg^{-1} (△) L-tryptophan injected 60 min later. Values are mean counts for each treatment group ($n = 8$). Two-way analysis of variance for activity data: $F(15, 168) = 7.8$, $P < 0.01$. Significant differences with respect to the amiflamine-saline group ($P < 0.01$, Dunnett's test): (a) △ > ○ at 91–120 min interval; ●, ◆, △ > ○ at 121–150 and 151–180 min intervals; (b) △ > ○ at 91–120 and 121–150 min intervals.

90 min after the L-tryptophan administration (Figure 4b). No effects on rearing behaviour were noted (Figure 4c).

Combination of clorgyline with L-tryptophan

Clorgyline was administered at two doses: 1 mg kg^{-1} and 5 mg kg^{-1}, i.p. The 1 mg kg^{-1} dose was without effect on any of the behavioural parameters measured over the first 60 min, whereas rearing and activity (but not locomotion) were decreased during this period after the 5 mg kg^{-1} dose (Figure 2). The combination of L-tryptophan, either at 25 or 100 mg kg^{-1}, i.p., with either dose of clorgyline did not produce any significant increase in rearing, locomotion or activity measures of spontaneous behaviour (data not shown). A small, non-significant increase of total activity was noted at the 91–120, 121–150 and 151–180 min intervals following the combination of clorgyline (5 mg kg^{-1}, i.p.) with L-tryptophan (100 mg kg^{-1}, i.p.) (Figure 5), whereas rearing behaviour was slightly decreased.

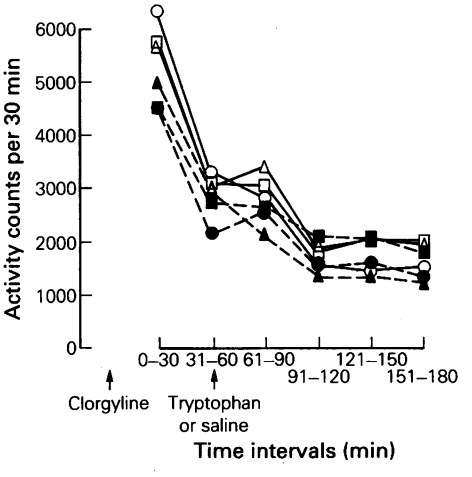

Figure 5 Total activity counts from the activity test boxes at various intervals following clorgyline and L-tryptophan administration. Rats were injected with clorgyline i.p. immediately before placement in the test boxes. Saline or L-tryptophan were injected 60 min later. Values are means ($n = 8$) for the following groups: clorgyline (1 mg kg^{-1}) plus saline (○); clorgyline (1 mg kg^{-1}) plus L-tryptophan (25 mg kg^{-1}) (△); clorgyline (1 mg kg^{-1}) plus tryptophan (100 mg kg^{-1}) (□); clorgyline (5 mg kg^{-1}) plus saline (●); clorgyline (5 mg kg^{-1}) plus L-tryptophan (25 mg kg^{-1}) (▲); clorgyline (5 mg kg^{-1}) plus L-tryptophan (100 mg kg^{-1}) (■). In no case were the values for the L-tryptophan-treated animals significantly different from the respective clorgyline-saline controls. ($P > 0.01$, Dunnett's test).

Discussion

From the data presented, it can be seen that the combination of amiflamine at either 2.5 mg kg^{-1} or 5.0 mg kg^{-1} i.p. with a high dose of L-tryptophan (100 mg kg^{-1} i.p.) produced notable increases in general activity and, to a lesser extent, locomotion. Amiflamine, by itself, produced significant decreases in locomotion, rearing and activity over the initial 60 min in the test boxes, but did not exert effects over the later time intervals. Thus amiflamine, despite its structural similarity to amphetamine, does not have any amphetamine-like effects on locomotion, rearing and activity. The combination of clorgyline (1.0 or 5.0 mg kg^{-1}, i.p.) and L-tryptophan did not produce potentiation of the activity or locomotion behaviour, in agreement with an earlier study (Green & Youdim, 1975).

From the data presented in Figures 1, 3 and 4 and Table 1, it may be noted that the increase in general activity found after the amiflamine plus L-tryptophan combination was far greater than the increase in locomotion. A large number of activity counts relative to locomotion counts reflects a measure of tremors and other 5-HT related behaviours. Thus, the data are consistent with the assumption that the activity increase found with amiflamine plus L-tryptophan was due to a large increase in the number of tremors observed. This is in agreement with the data of Jalfre *et al.* (1982) who demonstrated that a number of MAO inhibitors are able to potentiate the tremorogenic activity of L-tryptophan (200 mg kg^{-1} i.p.) in mice. These authors found clorgyline active in their test, but only at fairly high doses (ED_{50} value 42 mg kg^{-1} orally, compared with an ED_{50} value for antagonism of reserpine-induced ptosis in mice of 11 mg kg^{-1} orally) (Jalfre *et al.*, 1982).

A number of hypotheses have been put forward to explain the potentiation of behaviour produced by the combination of L-tryptophan and monoamine oxidase inhibitors. It has been suggested that the potentiation is due to accumulation of unmetabolised 5-HT but that both forms of MAO need to be inhibited before such accumulation is found (Green & Youdim, 1975). Alternatively, it has been suggested that some of the behavioural effects may be due to tryptamine involvement (Marsden & Curzon, 1978) whereby the increased tryptamine concentration found after treatment with L-tryptophan and an MAO inhibitor acts either directly on central 5-HT receptors or releases 5-HT (Marsden & Curzon, 1978, 1979). However, neither hypothesis *per se* can account for the difference between the results found with amiflamine and clorgyline, since both compounds are MAO-A selective inhibitors which, at the doses used, produce a similar degree of inhibition of MAO-A with little or no inhibition of MAO-B activity (see Green & Youdim, 1975; Ask *et al.*, 1982). It is thus clear that amiflamine and clorgyline must possess properties in addition to their MAO-A inhibitory properties which account for their different effects on activity when given in combination with L-tryptophan.

Amiflamine has been shown to exert its inhibitory actions on 5-HT deamination by selectively being taken up into 5-hydroxytryptaminergic nerve terminals by the neuronal uptake mechanisms (Ask *et al.*, 1983). This is found in rats with an ED_{50} of 0.4 mg kg^{-1} given orally, whereas MAO-A localised elsewhere (such as within noradrenergic neurones or extra-neuronally) is inhibited with an ED_{50} of > 1.4 mg kg^{-1} orally (Ask *et al.*, 1983). At doses of amiflamine of ca 3.5 mg kg^{-1} orally and higher, there appears to be a concomitant release of 5-HT from the neurones (Ögren *et al.*, 1981; Renyi, 1984). Thus, for the 5.0 mg kg^{-1} i.p. dose of amiflamine used in the present study, there is not only inhibition of both intra- and extraneuronal MAO-A but 5-HT release as well. Clorgyline, on the other hand, does not release 5-HT (Ögren *et al.*, 1981; Renyi, 1984). Thus, it is possible that the large potentiation of activity found after 5 mg kg^{-1} amiflamine plus L-tryptophan is a result of both MAO-A inhibition and 5-HT release produced by the amiflamine. Such a suggestion is in line with the study of Ashkenazi *et al.* (1983) who demonstrated that clorgyline *per se* did not induce the hyperactivity syndrome, whereas clorgyline plus LM 5008 (a selective 5-HT re-uptake inhibitor) did produce the syndrome. Thus for amiflamine plus L-tryptophan there is an increased extraneuronal 5-HT availability due to MAO-A inhibition and 5-HT release, and in clorgyline plus L-5008 due to MAO-A inhibition and inhibition of 5-HT reuptake.

From the above discussion, it would be expected that similar results would be found when amiflamine or clorgyline are given in combination with 5-hydroxytryptophan instead of L-tryptophan. This does not appear to be the case, since Ögren *et al.* (1981) have found that both amiflamine and clorgyline potentiate with roughly similar potencies head-twitch, tremor and abduction behaviours when given together with 5-hydroxytryptophan (90 mg kg^{-1}, i.v.). Waldmeier *et al.* (1981) have demonstrated that an acute dose of clorgyline (0.3 mg kg^{-1}, s.c.) decreases the formation of 5-hydroxytryptophan in the corpus striatum but not the brain stem of rats treated with the L-aromatic amino acid decarboxylase inhibitor, Ro-4-4602. Repeated administration with this dose of clorgyline for 7 days resulted in a decreased production of 5-hydroxytryptophan in the cortex, hippocampus and brain stem as well as the corpus striatum (Waldmeier *et al.*, 1981). Such findings are consistent with the hypothesis that the lack of potentiation of activity with clorgyline plus L-tryptophan is due to inhibition of striatal tryptophan hydroxylase, although this may not explain why

other MAO-A inhibitors, such as MD 780515, have weak potencies when given with L-tryptophan towards antagonism of reserpine-induced ptosis (Jalfre *et al.*, 1982).

In conclusion, it is apparent that none of the explanations listed above alone explains all the observed effects of clorgyline and amiflamine. It seems most likely that a combination of the 5-hydroxytryptamine releasing properties of amiflamine, the tryptophan hydroxylating inhibitory properties of clorgyline, and the monoamine oxidase-A inhibitory properties of both compounds explain the differences between the activity potentiating properties of clorgyline and amiflamine when given in combination with L-tryptophan.

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