Br. J. Pharmac. (1978), **64**, 341–345

THE INFLUENCE OF L-TRYPTOPHAN AND MONOAMINE OXIDASE INHIBITORS ON CATECHOLAMINE METABOLISM IN RAT BRAIN

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1 L-Tryptophan (100 mg/kg) was administered to rats with or without pretreatment with a monoamine oxidase inhibitor and the concentration of 5-hydroxyindoleacetic acid, homovanillic acid, dihydroxyphenylacetic acid, 3-methoxy 4-hydroxyphenyl glycol, normetanephrine, noradrenaline and dopamine measured in whole brain one hour later.

2 L-Tryptophan increased the concentration of 5-hydroxyindoleacetic acid, homovanillic acid, dihydroxyphenylacetic acid, 3-methoxy 4-hydroxyphenyl glycol and normetanephrine. The concentration of noradrenaline did not change whilst that of dopamine increased significantly.

3 In animals pretreated chronically with a monoamine oxidase inhibitor, tryptophan increased the concentration of dihydroxyphenylacetic acid and homovanillic acid compared to monoamine oxidase alone.

4 The results suggest either a release of dopamine and noradrenaline by 5-hydroxytryptamine, with a compensatory increase in their synthesis, or an increase in the firing of dopaminergic and noradrenergic neurones after L-tryptophan.

Introduction

The biogenic amine theory of the cause of affective illness remains the most attractive of current hypotheses. This states that mood is controlled by one or more of the neuronal systems which have the biogenic amines as their transmitter. Pathological change in neurotransmission in these systems either by way of altered transmitter release (Schildkraut, 1965) or a changed receptor sensitivity (Susler, Ventulani & Mobley, 1978) leads to the development of the symptoms seen in affective illness. There is still speculation as to the relative importance of the respective amines, noradrenaline (NA), 5-hydroxytryptamine (5-HT) and even dopamine. One piece of evidence in favour of 5-HT is the efficacy of the combination of L-tryptophan and a monoamine oxidase inhibitor (MAOI) in the treatment of depressive illness (for review see Murphy, Baker, Kotin & Bunney, 1973). Since L-tryptophan is the precursor of 5-HT and capable of causing an increase in turnover of this amine in brain (Ashcroft, Eccleston & Crawford, 1965) it is reasonable to assume that its antidepressant properties (Coppen, Shaw & Farrell, 1963) are mediated by way of the 5-HT neuronal system. However, evidence from animal experiments suggests that the amine systems do not function in isolation but are mutually interactive (see for example, Costall & Naylor, 1974). Our own unpublished observations in patients suggested that the combination of L-tryptophan and phenelzine produced symptoms in relation to subjective feelings of alertness and activity which suggested an amphetamine-like effect. Consequently we investigated, in rats, the result of the combination of tryptophan and phenelzine, not only on the turnover of 5-HT, but also on that of dopamine and NA.

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Methods

Drug administration

Male albino Wistar strain rats (weight about 200 g) were used. In the acute experiments phenelzine (20 mg/kg i.p.) was administered alone or 60 min before administration of L-tryptophan (100 mg/kg i.p.). The animals were killed by cervical fracture 1 h after tryptophan or 2 h following phenelzine. In the experiments on chronic treatment, animals were injected daily with phenelzine (10 mg/kg i.p.) for 15 days before a single dose of L-tryptophan (100 mg/kg i.p.) on the last day, 1 h after the last dose of the MAOI. The animals were killed 1 h after L-tryptophan. In

the experiment which examined normetanephrine concentrations, a single dose of pargyline (100 mg/kg) was given. Saline (0.9% w/v NaCl solution) was administered as a control for all injections.

Biochemical determinations

The rats were killed, decapitated and the whole brain removed. Each brain was treated separately and protein was precipitated with 0.4 N perchloric acid. The acid and neutral metabolites were separated from amines on a weak cation exchange resin as described by Eccleston, Ashcroft, Crawford & Loose (1966). Homovanillic acid (HVA), dihydroxyphenylacetic acid (DOPAC), 3-methoxy 4-hydroxyphenylglycol (MHPG), 5-hydroxyindoleacetic acid (5-HIAA) and tryptophan were assayed in the effluent from this column. 5-HT was measured in the eluate by the method of Bogdanski, Pletscher, Brodie & Udenfriend (1956), tryptophan was estimated by the procedure of Hess & Udenfriend (1959) and 5-HIAA by the spectrofluorimetric method of Ashcroft & Sharman (1962). HVA and DOPAC were estimated by the gas chromatographic technique of Pearson & Sharman (1975) and MHPG by the gas chromatographic method of Walter & Eccleston (1973). Normetanephrine was estimated by a modification of the radio-labelling method of Saavedra & Axelrod (1973).

Statistics

Student's t test was the statistical test used unless otherwise stated.

Results

Single administration of monoamine oxidase inhibitors and L-tryptophan

When a single dose of phenelzine was given there was a fall in the brain concentration of the amine metabolites 5-HIAA, HVA, DOPAC and MHPG (Table 1). Tryptophan alone produced a significant rise in the concentration of 5-HIAA, DOPAC and MHPG in brain. When the two drugs were given in combination, then the 5-HIAA concentration was greater than that occurring after phenelzine alone. Paradoxically the concentration of normetanephrine did not rise after administration of phenelzine (Table 2). A single dose of pargyline (100 mg/kg), on the other hand, gave a significant rise in the concentration of normetanephrine. L-Tryptophan (100 mg/kg) produced no change (Figure 1) in brain NA concentration but did produce a significant rise in the concentration of dopamine 1 h after administration.

Repeated administration of monoamine oxidase inhibitors

Phenelzine administered over a period of 15 days produced a significant fall in the concentration of 5-HIAA, HVA, DOPAC and MHPG in the brain (Table 3). Tryptophan (100 mg/kg) given after chronic administration of saline significantly increased the concentration of 5-HIAA, HVA, DOPAC and MHPG. In contrast to the effects of acute phenelzine, chronic administration of this drug followed by L-tryptophan produced a significant rise in DOPAC concentrations compared with phenelzine administra-

Table 1 The concentration of 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA), dihydroxyphenylacetic acid (DOPAC) and 3-methoxy 4-hydroxyphenyl glycol (MHPG) in rat brain following acute treatment with phenelzine or L-tryptophan

Treatment	*5-HIAA*	*HVA*	*DOPAC*	*MHPG*
Control	210 ± 10 (6)	144 ± 23 (13)	167 ± 15 (6)	69 ± 11 (5)
Phenelzine	90 ± 10 (4)**	74 ± 8 (4)**	61 ± 18 (4)**	21 ± 2 (3)**
Tryptophan	410 ± 50 (6)**	168 ± 31 (6)	219 ± 50 (6)*	94 ± 18 (6)*
Tryptophan plus phenelzine	140 ± 10 (4)†	99 ± 19 (4)	67 ± 17 (6)	20 ± 3 (4)

Amine transmitter metabolite concentrations (mean ± s.d.*(n)* ng/g) after phenelzine 20 mg/kg and/either tryptophan (100 mg/kg) or saline 1 h later. The animals were killed 1 h after tryptophan or saline administration.
Significantly different from the controls: * $P < 0.05$; ** $P < 0.001$. Significantly different from phenelzine alone: † $P < 0.001$.

Table 2 Rat brain normetanephrine concentration following acute or chronic monoamine oxidase inhibition or L-tryptophan

Treatment	*Normetanephrine*
Control	50.4 ± 8.9 (13)
Single dose phenelzine	49.7 ± 10.6 (5)
Single dose pargyline	62.8 ± 6.0 (7)*
Multiple dose phenelzine	63.6 ± 3.2 (8)**
Tryptophan	69.9 ± 7.1 (4)*
Single dose phenelzine plus tryptophan	41.9 ± 8.4 (6)

Concentration of normetanephrine (mean ± s.d.*(n)* ng/g) in whole brain following either acute (20 mg/kg) or a 15 day administration of phenelzine (10 mg/kg) or a single dose of pargyline (100 mg/kg). The animals were killed 2 h later. L-Tryptophan (100 mg/kg) was administered either alone or 1 h after phenelzine (20 mg/kg) the animals were killed 1 h later.

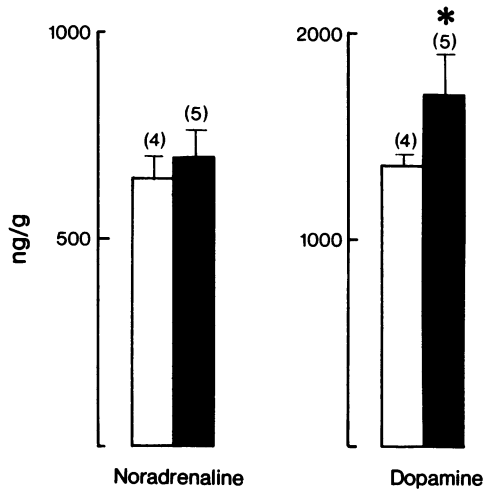

Figure 1 Concentration of noradrenaline and dopamine (ng/g) in whole brain 1 h following the administration of L-tryptophan (100 mg/kg); open columns: control; closed columns: after L-tryptophan. *$P < 0.05$.

tion alone. Analysis of variance applied to the data for HVA and DOPAC showed that phenelzine and L-tryptophan gave a significant rise ($P < 0.005$) in the concentration of these metabolites when compared with phenelzine alone. Normetanephrine concentrations significantly increased following treatment with phenelzine for fifteen days (Table 2).

Discussion

In animals the administration of a combination of L-tryptophan and a monoamine oxidase inhibitor leads to a characteristic behavioural syndrome (Hess & Doepfner, 1961; Grahame-Smith, 1971), which in rats may be subdivided into various components including hyperactivity, hyper-reactivity, reciprocal forepaw treading, headweaving and hind limb abduction. A dopaminergic component of the syndrome has been proposed (Green & Grahame-Smith, 1974) on the basis of the inhibition of the syndrome by pretreatment of the animals with α-methyl-*p*-tyrosine, a drug which blocks the synthesis of dopamine. However, Crow & Deakin (1977) suggested that the various facets of the syndrome (forepaw treading, headweaving and hind limb abduction) may be trypt-

Table 3 The concentration of monoamine metabolites in rat brain following chronic phenelzine administration with or without L-tryptophan

Treatment	*5-HIAA*	*HVA*	*DOPAC*	*MHPG*
Control	220 ± 10(3)	143 ± 18(6)	168 ± 15(6)	70 ± 14(3)
Phenelzine	60 ± 10(3)***	68 ± 13(3)***	62 ± 12(3)***	12 ± 1(3)***
Tryptophan	450 ± 50(3)***	177 ± 19(6)**	220 ± 25(6)***	100 ± 7(3)*
Tryptophan plus phenelzine	80 ± 10(3)	90 ± 13(3)	117 ± 20†(3)	16 ± 2(3)

Amine transmitter metabolite concentration (mean ± s.d.*(n)* ng/g) after phenelzine (10 mg/kg) for 15 days either with a single dose of L-tryptophan 100 mg/kg, or saline, 1 h later. Animals were also killed 1 h after a single dose of L-tryptophan. Metabolite abbreviations as shown in legend to Figure 1. Significantly different from control: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$. Significantly different from phenelzine: † $P < 0.025$.

aminergic, since they are abolished by administration of the 5-HT receptor blocking drug, methergoline, while the hyperactivity, which they believe is a separate part of the syndrome is either unchanged or increased. Deakin & Green (1978) also demonstrated the inhibition of part of the syndrome by the 5-HT antagonists methergoline and methysergide. In addition they found that propranolol, when given before tryptophan and tranylcypromine, blocked all components of the syndrome. It has been suggested that propranolol is a 5-HT antagonist (Middlemiss, Blakeborough & Leather, 1977) because of its ability to inhibit binding of 5-HT to rat brain synaptic membranes with a potency equivalent to that of methysergide.

Deakin & Green (1978) made specific chemical lesions of the 5-HT neurones in the spinal cord, and administered a 5-HT receptor agonist when sufficient time had elapsed for supersensitivity of the receptors to develop. Because headweaving, forepaw treading and limb abduction were increased in these animals they concluded that these facets of the syndrome were spinal while hyperactivity and hyper-reactivity were central in origin. They reasoned that propranolol could block 5-HT receptors in brain and spinal cord whilst methergoline and methysergide blocked only those in spinal cord. In our own experiments we are presumably looking at the biochemical changes which initiate the hyperactivity and hyper-reactivity. The finding of a change in the concentration of the metabolites not only of 5-HT but also of NA and dopamine after the administration of L-tryptophan suggests that there is a dopaminergic and noradrenergic component to this part of the syndrome. After a single dose of L-tryptophan, the concentration of MHPG, a major metabolite of NA, in brain increased as did the concentration of the dopamine metabolite DOPAC. Similar changes were found in control rats treated for 15 days with saline; L-tryptophan administration caused a rise in all the catecholamine metabolites examined, MHPG, HVA and DOPAC.

Pretreatment of the rats with a monoamine oxidase inhibitor reduced the neutral and acid metabolites. Nevertheless, after chronic administration of a MAOI, L-tryptophan administration resulted in a rise in the concentration of both HVA and DOPAC, compared with animals treated with the MAOI alone. This rise in the acid and neutral metabolites suggests three possibilities: an increase in turnover of the amines; release of the amine with subsequent deamination or competition between metabolites for the transport mechanisms removing them from the brain. The high 5-HIAA concentrations produced by the increased turnover of 5-HT after L-tryptophan could, for example, compete successfully with HVA at transport sites from brain resulting in a rise in the concentrations of HVA.

To try and clarify these points normetanephrine was also measured. Contrary to expectations, normetanephrine did not rise after acute phenelzine administration even though the MHPG concentration fell. This could be explained if a considerable proportion of the MHPG was formed from the methylation of DHPG and the concentration of this latter metabolite fell as a result of MAO inhibition. On the other hand, pargyline given alone did produce a rise in normetanephrine, as did phenelzine given over 15 days and the difference in the results following acute administration of these drugs may merely be due to the degree of MAO inhibition achieved. Certainly the significant rise in the concentration of normetanephrine after tryptophan alone suggests that the turnover of noradrenaline or its release is increased after administration of the amino acid and that the increase in the acid and neutral metabolites is not due simply to changes in efflux. Nevertheless it is difficult to explain why tryptophan plus phenelzine does not cause an increase in the concentration of normetanephrine, when compared with phenelzine alone.

The rise in the concentration of DOPAC in the acute experiment, and DOPAC and HVA in the chronic experiment suggests that dopamine turnover also increases after tryptophan. The fact that the concentration of noradrenaline did not fall and that the concentration of dopamine rose after L-tryptophan implies that, if the raised metabolites are a consequence of the release of dopamine and noradrenaline by 5-HT or tryptamine (Marsden & Curzon, 1978) then synthesis of the amines must have increased to keep pace with this release. Previous workers (Milson & Pycock, 1976) have shown that when 5-HT turnover is increased either by administration of tryptophan or 5-hydroxytryptophan there is an inhibition of contralateral turning induced by apomorphine in rats with unilateral lesions in the dopaminergic system (Ungerstedt, 1971). This inhibition of turning could be accounted for by release of dopamine from the intact striatal dopaminergic neurones which counteracted the turning induced by apomorphine via the supersensitive receptors. In keeping with this finding are our own (unpublished) observations that tryptophan and phenelzine in the same type of lesioned animal which had not been given apormorphine caused ipsilateral turning (towards the side of the lesion). This is the type of turning one would expect with amphetamine, a drug which releases dopamine.

The biochemical and behavioural findings suggest that tryptophan with or without a momoamine oxidase inhibitor causes an increase in noradrenergic and dopaminergic activity in brain either by release of these amines together with a compensatory increase in synthesis or by causing an increase in the firing rate of these groups of neurones. Certainly the thera-

peutic implications in man are that the efforts to potentiate a specific amine system may fail because of mutual dependency of function between the various systems.

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(Received December 19, 1977.
Revised April 10, 1978.)