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RELATIONSHIP OF BRAIN MONOAMINE AND LOCOMOTOR ACTIVITY IN RATS

ANDREW FOLDES and ERMINIO COSTA

Laboratory of Preclinical Pharmacology, National Institute of Mental Health, Saint Elizabeths Hospital, Washington, D.C., U.S.A.

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Abstract—The hyperactivity elicited by tryptophan injection in rats treated with monoamine oxidase (MAO) inhibitors was investigated. No correlation of the hyperactivity with the rates of brain serotonin (5-HT) accumulation was found under a variety of experimental conditions. D-Tryptophan was shown to elicit similar responses to the L-somer, albeit with a delayed onset, after either intraperitoneal or intracerebroventricular administration. Kynurenine, a major tryptophan metabolite, did not elicit hyperactivity. 6-Hydroxydopamine abolished the hyperactivity due to L-tryptophan loading. Another tryptophan metabolite, tryptamine (1–2 mg/kg), also elicits hyperactivity. This was antagonized by *p*-chlorophenylalanine, reserpine and alpha-methyltyrosine pretreatments. It is concluded that an indole metabolite other than 5-HT could trigger the motor activation, by acting on catecholaminergic neurons.

Short-term regulation of brain serotonin (5-HT) synthesis may be controlled by changes in the kinetic state of tryptophan hydroxylase (TPH) by availability of cofactors, or by availability of the dietary precursor tryptophan. If the substrate of TPH is experimentally increased by tryptophan loading in monoamine oxidase (MAO)-inhibited animals, a characteristic syndrome results [1], which includes hyperactivity.

It is not known whether or not in serotonergic neurons the synthesis of the transmitter is coupled to the release of 5-HT and to the stimulation of serotonergic receptors. It has been suggested that the rate of 5-HT synthesis exceeds the rate of neurotransmitter utilization, the excess being stored in a "non-functional pool" in vesicles protected from intraneuronal MAO which regulates the 5-HT levels in the "functional pool" [2–5]. This model appears to be supported by relationship between locomotor activity and brain 5-HT content measured in MAO-inhibited rats receiving high doses of tryptophan. In this proposal, the increase of locomotor activity was taken as an index of 5-HT receptor stimulation. Since the increase of locomotor activity elicited by tryptophan was abolished by the depletion of brain dopamine but not of brain norepinephrine, the model was expanded to include unidentified dopaminergic neurons which participate in the behavioral expression resulting from the 5-HT receptor stimulation [6].

We have investigated the validity of this model by studying the correlation between 5-HT accumulation rates and motor activation under a variety of experimental conditions. Our results suggest a possible alternative to the model proposed by Grahame-Smith and Green [6].

EXPERIMENTAL

Materials. Male Sprague–Dawley rats, weighing between 100 and 170 g, obtained from Zivic Miller Laboratories, Allison Park, Pa., were used. Tryptophan (uniformly labeled with tritium, 6·7 Ci/m-mole) was purchased from New England Nuclear Corp., Chicago, Ill., and its radiochemical purity was checked by thin-layer electrophoresis. D-Tryptophan, containing less than 1% of L-tryptophan contamination, was obtained from Schwarz-Mann, Orangeburg, N.Y. L-Kynurenine was also purchased from Schwarz-Mann. (+)-Tranylcypromine sulfate (TCP) was the generous gift of Smith, Kline & French Labs., Philadelphia, Pa. Pargyline HCl was obtained from Abbott Laboratories, North Chicago, Ill. Reserpine was purchased from Sigma Chemical Co., St. Louis, Mo. *p*-Chlorophenylalanine (PCPA) and 6-hydroxydopamine (60-HDA) were purchased from Regis Chemical Co., Morton Grove, Ill. Alpha-methyl-*p*-tyrosine methyl ester (α-MPT) was obtained from Aldrich Biochemicals, Cedar Knolls, N.J.

Methods. Motility studies were carried out by placing the treated animals individually in 40 × 32 cm cages on an infrared electronic motility meter, type 160 FC manufactured by Motor Produkter (Sweden). Horizontal motility was automatically recorded at 5- or 10-min intervals. At the conclusion of the motility study, the animals were decapitated and the brains rapidly frozen for subsequent 5-HT or tryptophan assay.

Whole brain 5-HT levels were assayed spectrofluorometrically according to the method of Curzon and Green [7], using an internal standard to calculate recovery, which varied between 50 and 70 per cent in these experiments.

Whole brain tryptophan concentrations were assayed by a modification of the method of Neff *et al.* [8]. The frozen brains were weighed, thawed and homogenized in 6 ml of ice-cold 0·4 M $HClO_4$. The supernatant was adjusted to pH 5–6 by the addition of 10 M potassium acetate and was again centrifuged at 0° (30,000 *g*, 10 min). Four ml of this supernatant was applied to a 0·8 × 2·5 cm column of Dowex 50W × 4 resin previously washed with 2 N HCl, water, 2 N NaOH, water, 1 M sodium acetate buffer, pH 6·0, and water. The effluent was discarded and tryptophan was eluted from the column with 10 ml of 0·5 M sodium acetate buffer, pH 6·0. In this eluate, recovery of ^{3}H-tryptophan added to a brain homogenate varied between 70 and 90 per cent. Washing

of the column with 0·1 M sodium acetate buffer, pH 4·5, and water before elution reduced the recovery slightly to 59 per cent, but had no effect on the subsequently assayed brain tryptophan levels.

The tryptophan content of the elutate was then assayed by a modification of the method of Denckla and Dewey [9], as described by Neff *et al.* [8].

Intraventricular (ivt.) injections were carried out according to the method of Noble *et al.* [10]. Routinely, 20 μl of a solution containing saline, D- or L-tryptophan or L-kynurenine was injected 30 min after intraperitoneal injection of 20 mg/kg of TCP. A similar time course was followed for intraperitoneal injections of D-, L-tryptophan or L-kynurenine after MAO inhibition. Animals were killed at various times up to 23·5 hr later.

Rat rectal temperatures were measured with a thermocouple probe (Yellow Springs Instrument Co., Inc.). The time and dosage schedules for the experiments with PCPA and α-MPT were identical to those used by Grahame-Smith [2, 5], tryptamine (1–2 mg/kg, i.p.) being substituted for L-tryptophan. For the reserpine experiments, rats were pretreated with 5 mg/kg of reserpine (i.p.) 18 hr before TCP injection.

6-OHDA (250 μg/brain) in saline containing 0·8% ascorbic acid was injected intraventricularly 9 and again 7 days before intraperitoneal injections of the MAO inhibitor and L-tryptophan. Doses of 6-OHDA were calculated as the free base.

RESULTS

TCP (20 mg/kg, i.p.) causes an immediate increase in motility (Fig. 1a) which is sustained for at least 3 hr. The steady state 5-HT level in brain is also increased by almost 100 per cent within 120 min (Fig. 1b) without a change in brain tryptophan concentration (Fig. 1c) or in body temperature (not shown).

L-Tryptophan treatment (100 mg/kg, i.p.) of TCP-pretreated rats causes an even greater increase in motility (Fig. 1a), commencing about 20 min after the injection of L-tryptophan and continuing until the animals are exhausted. The injection of L-tryptophan increases 5-HT levels to 300 per cent of saline controls within 2 hr (Fig. 1b).

Intraperitoneal D-tryptophan has similar effects to L-tryptophan on motility, body temperature, brain 5-HT and tryptophan concentrations, but the onset of motility is delayed. The steady state levels of both 5-HT and brain tryptophan are lower after administration of the D-isomer (Fig. 1) than after the L-isomer. The motility 30 min after tryptophan injection is increased only after the L- but not after the D-isomer, yet brain 5-HT concentrations are identical at that time. A similar lack of correlation between the increases in motility and 5-HT levels was noted under various experimental conditions throughout this study.

The dose-response curves for increased motility after i.p. administration of D- or L-tryptophan are different, while the corresponding curves for increased steady state levels of brain 5-HT are similar. After TCP pretreatment (Fig. 2), the i.p. dose of L-tryptophan required to elicit a significant increase of motility was much smaller (3·7 mg/kg) than that required to increase brain 5-HT (approximately 20 mg/kg).

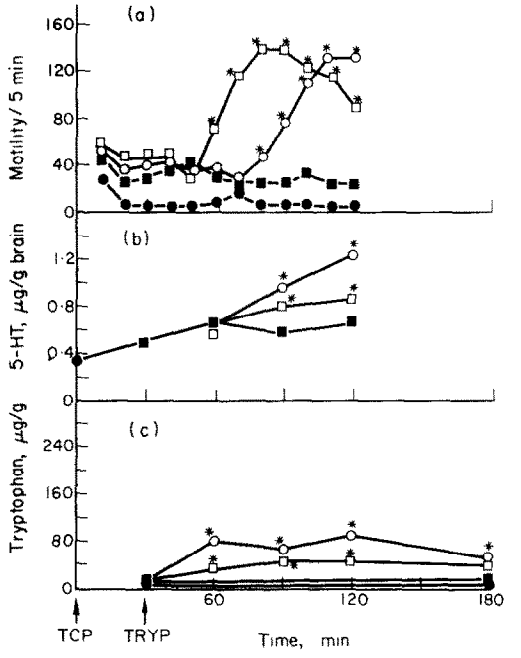

Fig. 1. Effects of intraperitoneally administered D- and L-tryptophan on motility, brain 5-HT and brain tryptophan levels after TCP. TCP (20 mg/kg, i.p.) or saline was administered to groups of three or four rats at time zero, and L- or D-tryptophan (100 mg/kg, i.p.) or saline was injected at 30 min as indicated by the arrows. Treatments: ● = saline; ■ = TCP + saline; □ = TCP + L-tryptophan; and ○ = TCP + D-tryptophan. All increases in motility after 50 min for L-, or 70 min for D-tryptophan are significantly different from TCP-treated animals. The motility with TCP is significantly higher than that with saline at all time points after 10 min. For panels (b) and (c) of the figure, * = $P < 0.05$ compared to TCP-treated animals.

Similarly, the corresponding doses for i.p. D-tryptophan were 10 mg/kg and about 100 mg/kg respectively.

Preliminary experiments indicated that the light ether anesthesia used for the intraventricular injections had no observable effect on the motility of the animals 5 min after injection. Intraventricular injection of D- or L-tryptophan (300 μg/brain) increases motility (Fig. 3a) to a similar level and with a similar time course as does intraperitoneal injection of 100 mg/kg. However, the increase in brain 5-HT content is much smaller than that after intraperitoneal injection of 100 mg of D- or L-tryptophan (compare Figs. 3b and 1b).

Intraperitoneal pargyline treatment (50 mg/kg), unlike TCP treatment, does not cause any increase in motility, but it does increase brain 5-HT levels, by an extent comparable to TCP (Fig. 4).

The rate of brain 5-HT accumulation (calculated from Figs. 1, 3 and 4) are shown in Table 1, and the lack of correlation of motility with these rates is illustrated by Fig. 5. It is evident that motility does not correlate with 5-HT accumulation rates.

No increase in either motility or brain 5-HT levels was seen after kynurenine injection (100 mg/kg, i.p., or 300 μg/brain, intraventricularly) (cf. Lapin [11]) in TCP- or pargyyline-pretreated rats.

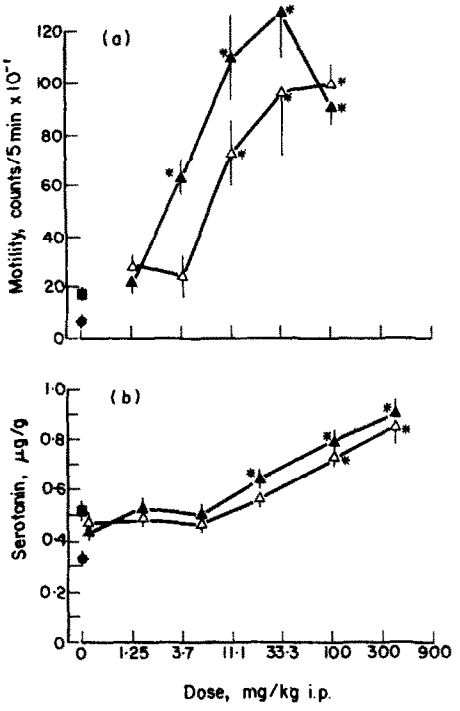

Fig. 2. Effects of increasing dosages of intraperitoneal L- and D-tryptophan on motility and brain serotonin levels. Panel (a) of the figure shows the effects of the two isomers on the motility of TCP-treated rats 2 hr after treatment. Panel (b) shows the corresponding increase in brain 5-HT levels. Treatments: ▲ = TCP + L-tryptophan; △ = TCP + D-tryptophan; ● = saline; and ■ = TCP. Standard errors of the mean of three animals are shown; * = $P < 0.05$ compared to TCP-treated animals.

Tryptamine in doses ranging from 0·5 to 2·0 mg/kg, i.p., was observed to cause an increase in the motility of TCP-pretreated rats. This motility increase was reduced 2 days after PCPA [12] (Fig. 6), when brain 5-HT concentrations were reduced to 26 per cent of control values. A single dose of PCPA (300 mg/kg, i.p.) given 4 hr before also abolished the tryptamine response. In addition, both schedules of PCPA injection, when followed by intraperitoneal TCP, resulted in enhanced motility over that obtained with TCP alone (Fig. 6).

A single dose of reserpine (5 mg/kg, i.p.) given 18 hr before TCP treatment also abolished the increase in motility due to tryptamine (Fig. 6). α-MPT (2 × 200 mg/kg, i.p.) given 18 and 16 hr before TCP treatment also abolished the motility effect due to tryptamine (1 mg/kg, i.p.). The activating effect of TCP alone was also blocked after α-MPT (Fig. 6).

Destruction of central sympathetic neurons with 6-OHDA prior to the administration of TCP (20 mg/kg, i.p.) and of L-tryptophan (100 mg/kg, i.p.) resulted in complete abolition of the hyperactivity due to tryptophan (Fig. 6), at doses of 6-OHDA which result in 70–80 per cent decreases in brain catecholamine levels [13]. At the same time (Table 1), there was no significant change in the rate of accumulation of brain 5-HT, despite a significant increase in brain 5-HT concentrations from 0·60 to 0·76 μg/g of brain 60 min after L-tryptophan.

DISCUSSION

The administration of L-tryptophan to TCP-pretreated rats causes an increase in motility, body temperature and steady state 5-HT concentration, whether the L-tryptophan is administered intraperitoneally or intraventricularly. In agreement with previous reports [3, 5], we find no correlation between the steady state levels of brain 5-HT and the increase in motility. However, we also find no correlation between the rates of synthesis or accumulation of 5-HT in whole brain and motor activation (Fig. 5). The normal synthesis rate of 0·3 μg/g/hr is in good agreement with the results of Neff *et al.* [8], but the rates of serotonin accumulation after the various treatments (Table 1) appear independent (Figs. 1, 3 and 4) of the motor response in each case.

The results obtained with D-tryptophan support this contention. In contrast to Yuwiler [14], we infer that significant conversion of D- to L-tryptophan occurs in rat brain. The delay in motor response after D-tryptophan administration (compared to the L-isomer) is consistent with the initial conversion of D- to L-tryptophan by D-amino acid oxidase and a transaminase both in the periphery [14] and the brain [15].

The involvement of indole metabolites other than 5-HT is suggested by the results shown in Fig. 1. After i.p. administration of TCP, the concentration of 5-HT

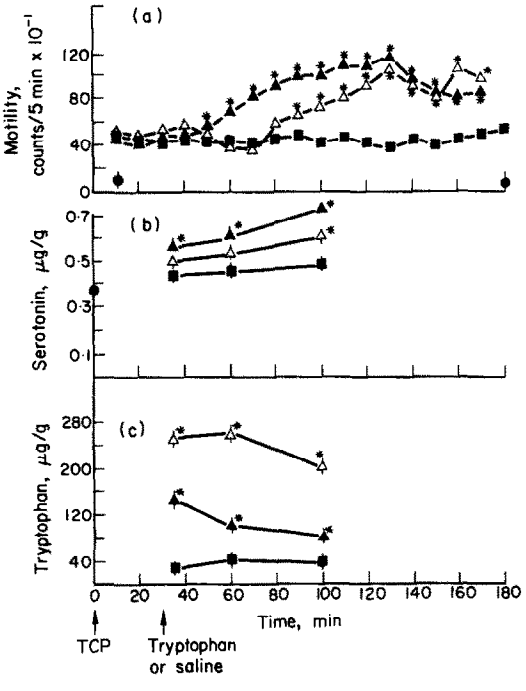

Fig. 3. Effects of intraventricularly administered L- and D-tryptophan on motility and concentrations of brain 5-HT and brain tryptophan. Intraventricular injections comprised 20 μl D- or L-tryptophan (15 mg/ml) or saline, 30 min after TCP (20 mg/kg, i.p.). The rats were sacrificed after 2 hr. Panel (a) of the figure shows the motility, panel (b) shows the effects on brain 5-HT and panel (c) shows the effects on brain tryptophan levels. Symbols are as previously described; all points are the means of three to four animals. The error bars, where shown, indicate the standard errors of the means; * = $P < 0.05$ compared to TCP-treated animals.

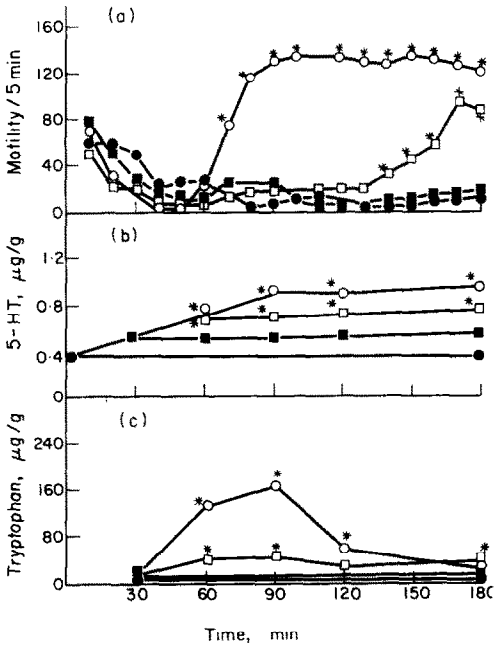

Fig. 4. Effects of intraperitoneally administered L- and D-tryptophan on motility and concentrations of brain 5-HT and tryptophan after pargyline. All symbols and experimental procedures are as in Fig. 1, with the exception that pargyline (50 mg/kg, i.p.) replaced TCP throughout. Increases in motility after 60 min for L-, and 130 min for D-tryptophan are significantly different from pargyline-treated animals. In panels (b) and (c) of the figure, * = $P < 0.05$ compared to pargyline-treated animals.

in the brain increases with time at the rate of 0·3 μg 5-HT/g of brain/hr, until a plateau is reached at a time when brain tryptophan levels are not decreased compared to controls. This plateau reflects an equilibrium between synthesis and removal of 5-HT.

After TCP and L-tryptophan, 5-HT accumulation continues at a higher rate and no plateau is reached in 3 hr. One possible explanation is that removal of 5-HT from the brain is now saturated. The rate of

Table 1. Rates of accumulation of brain 5-HT

Treatment	5-HT (μg/g brain/hr)
Tranylcypromine (TCP) (20 mg/kg, i.p.)	0·27 ± 0·026
Pargyline (50 mg/kg, i.p.)	0·33 ± 0·031
TCP + L-tryptophan (100 mg/kg, i.p.)	0·59 ± 0·042
TCP + D-tryptophan (100 mg/kg, i.p.)	0·28 ± 0·029
TCP + L-tryptophan (300 μg/brain, ivt.)	0·15 ± 0·011
TCP + D-tryptophan (300 μg/brain, ivt.)	0·11 ± 0·008
Pargyline + L-tryptophan (100 mg/kg, i.p.)	0·39 ± 0·038
Pargyline + D-tryptophan (100 mg/kg, i.p.)	0·36 ± 0·026
6-OHDA + TCP + L-tryptophan (100 mg/kg, i.p.)	0·27 ± 0·020

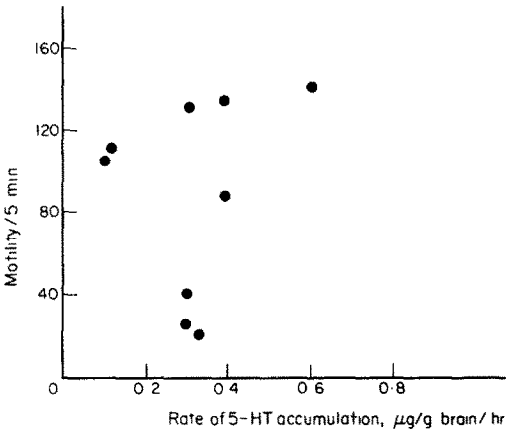

Fig. 5. Lack of correlation of 5-HT accumulation rates with increase in motility. The rates of 5-HT accumulation (μg/g/hr) in brain, under the various experimental conditions shown in Figs. 1, 3 and 4, are plotted against the maximal motility response elicited by each corresponding set of conditions.

accumulation appears to be independent of 5-HT concentration. This suggests that a saturable enzyme is involved in the removal of 5-HT from the brain. Possible candidates may be the *N* or *O*-methylating enzymes whose presence in brain is well established [16].

Kynurenine, the major peripheral tryptophan metabolite, also had no effect on either motility or brain 5-HT synthesis after intraperitoneal or intraventricular administration (cf. Lapin [11]), but the involvement of other indole metabolites has not been excluded.

Exogenous tryptamine in doses as low as 0·5 mg/kg, i.p., caused hyperactivity, 2·0 mg/kg resulting in the

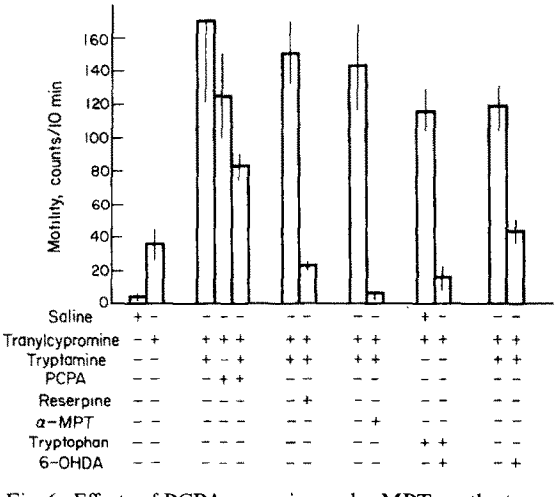

Fig. 6. Effects of PCPA, reserpine and α-MPT on the tryptamine-induced motility of TCP-treated rats. Rats were pretreated i.p. with reserpine (5 mg/kg) 18 hr, PCPA (2 × 300 mg/kg) 24 and 48 hr or α-MPT (2 × 200 mg/kg) 18 and 16 hr before TCP (20 mg/kg) injection. Tryptamine (1–2 mg/kg) was given 30 min after TCP. The last histograms show the effect of 6-OHDA pretreatment (2 × 250 μg/brain intraventricularly, 9 and 7 days before TCP) on the motility elicited by TCP and L-tryptophan (100 mg/kg, i.p.) or tryptamine (1 mg/kg, i.p.).

maximal tolerated response in TCP-treated rats (Fig. 6). After administration of 15 mg tryptamine/kg, i.p., Pletscher *et al.* [17] found 5 μg/g of the amine in the brain of MAO-inhibited rats. If similar conditions hold in our experiments, brain tryptamine levels may reach 0·16 to 0·66 μg/g. These levels are comparable to the 0·2 μg/g of brain reported by Eccleston *et al.* [18] after MAO inhibition and tryptamine loading and indicate that endogenously formed tryptamine could be present in a sufficient amount to cause hyperactivity under these conditions.

Grahame-Smith [2] used PCPA to show that the activation observed after tryptophan loading involves brain 5-HT. Our demonstration (Fig. 6). that PCPA pretreatment also reduces the hyperactivity due to exogenous tryptamine indicates that the involvement of endogenously formed tryptamine in the behavioral effects observed after tryptophan loading cannot be excluded on the basis of experiments with PCPA.

The mechanism of this reversal of tryptamine activation by PCPA is unclear. Possible mechanisms include:

(A) Tryptamine may be acting indirectly by releasing 5-HT. In the presence of PCPA, when brain 5-HT levels are only 26 per cent of control, the effect of tryptamine may be reduced due to the lack of 5-HT remaining to be released by tryptamine.

(B) Tryptamine may be acting directly at a specific receptor site [19, 20] to cause hyperactivity. PCPA, or more likely, endogenous PCPA metabolites, such as *p*-chlorophenylethylamine, in itself a known motor excitatory drug [21], may antagonize tryptamine action at the receptor site.

(C) Tryptamine may be acting indirectly by releasing a catecholamine from some adrenergic system. The results of Grahame-Smith and Green [6] and our own results with 6-OHDA and α-MPT indicate that a catecholamine, perhaps dopamine, could be involved in the behavioral response. Tryptophan loading could exert its action in MAO-inhibited rats via the formation of endogenous tryptamine which consequently releases dopamine. It is of interest to note that the increase motility due to TCP over that obtained with pargyline may be due to release of catecholamines by TCP (cf. Reigle *et al.* [22]). Moreover, the increased motility after TCP was prevented or diminished by pretreatment with α-MPT, indicating that the increased motility seen after TCP is due, in part, to the release of catecholamines [23]. The enhancement of TCP-induced motility by PCPA could result from protection of an active PCPA metabolite (possibly *p*-chlorophenylethylamine) from degradation by monoamine oxidase.

The results show that: (1) the hyperactivity does not correlate with brain 5-HT accumulation; (2) tryptamine evokes similar motor responses; and (3) agents which interfer with the catecholinergic system (e.g. 6-OHDA and α-MPT) abolish the motor responses without decreasing the rate of 5-HT accumulation in brain. These results suggest that tryptophan loading of MAO-inhibited rats may result in the accumulation of tryptamine or of other indole metabolites which affect some catecholarminergic, probably dopaminergic pathway, leading to the observed hyper-activity, which then would be catecholamine and not indoleamine mediated.

Grahame-Smith's model was invaluable as a basis for further investigation, but the involvement of 5-HT in the hyperactivity is not convincingly proven. First, the hyperactivation does not correlate with 5-HT synthesis or accumulation rates under various conditions. Secondly, the involvement of other indole metabolites, some of which are known to cause motor activation, has not been adequately considered. Thirdly, at least one of the drugs used, TCP, may have direct effects on brain 5-HT metabolism and indirect effects via other neurotransmitter systems; and fourth all of the data are at least equally consistent with other putative models, such as the one suggested here.

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