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DIFFERENTIAL EFFECTS OF TRANYLCYPROMINE AND PARGYLINE ON INDOLEAMINES IN BRAIN*

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Abstract—Investigations of the effects of tranylcypromine and pargyline on general activity and body temperature in mice demonstrated an increase in activity after tranylcypromine treatment and a decrease after pargyline treatment. Body temperature was unchanged with tranylcypromine and lowered with pargyline. At doses which similarly inhibited monoamine oxidase, tranylcypromine was shown to significantly raise brain tryptophan while pargyline had no effect. The rise in brain tryptophan was accompanied by increases in plasma-free tryptophan levels. The increase in brain tryptophan with tranylcypromine did not, however, lead to significant increases in accumulation of serotonin compared with pargyline-treated animals. Results were interpreted in terms of accumulation of indoleamines other than serotonin after tranylcypromine treatment.

Several drug treatments have been shown to alter brain tryptophan levels and to concomitantly increase brain serotonin turnover [1, 2]. The increase in serotonin turnover was postulated to be a result of the increased availability of substrate for tryptophan hydroxylase, the rate-limiting enzyme for serotonin synthesis [3]. Two recent reports also indicate that certain monoamine oxidase inhibitors (MAOI) may also produce changes in brain tryptophan levels [4, 5].

During the course of studies on alcohol withdrawal in animals, we found that administration of the MAOI tranylcypromine (TCP) produced a syndrome characterized by pronounced hyperexcitability and convulsions. On the other hand, pargyline, another MAOI, was found to tranquilize the animals. Our previous studies also demonstrated a significant hypothermia in mice treated with pargyline [6]. Foldes and Costa [7] observed an increased motility in rats treated with TCP, while pargyline-treated animals behaved similarly to those treated with saline. In addition, these authors reported that TCP administration had no effect on body temperature. Since temperature regulation [8] and motor activity [9] in rodents have been theorized to be controlled via intervention of serotonergic systems, we decided to compare the effects of two monoamine oxidase inhibitors (i.e. TCP and pargyline) on levels of tryptophan and the synthesis of serotonin in brain.

MATERIALS AND METHODS

Male C57Bl/6 mice were used throughout this study. Animals were purchased from ARS Sprague-Dawley and kept in our laboratories for 7 days under standard conditions of light, temperature and feeding [10] before use in these studies. All experiments were initiated between 900 and 1000 hr. Pargyline was supplied by Saber Laboratories, and ± tranylcypromine was purchased from Sigma Chemical Co. Serotonin-3-[^{14}C]creatinine sulfate was purchased from New England Nuclear. All injections were administered intraperitoneally (i.p.) in isotonic saline. Activity was measured with the aid of a Stoelting activity counter with a two-level detector. Measurements of activity were made on mice placed individually in plastic cages. Room environment was of constant temperature (23 ± 1°), and white noise was present to mask extraneous external stimuli. Bedding in the test cage was changed after each period of testing. Mice were each allowed to acclimate to the test cage for 10 min, placed back in their home cage, and then were injected with saline or drug. Starting 10 min after injection and at appropriate intervals thereafter (see Fig. 1), mice were returned to the test cage, allowed to orient themselves for 1 min, and then their activity was recorded for the following 3 min. Mice were divided into groups such that a saline-, a pargyline- and a TCP-injected animal were tested in parallel throughout the time points. For a particular animal, the activity during each test period was summed with activity during all preceding test periods, and the results were expressed as the mean of these cumulative activity scores. Body temperature was measured using a Yellow Springs YF-1 rectal thermometer. Measurements were made at an environmental temperature of 23° by inserting the probe 2.5 cm into the rectum.

Brain levels of tryptophan and serotonin were assayed after separation of these compounds by column chromatography. Mice were decapitated; the brains were removed quickly and homogenized in 0.4 N perchloric acid. After removal of precipitated protein, the pH of the resultant supernatant was adjusted to 6.5, the precipitated potassium perchlorate was removed, and serotonin was separated on Amberlite CG50. The effluent of the Amberlite column was pH adjusted to 3.0 and tryptophan separated on Dowex 50 [10]. Tryptophan was eluted from the Dowex using 0.5 N ammonium hydroxide. Serotonin was assayed as described by Maickel and Miller

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[11], while tryptophan concentrations were determined by a modification [12] of the procedure of Denckla and Dewey [13]. All values were corrected for recovery by processing standards through the entire procedure. The possible interference by TCP or pargyline with assays of tryptophan was assessed by adding 2–10 μg of these compounds to standards of brain tissue and carrying such samples through the assay procedure.

Total and free (ultrafiltrable) plasma tryptophan was determined by a modification of the method of Knott and Curzon [14] using Amicon CF50 Diaflo cones to prepare the ultrafiltrate.

Monoamine oxidase activity in brain tissue was determined by a modification of the method of Wurtman and Axelrod [15] as used previously in our laboratories [16]. Serotonin-3-[^{14}C]creatinine sulfate (5 mM; sp. act. 0.01 μCi/μmole) was used as substrate and the reaction mixtures were incubated for 30 min at pH 7.4. Alternatively, monoamine oxidase activity was monitored using *p*-dimethylaminobenzylamine (DAB) as substrate [17]. DAB has been shown to be preferentially deaminated by the type B monoamine oxidase activity [16].

RESULTS

Rectal temperatures measured 45 min after injection of pargyline (100 mg/kg) were significantly ($P < 0.01$) lower than those of TCP (25 mg/kg) or saline-injected animals. Temperatures of pargyline-treated mice were 33.6 ± 1.2° (N = 6), while temperatures of TCP-treated animals were 37.5 ± 0.7 (N = 5), and saline-treated mice were 37.2 ± 0.3° (N = 6).

On the other hand, cumulative activity scores increased at a greater rate in TCP-treated mice when compared to controls (Fig. 1). Differences in the mean values for these scores were significant at 60 min and thereafter. Activity in pargyline-treated mice was depressed at the initial test period and remained below control levels thereafter.

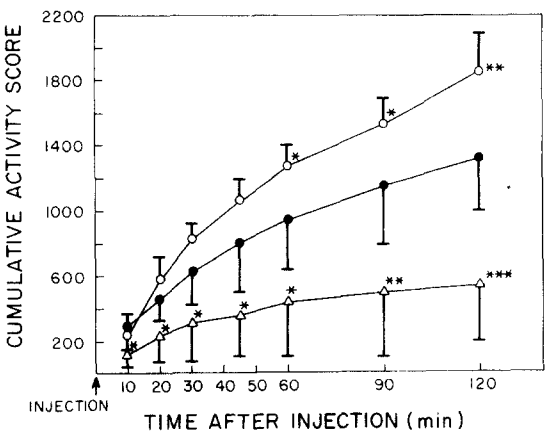

Fig. 1. Activity was measured as described in the text. Results are expressed as the mean of the cumulative activity score ± S. D. for seven mice for each drug or saline injection. Cumulative activity expresses the sum of all activity measured through all trials up to and including a particular time after an injection. Key: (—○—) TCP, 25 mg/kg; (—△—) pargyline, 100 mg/kg; and (—●—) saline. A single asterisk indicates $P < 0.1$, a double asterisk $P < 0.05$, and a triple asterisk $P < 0.02$ compared with saline controls (*t*-test).

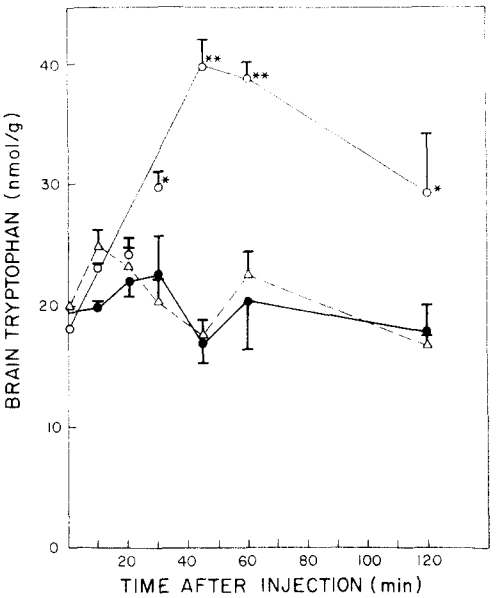

Fig. 2. Brain tryptophan levels were measured after injection of saline (—●—), TCP (25 mg/kg) (—○—), and pargyline (—△—). Results are expressed as nmoles/g of brain ± S. D. Three to six animals were used with each drug at each time point. A single asterisk indicates $P < 0.05$, a double asterisk $P < 0.005$ compared with saline-treated controls (*t*-test).

The injection of TCP (25 mg/kg) resulted in a significant elevation in brain tryptophan levels over those found in control animals (Fig. 2). No significant differences in brain tryptophan levels were found in animals treated with 100 mg/kg of pargyline at any of the times of testing in Fig. 2, and no difference in brain tryptophan was noted 45 min after injection of 200 mg/kg of pargyline compared to control animals ($P > 0.1$).

Increasing doses of TCP resulted in corresponding increases in brain tryptophan levels (Fig. 3). An analysis of variance for repeated measures using the mean values obtained at the various doses of TCP demonstrated a significant increase in tryptophan levels in brain as the dose was increased from 5 to 50 mg/kg ($P < 0.05$). On the other hand, no significant difference in brain levels of serotonin was found between animals treated with the 5 and 50 mg/kg doses of TCP.

Table 1 illustrates brain monoamine oxidase activity at various times after administration of TCP or pargyline. Monoamine oxidase activity, assayed using radioactive serotonin, was essentially totally inhibited within 10 min after 25 mg/kg of TCP or after 100 or 200 mg/kg of pargyline.

When MAO activity using DAB as substrate was measured at four time intervals between 5 and 45 min after the injection of 25 mg/kg of TCP or 100 mg/kg of pargyline, no MAO activity was measurable in brain at 5 min after the injection of the monoamine oxidase inhibitors. In addition, no MAO activity, using this substrate, was measured at

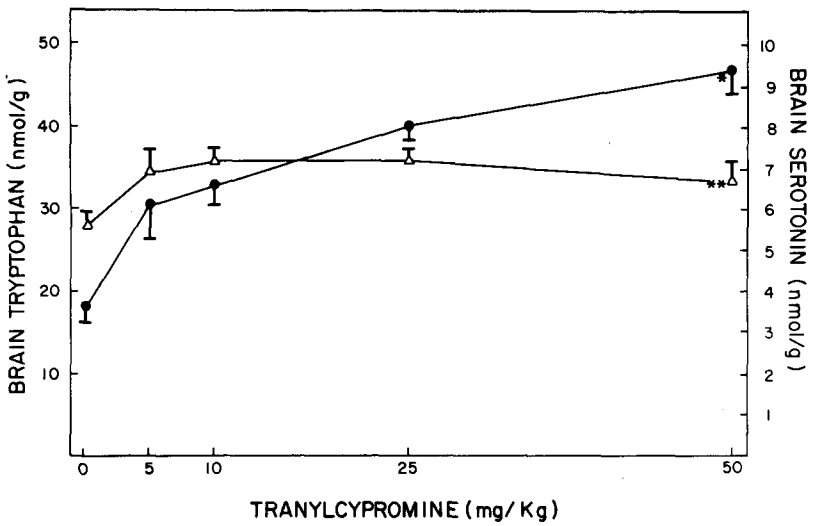

Fig. 3. Brain levels of serotonin (—△—) and tryptophan (—●—) were measured 45 min after the injection of increasing doses of TCP or injection of saline. Results are expressed as nmoles/g of brain ± S. D. Three to six animals were used for each dose. A single asterisk indicates $P < 0.05$, comparison between tryptophan levels after 5 mg/kg of TCP and 50 mg/kg of TCP (*t*-test). A double asterisk indicates $P > 0.2$, comparison between serotonin levels after 5 mg/kg of TCP and 50 mg/kg of TCP (*t*-test).

the other three time points. MAO activity using DAB in brains of saline-injected animals was shown to be 6.62 ± 0.74 nmoles DAB deaminated/min/g of brain.

Brain serotonin levels assayed at various times after the administration of pargyline (100 mg/kg) were found to be similar to those levels found in animals treated with TCP (25 mg/kg) (Fig. 4). The regression coefficients for lines fitted to the data by the method of least squares were $r = 0.93$ for animals treated with TCP and $r = 0.99$ for animals treated with pargyline. No significant difference in the slope of the lines ($m = 0.040$ for pargyline, $m = 0.049$ for TCP, expressed as nmoles/min) could be found. The slope of the regression lines would also represent the turnover of brain serotonin as measured by the method of Neff and Tozer [18].

Total plasma tryptophan levels were similar between control (saline-injected) and TCP-treated

Table 1. Brain monoamine oxidase activity after administration of tranylcypromine and pargyline*

Time after injection (min)	MAO activity	Per cent of control activity Tranylcypromine	
		(5 mg/kg)	(25 mg/kg)
0	10.6 ± 3.8		
10		15.8 ± 2.9	2.1 ± 1.9
20		3.3 ± 4.7	0.9 ± 1.5
30		1.3 ± 1.9	0
45		2.0 ± 2.8	0
60		1.3 ± 1.9	0
120		2.0 ± 1.0	0
		Pargyline	
		(100 mg/kg)	(200 mg/kg)
0	12.9 ± 2.3		
10		7.0 ± 9.9	0
20		2.1 ± 1.6	0
30		1.1 ± 1.6	0
45		1.0 ± 1.8	0
60		2.6 ± 2.4	0
120		1.6 ± 1.4	0

* Monoamine oxidase (MAO) activity was measured in brain as described in the text at various times after drug injection. Results are expressed as per cent activity ± S. D. of the zero time levels. The zero time MAO activity was obtained by decapitating animals immediately after the injection and is expressed as nmoles of deaminated products formed from [^{14}C]serotonin/min/g of brain. Three to six animals were used at each time for each dose of drug.

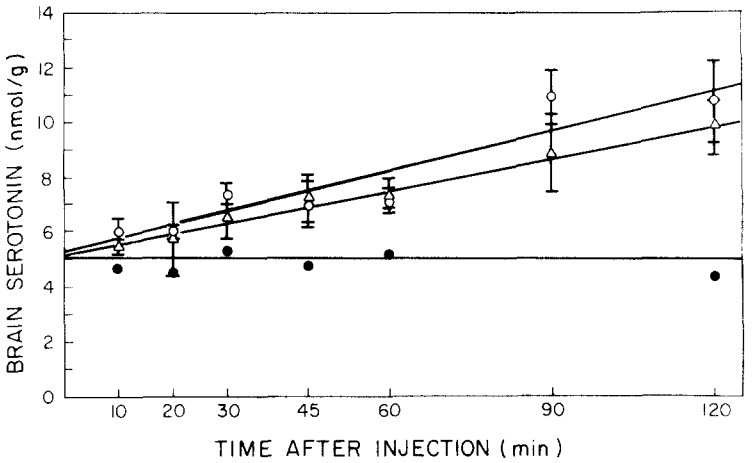

Fig. 4. Brain serotonin levels were determined at the various times after injection of saline (—●—), TCP (25 mg/kg) (—○—), or pargyline (100 mg/kg) (--△--). Five to six animals were used at each time with each drug and two animals with saline. Results are expressed as nmoles serotonin/g of brain ± S. D. for the drug-treated mice and as mean nmoles serotonin/g of brain for saline-injected animals.

animals, while pargyline-treated animals had slightly lower total plasma tryptophan levels (Table 2). On the other hand, ultrafiltrable tryptophan was significantly elevated in TCP-pretreated animals compared with control animals, while no significant differences in plasma-ultrafiltrable tryptophan were noted between pargyline- and saline-injected animals (Table 2).

DISCUSSION

The increased brain serotonin levels after inhibition of monoamine oxidase in pargyline-treated animals may be primarily responsible for akinesia [19] and hypothermia [20]. However, one must then assume that the administration of TCP alters systems unaffected by pargyline, since brain serotonin levels were also elevated in TCP-treated mice but the mice behaved quite differently. Some of these systems which could contribute to the effects of TCP may be related to the increased levels of brain tryptophan witnessed in our studies. Grahame-Smith [4] also reported a significant elevation in rat brain tryptophan levels 90 min after TCP.

The rise in brain tryptophan after TCP would be expected to lead to a progressive increase in the rate of serotonin synthesis if brain tryptophan hydroxylase was much below saturation with substrate [3]. However, recent studies by Friedman *et al.* [21] suggest that the K_m of tryptophan hydroxylase for tryptophan is considerably lower than previous estimates [3] if the "natural" cofactor (tetrahydrobiopterin) is used to assay enzyme activity.

Significant differences in accumulation of serotonin or corresponding increases in levels of serotonin after various doses of TCP (Fig. 3), which increase brain tryptophan levels, were not found in our studies. One must consider, however, that the increases in brain tryptophan reported here are relatively small compared to those noted after tryptophan loading in animals [22]. Thus, the increases in serotonin accumulation with increasing brain tryptophan levels in the MAOI-pretreated animals noted by others [4] may reflect the fact that larger changes in brain tryptophan concentrations are necessary to produce increases in serotonin synthesis. As brain levels of tryptophan increase due to changes in the plasma ratio of free to bound tryptophan (Table 2) [14] or after tryptophan

Table 2. Plasma tryptophan levels after injection of pargyline, tranylcypromine or saline*

Drug	Plasma tryptophan	
	Total (nmoles/ml)	Ultrafiltrable (nmoles/ml)
None	108.7 ± 15.2 (10)	16.2 ± 2.4 (6)
Pargyline (100 mg/kg)	84.7 ± 8.8† (7)	16.6 ± 4.4 (5)
Tranylcypromine (25 mg/kg)	100.4 ± 6.4 (8)	21.5 ± 2.9† (5)

* Plasma tryptophan levels (total and ultrafiltrable) were determined, as described in the text, 45 min after injection of drug or saline. Blood from two animals was pooled for each determination. The number of such determinations is shown in parentheses, and the results are expressed as the mean ± S. D.

† $P < 0.05$ compared to saline-injected controls.

loading, an increased uptake of tryptophan would be expected into both "functional" and "non-functional" pools. Such pools would be exemplified by compartments within serotonergic neurons as well as tryptophan pools in cells which contain no serotonin.

Tryptophan hydroxylase has been shown to be located primarily in serotonergic cell bodies and terminals [23, 24], while aromatic amino acid decarboxylase is ubiquitous in all monoaminergic neurons [25], and thus tryptophan, which enters catecholamine neurons, may under certain conditions be decarboxylated to tryptamine. Although the K_m for dihydroxyphenylalanine (dopa) has been estimated to be 5-fold lower than the K_m for tryptophan with aromatic amino acid decarboxylase [26], the low levels of dopa normally present in brain [27] would allow for significant competition for decarboxylase by tryptophan when the levels of this amino acid are elevated in brain. It is of interest that pretreatment of animals with a decarboxylase inhibitor has been shown to block the tryptophan/MAOI-induced increase in locomotion [4]. Tryptamine formed in the presence of a MAOI would be expected to accumulate at the sie of formation. On the other hand, since the tryptamine is an excellent substrate for monoamine oxidase [28, 29], one would expect that it would be rapidly metabolized if monoamine oxidase was not inhibited. This could explain the observation that tryptophan administered without a MAOI did not result in increases in locomotor activity in rats [4].

Under our experimental conditions, monoamine oxidase in mouse brain was essentially totally inhibited by both TCP and parglyine (see Table 1 and Results). Similar results were obtained when substrates for either the A or B form of the enzyme were used to assay MAO activity. The increase in brain tryptophan in the presence of TCP could therefore lead to accumulation of tryptamine in non-serotonergic neurons by mechanisms described above. An increase in tryptamine would be less evident in the pargyline-treated animals since brain tryptophan was not elevated. It has been shown, however, that the administration of tryptophan to pargyline-treated animals produces a syndrome quite similar to that produced by TCP alone [7]. Low doses of tryptamine administered to rats also produced increases in locomotor activity [7]. Tryptamine has been shown to be a normal constituent of brain [30], but systematic studies of changes in tryptamine concentration under conditions similar to those used in our work have not been performed.* The block of the tryptophan/MAOI-dependent locomotor activation produced by pretreatment of animals with 6-hydroxydopamine and α-methyl-*p*-tyrosine [7] suggests the involvement of catecholamine neurons in mediating this effect. Tryptamine may release catecholamines from neuronal pools or may itself be released from these areas to act either as a false transmitter or a true agonist on receptors for tryptamine [31]. Dewhurst [31] predicted amines such as tryptamine would act directly on specific receptors to increase alertness and motor activity.

Our studies are consistent with recent postulates [7] on the involvement of tryptamine in the tryptophan/MAOI syndrome of hyperactivity. Furthermore, the lack of hypothermia in the presence of monoamine oxidase inhibition and accumulation of serotonin seen with TCP also suggests the activation of catecholamine systems either directly by TCP [32] or by intervention of tryptamine. Norepinephrine has been shown to inhibit the heat loss systems activated by serotonin [20]. Our studies are not consistent with a hypothesis that serotonin mediates the hyperactivity produced by TCP; however, the increased brain tryptophan levels noted after TCP treatment may be the underlying phenomenon responsible for some of the unique clinical features of this monoamine oxidase inhibitor [33].

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*Recent work in collaboration with S. R. Phillips and A. A. Boulton (B. Tabakoff, F. Moses, S. R. Phillips and A. A. Boulton, manuscript in preparation) has demonstrated our predicted increase in brain tryptamine after administration of TCP. Although increases in tryptamine were also evident in pargyline-treated mice, the magnitude of these changes was significantly less than those seen in TCP-treated animals.

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