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Drug-Induced Changes in Motor Activity After Selective MAO Inhibition

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GIANUTSOS, G., G. M. CARLSON AND J. G. GODFREY. *Drug-induced changes in motor activity after selective MAO inhibition.* PHARMACOL BIOCHEM BEHAV **19**(2)263–268, 1983.—The increase in motor activity produced in mice by phenylethylamine (PEA), L-DOPA and amphetamine was evaluated after selective inhibition of MAO Type A (by clorgyline) or Type B (by low doses of pargyline). PEA-induced motor stimulation was intensified in the presence of MAO-B inhibition, but not when MAO-A was inhibited. This was paralleled by higher concentrations of brain and plasma PEA (after injection) in mice in which there was inhibition of MAO-B compared with control or MAO-A inhibition. Conversely, L-DOPA produced significant stimulation only when MAO-A was inhibited. The clorgyline pretreatment resulted in larger increases in brain dopamine concentrations (in the striatum, olfactory tubercles and in the area containing the substantia nigra) than did MAO-B inhibition. This effect occurred both in mice receiving L-DOPA + inhibitor and in mice receiving the inhibitor alone. Amphetamine-induced stimulation was increased following the inhibition of either form of MAO, and this was not the result of changes in the distribution or metabolism of amphetamine. These results support the concept that MAO-A and MAO-B deaminate different substrates in the rodent CNS and that amphetamine may utilize either dopamine or PEA in producing its stimulant effects.

Motor activity MAO inhibition Drug interaction

MONOAMINE oxidase (MAO) oxidatively deaminates several important monoamines in the nervous system. This enzyme is now believed to exist as two subtypes, designated A and B, on the basis of substrate preference and inhibitor selectivities (see [13,19] for review). MAO-A is generally considered to have a substrate preference for serotonin and norepinephrine while phenylethylamine (PEA) is a selective substrate for MAO-B *in vitro*. Dopamine (DA) is metabolized by either form of the enzyme with species differences playing a significant part. In rodents, MAO-A is considered the primary enzyme for DA inactivation [5], while in humans, MAO-B may be more important [9]. Similarly, MAO inhibitors with selective affinity for one form of the enzyme have been developed, including clorgyline which selectively inhibits MAO-A [10] and deprenyl which is an inhibitor of MAO-B [11].

MAO inhibition has long been known to alter the pharmacological effects of certain drugs which are dependent on monoamine substrates for their pharmacological activity (e.g., [14]), but the effect of more selective MAO inhibition is less well described. We chose to examine the effects of three substances, PEA, L-DOPA and amphetamine, whose effects might be expected to differ based on in vitro results, in the presence of selective MAO inhibition and have found some major differences among the drugs.

METHOD

Animals

Male CD-1 mice (obtained from Charles River Farms, Wilmington, MA) were used in all experiments. The mice were housed in groups of 8 on a 12 hr light:dark cycle (lights on 7 a.m.) in environmentally controlled facilities and were allowed free access to food (Purina) and water. All experiments were performed during the morning.

Activity

Motor activity was measured using a Stoelting Activity Monitor; under the conditions of the experiment, locomotor activity rather than stereotypy was measured. Mice were placed individually into a cage for a 20 min period of acclimation prior to receiving an SC injection of saline or one of the test compounds. The mice were returned to the chamber and activity was measured for 1–2 hr. "Counts" recorded by the monitor were accumulated and are expressed as total counts.

When an MAO inhibitor was used, it was injected IP, 20 hr before the test. Challenge drugs consisted of PEA (25 mg/kg), L-DOPA (75 mg/kg) or amphetamine (1 mg/kg), which were injected SC.

MAO Activity

Inhibition of MAO was measured 20 hr after an IP injection of clorgyline or pargyline. Brains were homogenized in 80 mM phosphate buffer (pH=7.2) and a 50 μL aliquot was used for the assay as described by Campbell and coworkers [3]. Briefly, the tissue was incubated for 60 min at 37°C in buffer containing EDTA and ascorbate along with ^{14}C-serotonin (500 μM) for the measurement of MAO-A activity

TABLE 1
EFFECT OF MAO INHIBITION ON MAO ACTIVITY AND MOTOR ACTIVITY

Pretreatment*	MAO Activity (% Inhibition)* Type A	Type B	Motor Activity (mean counts ± SEM)
Saline	0 ± 5	0 ± 3	1924 ± 272
Clorgyline (1)	75 ± 7	0 ± 3	2039 ± 396
Pargyline (5)	9 ± 3	81 ± 7	2175 ± 483
Pargyline (50)	86 ± 5	92 ± 7	2188 ± 253

*Mice were pretreated with inhibitor (doses in parentheses in mg/kg) and were sacrificed 20 hr later for MAO measurement (see text). Percent inhibition represents the difference in enzymatic activity compared with activity in brains from saline-pretreated mice (N=6). Motor activity was also measured for 1 hr, 20 hr after injection and represents means obtained from 12 mice (see text).

and ^{14}C-PEA (20 μM) for measurement of MAO-B. Deaminated, radiolabelled metabolites were seperated by ion-exchange on Amberlite CG-50 columns and were quantified by liquid scintillation spectrometry. A boiled tissue sample served as the blank. Enzyme inhibition was determined by comparison with activity in brains obtained from saline-treated mice.

PEA Accumulation

The effect of MAO inhibition on the availability of injected PEA was determined essentially as described by Fuller and Roush [8]. Mice were injected SC with PEA (25 mg/kg) spiked with 20 μCi/kg ^{14}C-PEA. After 30 min, the mice were sacrificed and the amount of labelled PEA in the brain and plasma was determined. In brief, tissue was deproteinized and the clear supernate was adjusted to pH 11, saturated with NaCl and extracted with benzene. The organic layer was separated, washed with 0.1 N NaOH and the radioactivity remaining in the organic layer was quantified by liquid scintillation spectrometry. Samples were corrected for recovery by the addition of known amounts of ^{14}C-PEA to perchlorate extracts of brains from untreated mice.

Dopamine

Dopamine was measured by the radioenzymatic method of Cuello and coworkers [4]. This method depends on the enzymatic conversion of DA *in vitro* to radiolabelled 3-methoxytyramine by COMT using ^{3}H-S-adenosyl methionine (SAM) as the methyl donor. Briefly, tissue was dissected on ice and homogenized in 0.2 N perchloric acid. The area containing the substantia nigra was removed from the ventral surface of a slice made by cutting at the level of the mammillary bodies and the dorsal pons, as suggested by Westerink and Korf [17] and included some adjacent structures. An aliquot of the homogenate is incubated with COMT and SAM and the labelled product is separated by organic extraction and paper chromatography and quantified by liquid scintillation spectrometry.

Amphetamine

Brain and plasma amphetamine concentration after injection of amphetamine was measured essentially as described by Maickel and coworkers [12]. Mice were injected SC with amphetamine spiked with ^{14}C-amphetamine (10 μCi/kg) and were sacrificed after 30 min. Plasma and brain (homogenized in 0.01 N HCl) were alkalinized and labelled amphetamine was extracted with benzene. The organic layer was washed with 0.5 N NaOH and the labelled drug was back extracted into acid. The radioactivity in an aliquot of the acid layer was quantified by liquid scintillation.

Statistics

For behavioral experiments, the results were analyzed by Analysis of Variance, followed by Dunnet's Test for comparison with control. In the biochemical experiments, Student's *t*-test was used. In all cases, the level of significance was chosen as $p<0.05$.

RESULTS

MAO Activity

The effect of injection of clorgyline or pargyline on MAO activity is summarized in Table 1. Clorgyline inhibited the A form of MAO by 50% at a dose of 0.5 mg/kg but failed to produce significant inhibition of MAO-B at doses up to 5 mg/kg. Pargyline inhibited MAO-B by 50% at a dose of 1.5 mg/kg while a 20-fold higher dose was needed to affect MAO-A to the same extent. On the basis of these experiments, subsequent studies utilized clorgyline at 1 mg/kg to inhibit MAO-A (75% inhibition), pargyline at 5 mg/kg to inhibit MAO-B and pargyline at 50 mg/kg when it was necessary to inhibit both forms of the enzyme. The degree of enzyme inhibition produced by the high dose of pargyline was essentially the same as that produced by the combined administration of clorgyline (1 mg/kg) plus pargyline (5 mg/kg).

Motor Activity

Pretreatment of the mice with either MAO inhibitor at the doses described above failed to significantly alter motor activity when compared with saline-pretreated mice (see Table 1). However, when mice were pretreated with these compounds, the motor stimulation produced by PEA, DOPA or amphetamine was altered. As depicted in Fig. 1, pretreatment with pargyline at doses which inhibited MAO-B, significantly potentiated the stimulation of motor activity produced by PEA administration. This dose of PEA was just sub-threshold in altering motor activity in controls (i.e., no significant effect in saline-pretreated mice), but resulted in

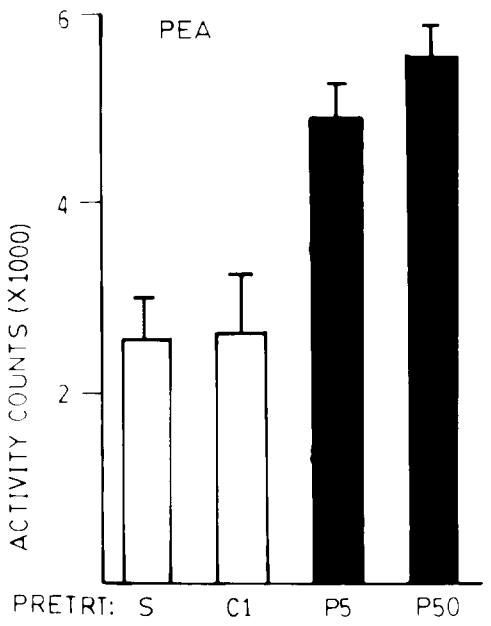

FIG. 1. Effect of PEA on Motor Activity after MAO Inhibition. Motor activity was measured for 1 hr after an SC injection of PEA (25 mg/kg) in mice pretreated 20 hr earlier with saline (S), clorgyline (1 mg/kg, C1), or pargyline (5 mg/kg, P5 or 50 mg/kg, P50). Activity is expressed as total "counts" for the period. Filled bars represent values which are significantly different ($p<0.05$) from saline-pretreated mice, Dunnet's Test following ANOVA, $F(3,36)=5.91$.

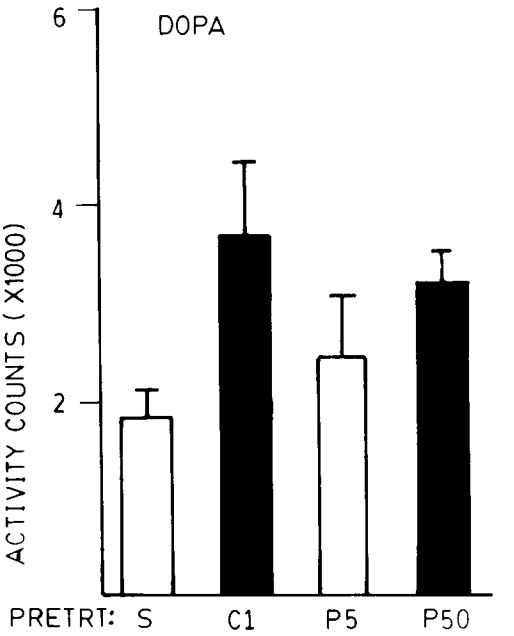

FIG. 2. Effect of L-DOPA on Motor Activity after MAO Inhibition. Motor activity was measured for 1 hr after injection of L-DOPA (75 mg/kg) in mice pretreated as described in Fig. 1. Filled bars represent values significantly different from saline-pretreated mice, Dunnet's Test following ANOVA, $F(3,20)=3.18$.

significant stimulation in the presence of MAO-B inhibition. On the other hand, inhibition of MAO-A by clorgyline failed to alter the pharmacological effect of PEA. In order to ensure that the small (8–10%) inhibition of MAO-A by this dose of pargyline did not affect the results, a separate group of mice was tested after injection of pargyline (50 mg/kg) plus harmaline (30 mg/kg). Harmaline is a reversible inhibitor of MAO-A and has been shown by Fuller and Hemrick [7] to protect MAO-A from inhibition by pargyline. Under these conditions, MAO-B is selectively inhibited. As summarized in Table 2, the stimulatory effect of PEA was potentiated by the combined treatment and this was not different from the effect produced by pargyline alone.

In contrast to these results, clorgyline pretreatment did result in significant stimulation of activity when combined with a normally-ineffective dose of L-DOPA, as illustrated in Fig. 2. In this case inhibition of MAO-B alone failed to significantly potentiate the pharmacological effects of L-DOPA (which presumably are due to its conversion to DA).

As depicted in Fig. 3, the effect of amphetamine was intensified when either form of MAO was inhibited. This dose of amphetamine was found to be a sub-threshold dose for producing stimulation in normal mice, but produced significant stimulation in the presence of MAO-A or MAO-B inhibition. The combined inhibition produced by the large dose of pargyline was not different from the effect produced by inhibition of one form of the enzyme; the reason for this effect remains to be elucidated.

PEA

Brain and plasma concentrations of PEA after injection of the drug (25 mg/kg) are illustrated in Table 3. Inhibition of MAO-B by pargyline leads to higher concentrations of available PEA (presumably due to interference with its metabolism by MAO-B) in both brain and plasma. On the other hand, inhibition of MAO-A fails to alter the concentration of PEA after exogenous administration. These results are in good agreement with the effect of MAO-inhibitor pretreatment on motor activity in which MAO-B inhibition potentiated the effect of PEA while MAO-A inhibition was inactive.

TABLE 2

PEA-INDUCED CHANGES IN MOTOR ACTIVITY AFTER PARGYLINE AND OR HARMALINE†

Pretreatment	Activity (mean counts/hr ± SEM)
Saline	1908 ± 305
Pargyline	6487 ± 482*
Harmaline	2035 ± 419
Pargyline + Harmaline	5603 ± 550*

*Represents values significantly different ($p<0.05$) from saline control, Dunnet's Test following ANOVA, $F(3,20)=5.10$.

†Mice were pretreated with pargyline (5 mg/kg) and/or harmaline (30 mg/kg) 20 hr before challenge with PEA (25 mg/kg), N=6.

DA Measurements

The effect of inhibition of MAO on regional brain DA concentrations and on the change in DA induced by treat-

TABLE 3

EFFECT OF MAO INHIBITION ON THE AVAILABILITY OF PEA

Pretreatment†	PEA Concentration (μg/g or ml;mean ± SEM) Brain	Plasma
Saline	1.29 ± 0.09	0.45 ± 0.12
Clorgyline (1)	1.43 ± 0.19	0.49 ± 0.10
Pargyline (5)	5.71 ± 1.55*	0.83 ± 0.20*

*Values significantly different ($p<0.05$) from saline controls.

†Mice (N=8) pretreated with saline or inhibitor (dose in mg/kg in parentheses) 20 hr before injection of PEA (25 mg/kg) and were sacrificed 30 min after the second injection for PEA determination. (See text for further details.)

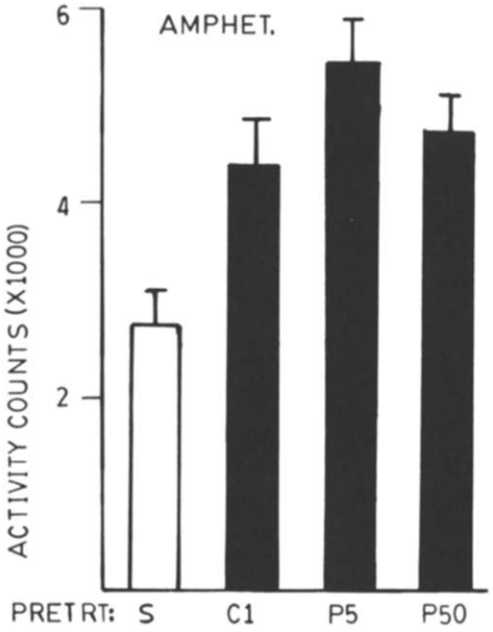

FIG. 3. Effect of Amphetamine on Motor Activity after MAO Inhibition. Motor activity was measured for 1 hr after SC injection of amphetamine (1 mg/kg) in mice pretreated as described in Fig. 1. Filled bars represent values significantly different from saline-pretreated mice, Dunnet's Test following ANOVA, F(3,28)=3.77.

TABLE 4

EFFECT OF MAO INHIBITION ON DOPA-INDUCED CHANGES IN BRAIN DOPAMINE

Pretreatment†	Dopamine Content (ng/mg protein; mean ± SEM) Striatum	Olfactory Tubercle	Substantia Nigra Area
A. No L-DOPA‡			
Saline	100 ± 6	74 ± 4	15 ± 3
Pargyline (5)	123 ± 6*	78 ± 6	17 ± 2
Clorgyline (1)	134 ± 12*	114 ± 7*	23 ± 3*
B. Plus L-DOPA‡			
Saline	171 ± 25	113 ± 11	29 ± 9
Pargyline (5)	184 ± 17	131 ± 12	38 ± 7
Clorgyline (1)	241 ± 18*	193 ± 14*	65 ± 11*

*Indicates values significantly different ($p<0.05$) from corresponding saline control.

†Mice received injection of saline or inhibitor (dose in parentheses) 20 hr before receiving second injection. N=8.

‡Mice were injected with carbidopa (50 mg/kg) and 20 min later received saline (part A) or L-DOPA (part B; 50 mg/kg) and were sacrificed 60 min after the last injection for the measurement of DA (see text for further details). N=8.

ment with L-DOPA are summarized in Table 4. DOPA, as expected [6], significantly increased brain DA concentrations when administered alone, presumably via its conversion to DA. This effect occurred in the striatum, olfactory tubercles and the mesencephalic area containing the substantia nigra. Similarly, inhibition of MAO-A by clorgyline also resulted in a significant increase in DA in all 3 regions. However, pretreatment with pargyline resulted in much smaller increases and these tended to be localized to the striatum. When DOPA was administered in the presence of MAO-inhibition, an even larger increase in brain DA was produced in all 3 regions; clorgyline pretreatment was particularly effective in this regard.

Amphetamine

The influence of MAO inhibition on the concentration of amphetamine is summarized in Table 5. Deamination is a possible, although minor, route of amphetamine metabolism [18], and these experiments were performed in order to ensure that the potentiation of amphetamine in the presence of MAO-inhibition was due to alterations in monoamine substrates rather than to pharmacokinetic factors. It can be seen that plasma and brain concentrations of amphetamine were the same in the presence or absence of either type of MAO inhibition.

DISCUSSION

The pharmacological effects of PEA, L-DOPA and amphetamine were found to be potentiated in the presence of MAO inhibition, but the type of inhibition was a critical factor in defining their pharmacological activity. In the presence of MAO-B inhibition, the stimulatory effects of PEA were

TABLE 5

EFFECT OF MAO INHIBITION ON AMPHETAMINE CONCENTRATION

Pretreatment*	Amphetamine Concentration (μg/g or ml; mean ± SEM) Brain	Plasma
Saline	1.68 ± 0.26	0.27 ± 0.03
Clorgyline (1)	1.77 ± 0.15	0.22 ± 0.02
Pargyline (5)	1.61 ± 0.29	0.20 ± 0.04

*Mice (N=6) pretreated with saline or inhibitor (dose in mg/kg in parentheses) 20 hr before injection of amphetamine (1 mg/kg) and were sacrificed 30 min after the second injection for amphetamine determination. (See text for further details.)

increased, along with the blood and brain concentration of PEA. These effects did not occur when MAO-A was selectively inhibited. The behavioral results are consistent with those reported by Braestrup and coworkers [2] who found that the stereotypy produced by PEA was potentiated by deprenyl but not by clorgyline. In contrast, MAO-A inhibition potentiated the behavioral effects of L-DOPA and resulted in substantially higher concentrations of DA than did MAO-B inhibition. These effects were noted in all three dopaminergic areas studied (striatum, olfactory tubercle and the mesencephalic region which included the DA cell bodies located in the substantia nigra). These results support previous suggestions [5,16] that the A form of MAO is primarily responsible for the metabolism of dopamine in the rodent CNS, while MAO-B is likely to be responsible for the metabolism of exogenous or endogenous PEA.

Clearly, an increase in DA content alone was not sufficient to produce an increase in motor activity, since both L-DOPA and clorgyline alone significantly increased DA all brain regions studied but did not alter motor activity. An additional stimulus which causes the release of presynaptic DA, or possibly an increase in DA above some critical threshold level, is necessary to produce motor stimulation.

Of further interest is the influence of MAO inhibition on the stimulation induced by amphetamine. While it has long been known that non-selective MAO inhibition increases the effects of amphetamine [14], it was found that inhibition of either form of MAO would lead to this effect. Since this was not due to alterations in amphetamine metabolism or distribution, it suggests that the monoamines which are substrates for these forms of MAO play a role in defining the pharmacological effects of the drug. Thornburg and Moore [15], using selective inhibitors of catecholamine synthesis, found that amphetamine is dependent on DA for its effects, presumably by facilitating release. The potentiation of amphetamine by clorgyline would be consistent with this hypothesis by sparing releasable DA from degradation. On the other hand, Borison and coworkers [1] suggested that amphetamine acts via endogenous PEA to produce stimulation based upon a series of pharmacological tests in the rabbit, which included an amphetamine-induced reduction of brain PEA concentrations. A decrease in PEA metabolism in the presence of MAO-B inhibition could account for the potentiation produced by pargyline. These results suggest that amphetamine may utilize either DA or PEA to produce its pharmacological effects *in vivo*.

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