Table 3. *Monoamine oxidase activity in rat brain after single and repeated administration of phenelzine and bromocriptine.*

Drug	t (h)	n	MAO-activity μmol min^{-1} g^{-1}	%
None		4	201 s.d. 6	100 s.d. 3
Phenelzine	1	4	56 s.d. 25	28 s.d. 12***
1 day	2	4	46 s.d. 27	23 s.d. 13***
(17 mg kg^{-1}, i.p.)	4	4	46 s.d. 4	23 s.d. 2***
	8	4	37 s.d. 9	18 s.d. 4***
None		10	207 s.d. 9	100 s.d. 4
Bromocriptine	1	5	216 s.d. 8	104 s.d. 4
1 day	2	5	215 s.d. 4	104 s.d. 2
(32 mg kg^{-1}, s.c.)	4	5	215 s.d. 5	104 s.d. 2
Bromocriptine	1	5	207 s.d. 6	100 s.d. 3
7 days	2	5	208 s.d. 5	100 s.d. 2
(10 mg kg^{-1}, s.c.)	4	5	209 s.d. 6	101 s.d. 3

t is the time in h between the last administration of the drugs and killing of the animals.
n represents the number of rats used. Statistical comparison with *t*-test: *** $P < 0.001$.

metabolic changes which take several days to yield biologically significant changes, it was of interest to measure MAO-activity in whole rat brain after repeated administration of bromocriptine. As shown in Table 3, a single dose of phenelzine (17 mg kg^{-1}, i.p.) caused a pronounced reduction in brain MAO-activity, lasting for several hours. In contrast, no significant changes in brain MAO-activity were observed when bromocriptine was administered either as a single dose of 32 mg kg^{-1} (s.c.) or for 7 consecutive days at a dose of 10 mg kg^{-1} (s.c.). These data do not permit the conclusion that bromocriptine affects the activity of brain MAO indirectly via metabolic changes. Therefore, bromocriptine seems to differ from L-dopa in that it does not affect the activity of MAO either by a direct action or indirectly via metabolic changes.

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Inhibition of mouse brain monoamine oxidase by (+)-amphetamine *in vivo*

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The biochemical basis of amphetamine's central stimulant action is still uncertain (see review by Groves & Rebec, 1976), but the possibility that it might act partly by inhibiting monoamine oxidase (Mann & Quastel, 1940) is nowadays usually discounted. Attempts to demonstrate monoamine oxidase inhibition directly in amphetamine-treated animals by excising the brains, homogenizing them and adding substrate, have failed (Randall & Bagdon, 1959; Parmar, Poulose & Bhargava, 1967). However, amphetamine is a reversible inhibitor of monoamine oxidase, and, as pointed out by Planz, Palm & Quiring (1973), the dilution of the tissue which occurs when the brain is homogenized will also dilute the inhibitor. This will result in a level of inhibition in the subsequent assay which is much lower than that which existed in the original brain. This effect will be accentuated if, as is usually the case, the assay is conducted using a high substrate concentration. An alternative way of showing that competitive, reversible inhibitors inhibit monoamine oxidase *in vivo* is to demonstrate that the compound can prevent the inhibitory action of a labile, irreversible inhibitor (Horita & McGrath, 1960; Kuntzman & Jacobson, 1963; Horita & Chinn, 1964; Horita, 1965; Pletscher & Besendorf, 1959; Planz & others 1973). Amphetamine has been shown to act in this way *in vitro* (Green, 1964; Parmar, 1966), but not so far *in vivo*. In this communication, (+)-amphetamine is shown to reduce the level of brain monoamine oxidase inhibition produced by phenelzine *in vivo*. Phenelzine is an irreversible inhibitor producing inhibition lasting several days, but the active form of the drug itself is short-lived in the brain, disappearing within about 30 min from the time of injection. (+)-Amphetamine has a half-life in mouse brain of about 1 h (Fuller & Hines, 1967a).

Pairs of male mice (20 to 30 g, CBA strain) were given (+)-amphetamine sulphate or 0·9% NaCl 15 min before phenelzine hydrogen sulphate. The drugs were dissolved in 0·9% NaCl and injected subcutaneously in a volume of 10 ml kg^{-1}; the phenelzine hydrogen sulphate was also neutralized with sodium bicarbonate. Mice given amphetamine were kept in boxes singly to minimize the degree of central excitement. After 24 h the mice were killed and the brains homogenized in 0·1 M sodium phosphate buffer (pH 7·4). Monoamine oxidase was assayed essentially as described by Otsuka & Kobayashi (1964) except that the substrate was [^{14}C]5-hydroxytryptamine (11·5 μM) instead of tyramine.

Fig. 1 shows the effect of pretreatment with 2 or 5 mg kg^{-1} of (+)-amphetamine sulphate on the extent of brain

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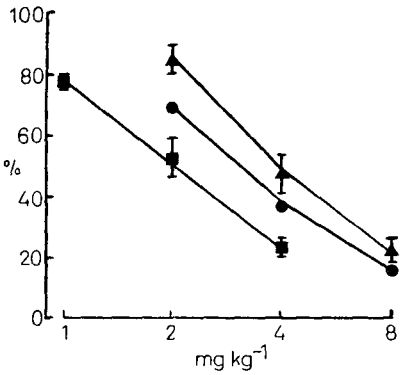

FIG. 1. Effect of pretreatment with 0·9% NaCl (■), or (+)-amphetamine sulphate [2 mg kg^{-1} (●); 5 mg kg^{-1} (▲)] on inhibition of mouse brain monoamine oxidase by phenelzine *in vivo*. All points are the means (± s.e.m.) from assays on at least 4 pairs of mice. For clarity, error bars are omitted from the points for the lower dose of (+)-amphetamine. Ordinate: Brain monoamine oxidase (% of control). Abscissa: Phenelzine hydrogen sulphate (mg kg^{-1}).

monoamine oxidase inhibition produced 24 h after various doses of phenelzine. The measured brain monoamine oxidase activity of mice treated with these doses of (+)-amphetamine alone under these conditions did not significantly differ from that of saline-treated controls. The dose of phenelzine required to cause 50% inhibition was approximately doubled in mice pretreated with 5 mg kg^{-1} of (+)-amphetamine sulphate. This suggests that the (+)-amphetamine has halved the number of active centres available for reaction with phenelzine; that is, the (+)-amphetamine has itself caused about 50% inhibition of brain monoamine oxidase.

Fuller & Hines (1967a) showed that 1 h after 5 mg kg^{-1} of (+)-amphetamine sulphate administered intraperitoneally in mice, the brain concentration was about 16 μmol kg^{-1}. The same workers (1967b) also showed that about a third of this material was protein-bound, thus the concentration of free (+)-amphetamine would probably be about 10 μmol kg^{-1}. The I50 value for inhibition by (+)-amphetamine of monoamine oxidase in mouse brain homogenate assayed *in vitro*, using the same assay procedure, was about 6 μM. This is in close agreement with the value reported previously (Green, 1970) for inhibition by (+)-amphetamine of monoamine oxidase in isolated mitochondrial or microsomal fractions from rat or mouse brain with 5-HT, noradrenaline or tyramine as substrates.

Our experiments thus indicate that (+)-amphetamine at pharmacologically-active doses can cause appreciable inhibition of mouse brain monoamine oxidase *in vivo*. This action might account for the ability of (+)-amphetamine to cause a rise in brain 5-HT and a fall in the concentration of 5-hydroxyindoleacetic acid (Hitzemann, Loh & Domino, 1971), and for its ability to shift the distribution of brain catecholamine metabolites from deaminated to *O*-methylated derivatives (Glowinski, Axelrod & Iversen, 1966; Leitz & Stefano, 1971). In so far as monoamine oxidase inhibition may preserve from destruction those biogenic amines released by (+)-amphetamine from their intraneuronal storage sites in the brain (Groves & Rebec, 1976), this action may also contribute to some of the stimulant effects of amphetamine on the central nervous system.

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