Lack of effect of bromocriptine on the activity of monoamine oxidase in rat brain

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Recently, Zeller, Boshes & others (1976) showed that the activity of monoamine oxidase (MAO) in platelets of patients suffering from parkinsonism was reduced when the patients were treated with L-dopa. Extrapolating from platelet to cerebral MAO, it was suggested that L-dopa may not only provide an increase in dopamine supply but may also delay the degradation of this neurotransmitter by reducing MAO-activity. L-Dopa was postulated to affect platelet MAO-activity indirectly via metabolic changes, a process which would take several days to yield biologically significant changes in the activity of this enzyme. Since it was of interest to see whether these findings could be extended to other drugs used in the treatment of parkinsonism, the effect of the ergot derivative bromocriptine on the activity of MAO in rat brain was investigated. Possible direct effects of bromocriptine on MAO-activity were investigated by measuring MAO-activity in whole brain homogenate and the concentration of the noradrenaline metabolite 3-methoxy-4-hydroxyphenylglycol sulphate (MOPEG-SO_4) in the brain stem after subcutaneous administration of the drug. To assess possible indirect effects of bromocriptine, the activity of brain MAO was measured after administration of the agent for one week. The MAO-inhibitor phenelzine was used as a reference compound.

Male Sprague-Dawley rats, 150–250 g were used. MAO-activity was measured by the method of Weissbach, Smith & others (1960). After decapitation of the rats, the brains were rapidly homogenized in ice-cold 0·32 M sucrose solution, using a Teflon pestle (Thomas, Philadelphia). Each enzyme assay contained 1 mg rat brain, 5×10^{-5} M kynuramine dihydrobromide and 0·1 M tris buffer pH 8·5 in a total volume of 0·6 ml. After 20 min incubation at 37° the reaction was stopped by addition of 1M sodium hydroxide solution, and the amount of 4-hydroxyquinoline formed was measured fluorimetrically. For the determination of MOPEG-SO_4, the pooled brain stems of 2 rats were homogenized in 0·4 M perchloric acid, and the homogenates were centrifuged at 12800 *g* for 10 min at 0–4°. MOPEG-SO_4 was isolated from the neutralized perchloric acid extracts by adsorption onto Dowex 1 × 4 100–200 mesh, eluted with dilute perchloric acid and measured fluorimetrically (Meek & Neff, 1972). Bromocriptine methanesulphonate was dissolved in tartaric acid and diluted with 5% glucose solution or water. Phenelzine sulphate (Aldrich) was dissolved in water. For the *in vivo* experiments (Tables 2 and 3), doses of bromocriptine and phenelzine were used which in previous experiments were found to lower the concentration of striatal 3,4-dihydroxyphenylacetic acid, a major metabolite of dopamine, by at least 30% (unpublished observations).

Table 1. *Effects of phenelzine and bromocriptine on the activity of monoamine oxidase in whole rat brain homogenate.*

Inhib. concn (M)	MAO activity (μmol min^{-1} g^{-1}) With phenelzine	%	With bromocriptine	%
none	0·191	100	0·198	100
10^{-5}	0	0	0·187	94
10^{-6}	0·002	1	0·192	97
10^{-7}	0·040	21	0·192	97
10^{-8}	0·137	72	0·195	98

Table 2. *Effects of phenelzine and bromocriptine on the content of 3-methoxy-4-hydroxyphenylglycol sulphate in rat brain stem.*

Drug	t (h)	n	MOPEG-SO_4 content ng g^{-1}	%
None		9	271 s.d. 55	100 s.d. 20
Phenelzine (17 mg kg^{-1}, i.p.)	1	6	245 s.d. 41	90 s.d. 15
	2	5	195 s.d. 56	72 s.d. 21*
	4	8	167 s.d. 33	62 s.d. 12***
	8	5	165 s.d. 30	61 s.d. 11***
None		9	271 s.d. 18	100 s.d. 7
Bromocriptine (10 mg kg^{-1}, s.c.)	1	7	267 s.d. 36	99 s.d. 13
	2	8	264 s.d. 57	97 s.d. 21
	4	7	358 s.d. 52	132 s.d. 19***
	8	6	396 s.d. 59	146 s.d. 22***

t is the time in h between drug administration and killing the animals. n represents the number of determinations, each performed on the pooled homogenates of brain stems of 2 rats. Statistical comparison with *t*-test: * $P < 0.05$, *** $P < 0.001$.

As expected, phenelzine, added directly to the brain homogenates, strongly inhibited MAO with a pI50 of about 7·6 (Table 1). In contrast, no significant inhibition of MAO-activity could be demonstrated after the addition of 10^{-8} to 10^{-5} M bromocriptine. Further evidence indicating that bromocriptine has no direct action on MAO-activity was obtained when the concentration of brainstem MOPEG-SO_4 was measured (Table 2). Formation of this noradrenaline metabolite requires MAO-activity. Inhibition of MAO-activity by a single dose of phenelzine (17 mg kg^{-1}, i.p.) led to a highly significant decrease in the concentration of MOPEG-SO_4 which lasted for several hours. Administration of bromocriptine (10 mg kg^{-1}, s.c.), on the other hand, had no effect on the concentration of this metabolite up to 2 h after administration, and increased it after 4 h and more. The failure of bromocriptine to decrease the concentration of MOPEG-SO_4 is further evidence that this drug, even at high doses, has no direct effect on brain MAO-activity.

Since it was suggested (Zeller & others, 1976) that L-dopa affected platelet MAO-activity indirectly via

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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