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Amphetamine, *p*-hydroxyamphetamine and β-phenethylamine in mouse brain and urine after (−)- and (+)-deprenyl administration

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Deprenyl selectively inhibits the B form of monoamine oxidase (MAO) (Christmas et al 1972; Knoll & Magyar 1972) and evokes in animals an acute central nervous excitant effect similar to that of amphetamine (Knoll et al 1965; Braestrup et al 1975). Both methamphetamine and amphetamine are present in the urine of normal human males receiving 5–10 mg of (−)-deprenyl daily (Reynolds et al 1978a), while amphetamine has been found post mortem in the brains of similarly treated Parkinson patients (Reynolds et al 1978b, 1979). The experiments described here confirm the presence of amphetamine in the brain and urine of mice treated with relatively low doses of either (−)- or (+)-deprenyl. The brain concentration and urinary excretion of phenethylamine (PE) were also measured to assess the MAO inhibitory effects of the doses of deprenyl administered.

Materials and methods

Brain tissue analysis. Male Swiss mice (ca 24 g) were injected with (−)- or (+)-deprenyl hydrochloride in 0·9% NaCl (saline) (i.p., 0·1 or 1·0 mg kg^{-1}), or for controls, with saline alone, and killed at times ranging from 15 min to 6 h later. The brains were quickly removed, frozen on dry ice, weighed, and homogenized in 2 ml of 15% sodium carbonate (w/v) containing deuterated (±)-amphetamine (50 or 100 ng), *p*-hydroxyamphetamine (*p*-OH amphetamine, 25 ng) and PE (25 ng). The amines were extracted into two 2 ml portions of distilled acetone, then reacted overnight at room temperature (20 °C) with 0·5 ml of dansyl chloride reagent (8 mg ml^{-1} acetone). Acetone was evaporated under a stream of nitrogen, and the dansyl amines were extracted into benzene and isolated on silica gel thin layer chromatography plates. *p*-OH amphetamine was separated from amphetamine and PE in the solvent system chloroform–butyl acetate (5:1 v/v). *p*-OHamphetamine was further purified, and amphetamine and PE were separated from each other on a second chromatogram developed consecutively with benzene–triethylamine (8:1 v/v), then carbon tetrachloride–triethylamine (5:1 v/v). No attempt was made to identify optical isomers. The amines were measured mass spectrometrically (Durden et al 1973; Danielson & Boulton 1976).

To examine the possibility that amphetamine might arise from deprenyl as an artifact of the extraction procedure, duplicate 0·1 mg samples of each isomer of deprenyl were added to 2 ml of 15% sodium carbonate containing deuterated amphetamine (50 ng) and *p*-OHamphetamine (25 ng) and processed as indicated.

Urine analysis. Amphetamine, *p*-OHamphetamine and PE were determined in the urine of mice injected with (−)- or (+)-deprenyl hydrochloride in saline (0·1 or 1·0 mg kg^{-1}), or with saline solution alone (controls). The animals were placed in porcelain Buchner funnels (85 mm diameter) fitted with stainless steel screen tops, and fasted but allowed free access to water for 24 h. Urine was collected into tubes containing 2 drops of concentrated hydrochloric acid. At the end of the collection period, the funnels were rinsed with distilled water and the volume of diluted urine adjusted to 20 ml. A 2-ml sample was removed for analysis and deuterated amphetamine (50 or 500 ng, depending on the dose of deprenyl), *p*-OHamphetamine (25 ng) and PE (100 ng) were added. For control samples, 10 ml of diluted urine was lyophilized, then re-dissolved in 2 ml of distilled water. The samples were saturated with sodium carbonate and processed as described above.

Results

Brain amine concentrations. Amphetamine was observed in the brain following administration of either (−)- or (+)-deprenyl, the amount increasing in approximate proportion to the dose of the drug (Table 1). The maximum concentration observed occurred 15 min after drug administration, the shortest time examined in these experiments, and decreased to less than 5% of that level at 6 h. Only traces of *p*-OHamphetamine could be detected in the brain (Table 2). Neither amphetamine nor *p*-OHamphetamine could be detected in samples that contained deprenyl but no tissue. At 0·1 mg kg^{-1}, neither (−)- nor (+)-deprenyl increased brain PE significantly, but surprisingly, significant decreases were observed 6 h after (−)-deprenyl, and 4–6 h after (+)-deprenyl (Table 3). A similar decrease occurred 6 h after 1·0 mg kg^{-1} of (+)-deprenyl, but at most other times examined, that dose of either (−)- or (+)-deprenyl significantly increased brain PE.

Table 1. Concentration of amphetamine in mouse whole brain after deprenyl administration. Values are the means ± s.e.m. of 6–12 values and are expressed as ng g^{-1} fresh tissue.

Time h	(−)-Deprenyl 0·1 mg kg^{-1}	(−)-Deprenyl 1·0 mg kg^{-1}	(+)-Deprenyl 0·1 mg kg^{-1}	(+)-Deprenyl 1·0 mg kg^{-1}
Control	<0·01	<0·01	<0·01	<0·01
0·25	6·23 ± 0·45	84·4 ± 10·9	8·12 ± 0·84	69·5 ± 6·4
0·50	5·36 ± 0·65	75·7 ± 7·8	6·60 ± 0·51	70·0 ± 5·2
1	2·96 ± 0·27	43·0 ± 3·6	3·20 ± 0·32	28·9 ± 1·8
2	1·15 ± 0·11	15·0 ± 1·9	1·10 ± 0·17	7·42 ± 1·07
4	0·40 ± 0·07	3·69 ± 0·24	0·68 ± 0·14	2·62 ± 0·43
6	0·28 ± 0·05	2·20 ± 0·23	0·38 ± 0·09	1·79 ± 0·47

Table 2. Concentration of *p*-hydroxyamphetamine in mouse whole brain after deprenyl administration. Values are the means ± s.e.m. of 3–7 values and are expressed as ng g^{-1} fresh tissue.

Time h	(−)-Deprenyl 0·1 mg kg^{-1}	(−)-Deprenyl 1·0 mg kg^{-1}	(+)-Deprenyl 0·1 mg kg^{-1}	(+)-Deprenyl 1·0 mg kg^{-1}
Control	<0·01	<0·01	<0·01	<0·01
0·25	0·08 ± 0·03	0·05 ± 0·01	0·01 ± 0·01	<0·01
0·50	0·10 ± 0·03	0·08 ± 0·04	<0·01	<0·01
1	0·04 ± 0·03	0·04 ± 0·02	0·02 ± 0·02	0·07 ± 0·05
2	0·07 ± 0·02	0·06 ± 0·02	0·01 ± 0·01	<0·01
4	0·09 ± 0·03	0·02 ± 0·01	<0·01	0·03 ± 0·03
6	0·12 ± 0·05	0·03 ± 0·02	<0·01	0·02 ± 0·02

Urinary excretion of amines. Similar and dose-dependent amounts of amphetamine were excreted in the urine after administration of either isomer of deprenyl (Table 4). Approximately 3·5% of the amphetamine theoretically available from deprenyl was excreted within 24 h. Small amounts of *p*-OHamphetamine were also found, the amount excreted after (+)-deprenyl being almost twice that excreted after (−)-deprenyl (Table 4). Although neither 0·1 nor 1·0 mg kg^{-1} doses of (+)-deprenyl affected the excretion of PE, significant increases were observed after identical doses of (−)-deprenyl.

Discussion

The occurrence of appreciable concentrations of amphetamine in the brain and urine of deprenyl-treated mice is in agreement with previous observations in man (Reynolds et al 1978a,b, 1979). In the mouse, brain amphetamine concentrations reached 6 ng g^{-1} after 0·1 mg kg^{-1} of (−)-deprenyl. Urinary PE excretion increased significantly but brain PE was not affected. These results indicate that the inhibition of tissue MAO was not sufficient to increase tissue PE concentrations. Even after a 1·0 mg kg^{-1} dose of either (−)- or (+)-deprenyl, the brain PE concentration was only about twice that in control animals. Much greater increases have previously been obtained by using higher doses of deprenyl or other MAO inhibitors (Philips & Boulton 1979).

In man, a daily dose of (−)-deprenyl equivalent to 0·1 mg kg^{-1} in the mouse (approximately 6·8 mg) strongly inhibited platelet MAO activity and increased urinary PE excretion (Elsworth et al 1978), and has recently been used in conjunction with L-dopa and a peripheral decarboxylase inhibitor to treat Parkinson's disease (Birkmayer et al 1977; Lees et al 1977). It has been suggested that the pharmacological effects of (−)-deprenyl in man may arise not only from its ability to block MAO-B and thus to inhibit dopamine oxidation, but also, or alternatively, from the production of amphetamine metabolites or from the elevated levels of PE which arise as a result of MAO-B inhibition (Reynolds et al 1979). The relatively small increases in PE observed in mouse brain suggest, however, that MAO-B is not extensively inhibited by deprenyl at doses of 1·0 mg kg^{-1} or lower, although these doses are capable of producing substantial concentrations of amphetamine in the brain. If similar conditions occur in man, it is quite possible that much of the clinical effect of deprenyl is due to its amphetamine metabolite rather than to its MAO inhibitory effect. (−)-Deprenyl, which is a more effective MAO inhibitor than (+)-deprenyl (Knoll & Magyar 1972) and is used clinically, is likely to be metabolized to (−)-amphetamine. Although (−)-amphetamine is less active than its (+)-isomer in inducing motor hyperactivity and stereotyped behaviour (Taylor & Snyder 1970; Thornburg & Moore 1972; North et al 1974), amphetamine-like effects have nevertheless been reported in patients treated with (−)-deprenyl (Birkmayer et al 1977; Elsworth et al 1978). The fact that amphetamine metabolites may accumulate in patients receiving long term treatment with (−)-deprenyl (Reynolds et al 1978b, 1979) and that chronic ingestion of amphetamine by man is known to produce a syndrome resembling that of paranoid schizophrenia (Connell 1958) suggests that the long term use of deprenyl as a clinical agent should be undertaken with some caution.

Table 3. Concentration of β-phenethylamine in mouse whole brain after deprenyl administration. Values are the means ± s.e.m. of 6–12 values (except control, n = 29) and are expressed as ng g^{-1} fresh tissue. PE concentrations significantly different from control values, as determined by Student's *t*-test: $^{a}P < 0·05$; $^{b}P < 0·01$; $^{c}P < 0·001$.

Time h	(−)-Deprenyl 0·1 mg kg^{-1}	(−)-Deprenyl 1·0 mg kg^{-1}	(+)-Deprenyl 0·1 mg kg^{-1}	(+)-Deprenyl 1·0 mg kg^{-1}
Control	1·76 ± 0·16	1·76 ± 0·16	1·76 ± 0·16	1·76 ± 0·16
0·25	1·56 ± 0·18	2·62 ± 0·27^{b}	2·00 ± 0·28	4·02 ± 0·69^{b}
0·50	1·36 ± 0·15	2·73 ± 0·17^{c}	1·64 ± 0·19	3·23 ± 0·43^{b}
1	1·43 ± 0·12	2·66 ± 0·20^{b}	1·51 ± 0·16	2·44 ± 0·16^{b}
2	1·34 ± 0·26	2·60 ± 0·28^{a}	1·38 ± 0·18	2·07 ± 0·17
4	1·38 ± 0·27	2·64 ± 0·27^{b}	1·01 ± 0·09^{c}	1·91 ± 0·25
6	0·56 ± 0·07^{c}	1·77 ± 0·24	0·98 ± 0·14^{c}	1·32 ± 0·09^{a}

Table 4. Urinary excretion of amphetamine, *p*-hydroxyamphetamine and β-phenethylamine by the deprenyl-treated mouse. Values are the means ± s.e.m. of 6–10 values and are expressed as ng per day. PE values significantly different from control values, as determined by Student's *t*-test: $^{a}P < 0·01$; $^{b}P < 0·001$.

Drug and dose (mg kg^{-1})	β-Phenethylamine	Amphetamine	*p*-Hydroxyamphetamine
(−)-Deprenyl Control	158 ± 10	<0·01	<0·01
0·1	230 ± 21^{a}	77·9 ± 7·3	1·31 ± 0·35
1·0	298 ± 20^{b}	709 ± 91	9·80 ± 1·27
(+)-Deprenyl Control	158 ± 10	<0·01	<0·01
0·1	168 ± 15	87·8 ± 18·5	2·55 ± 0·27
1·0	132 ± 11	819 ± 147	16·7 ± 1·4

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Cinromide (3-bromo-*N*-ethylcinnanamide), a novel anticonvulsant agent

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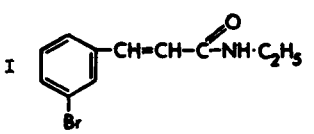
In a review article on antiepileptic drug development, Krall et al (1978) stated that despite optimal use of the 16 antiepileptic drugs marketed in the United States many patients with epilepsy fail to experience seizure control and others do so only at the expense of significant toxic side effects. In addition, multiple drug therapy, which has a number of obvious dangers such as drug interactions, is currently being practiced by many physicians in an attempt to control various types of seizures occurring within the same patient and also to control specific refractory seizure types in given patients.

We, therefore, have been seeking an agent that coupled a broad spectrum of anticonvulsant activity in a variety of animal models with low toxicity with the aim that such an agent might remove the need for multiple drug therapy. Cinromide appears to meet these efficacy and safety criteria and has the structure I.



The pharmacological properties of cinromide to date have been reported only in abstract form (Welch et al 1978; Soroko et al 1979). It appears to be a promising new antiepileptic drug as evidenced by suppression of highly refractory seizures of the Lennox-Gastaut syndrome in children (Lockman et al 1981). Two other open clinical studies suggest that cinromide is safe and has antiepileptic activity when used as adjunct therapy in patients with refractory partial seizures (Peck et al 1981) and in patients with uncontrolled primary and secondary generalized seizures (Ramsay et al 1981).

Anticonvulsant actions. In mice, cinromide afforded protection against maximal electroshock convulsions. The anticonvulsant activity was dose-related following both i.p. and oral administration (Fig. 1A). The ED50 values were 60 ± 11 mg kg^{-1} and 80 ± 15 mg kg^{-1} i.p. and orally, respectively. Similarly, cinromide afforded protection against maximal electroshock convulsions in the rat (Fig. 1B). The ED50 values were 58 ± 12 and 26 ± 6 mg kg^{-1}, i.p. and orally, respectively. In the rat following 50 mg kg^{-1} (ED84) orally the peak effect occurred at 60 min with a duration of 4–5 h. In addition, cinromide was 100% effective in blocking low-frequency (6 Hz) minimal electroshock seizures (Swinyard et al 1962) in mice at 400 mg kg^{-1} orally.

Cinromide afforded protection against leptazol (pentetrazol)-induced convulsions in mice. The i.p. and oral ED50 values were 90 ± 15 and 300 ± 61 mg kg^{-1}, respectively (Fig. 1C). In rats, cinromide administered i.p. produced a dose-related antileptazol activity with an ED50 value of 58 ± 11 mg kg^{-1} (Fig. 1D). Administered orally to rats, cinromide afforded protection to only 50% of the animals at doses as high as 600 mg kg^{-1}. In the intravenously infused leptazol-threshold test in rats, cinromide, at 75 mg kg^{-1} orally, significantly ($P = 0.01$) elevated the amount of leptazol needed to induce clonic seizures.

Table 1 summarizes the oral ED50 values obtained 1 h post-administration for cinromide and a variety of clinically effective anticonvulsants. Against maximal electroshock seizures in the mouse, cinromide was more effective than either trimethadione, phensuximide or valproic acid and

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