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COMMUNICATIONS

The effect of some tricyclic antidepressants on the inhibition of mouse brain monoamine oxidase in-vivo by phenelzine

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Abstract—Four tricyclic antidepressants, amitriptyline, imipramine, desipramine and iprindole have been shown to partially protect mouse brain monoamine oxidase in-vivo from the irreversible enzyme inhibition produced by subsequent injection of phenelzine. Levels of protection were similar when the enzyme was assayed with selective substrates (5-hydroxytryptamine and phenethylamine) for both the A and B forms of the enzyme. Although other explanations cannot at this stage be ruled out, these observations are consistent with the tricyclic antidepressants acting as reversible inhibitors of brain monoamine oxidase in-vivo.

A variety of tricyclic antidepressants have been shown to be moderately active inhibitors of brain monoamine oxidase (EC 1.4.3.4., MAO) in-vitro (Roth & Gillis 1975; Roth 1978; Achee & Gabay 1979; Green & McGachy 1987), but it is still unclear whether this inhibition is relevant to their clinical effectiveness (Sulser & Mobley 1980). Regular administration of some of these compounds has been reported to cause slight inhibition of brain MAO in rats (Arora & Meltzer 1976) and to reduce blood platelet MAO in man (Sullivan et al 1977). However, since these drugs are reversible inhibitors of the enzyme, assay of MAO in tissue extracts prepared from animals or man is likely to underrate the true level of inhibition in-vivo (Green 1984). A method has been described for measuring the extent of MAO inhibition by reversible inhibitors in-vivo based on their ability to prevent the irreversible inhibition produced by subsequent injection of a labile irreversible inhibitor (Green & El Hait 1980). We have attempted to assess the inhibition of mouse brain MAO in-vivo by amitriptyline, imipramine, desipramine and iprindole from the extent to which they protect the enzyme from inhibition by phenelzine.

Methods

Groups of four male mice (BKA strain, ca 30 g) were given a subcutaneous injection of 0·9% NaCl (saline) or one of the tricyclic antidepressants (at a dose of 20 mg kg^{-1}) 30 min before a subcutaneous injection of phenelzine hydrogen sulphate (4 mg kg^{-1}). All the compounds were dissolved in saline and injected in a volume of 10 mL kg^{-1}; the phenelzine hydrogen sulphate was also neutralized with sodium bicarbonate. After 24 h the mice were killed and their brains were homogenized in 0·1 M sodium phosphate buffer (pH 7·4). MAO was assayed using [^{14}C]5-HT (11·5 μM) or [^{14}C]2-phenethylamine (PEA) (5μM) as substrates as described by Green & McGachy (1987). Each assay mixture (total volume 2 mL) contained 5 mg brain tissue when 5-hydroxytryptamine (5-HT) was used, but only 0·5 mg when phenethylamine was used. The absolute MAO activities (in nmol product h^{-1} mg^{-1} tissue) of control mouse brain homogenates were approximately 1·5 with 5-HT and 4 with PEA. At the substrate concentrations used here, activity with 5-HT is almost exclusively due to MAO-A (Fowler & Tipton 1982) and that with PEA to MAO-B (Kinemuchi et al 1980). Four groups of mice were used in each experiment, one given two injections of saline for use as a control, one given saline and phenelzine and two given an antidepressant before the phenelzine. All MAO assays were carried out in triplicate. Brain MAO in mice 24 h after being given one of the antidepressants alone did not differ significantly from that in the brains of control mice.

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Results

The results are summarised in Table 1. The statistical significances of the differences between the levels of inhibition found after the antidepressant plus phenelzine and phenelzine alone were calculated from the measured enzyme activities using Student's *t*-test. The calculated extent of MAO inhibition due to the tricyclic antidepressants themselves, shown in the final two columns of Table 1, was derived using the equation given by Green & El Hait (1980). Although there was some variation in the results from day to day, in every experiment the level of MAO inhibition due to the phenelzine was reduced in the mice pretreated with one of the antidepressants.

The brain levels of the tricyclic antidepressants reached in these experiments were not measured, but the brain levels of some chlorpromazine analogues given subcutaneously to mice have been shown to reach a maximum corresponding to roughly uniform distribution of the injected dose (Green 1967). Similar results were obtained for brain levels of imipramine given intraperitoneally to rats (Bickel & Weder 1968). Assuming this is also true in mice for these antidepressants of broadly similar structure, the expected maximum brain levels from a dose of 20 mg kg^{-1} would be around 60 μM. At such concentrations in-vitro these compounds would be expected to produce around 30% inhibition of 5-HT oxidation but around 70% or more inhibition of PEA oxidation (Green & McGachy 1987).

Discussion

It is clear that all four of these tricyclic antidepressants exert a protective action against inhibition of brain MAO by phenelzine in-vivo with both substrates. The order of potency corresponds roughly with their potency as MAO inhibitors in-vitro, particularly of the B form of the enzyme. While these results are consistent with these compounds inhibiting brain MAO-A and MAO-B in-vivo, other explanations for the observed protective action cannot at this stage be ruled out. If the observed protective effect is solely due to occupancy of the active sites of

Table 1. Effect of pretreatment with tricyclic antidepressants on the MAO activity of mouse brain homogenates 24 h after injection of phenelzine, and calculated MAO inhibition produced by the antidepressants alone.

	MAO activity (% of control, mean ± s.e.) with				Calculated % MAO inhibition by the tricyclic antidepressant†	
	5-HT (11·5 μM)		Phenethylamine (5 μM)			
Tricyclic antidepressants	Phenelzine alone	Antidepressant + phenelzine	Phenelzine alone	Antidepressant + phenelzine	5-HT	Phenethylamine
Amitriptyline	26·6 ± 1·9(4)	53·4 ± 2·9(4)***	33·5 ± 1·6(4)	53·1 ± 4·1(4)***	53	42
Imipramine	26·7 ± 1·9(4)	41·3 ± 0·6(4)***	35·2 ± 1·3(4)	50·9 ± 3·3(4)***	33	35
Desipramine	26·8 ± 2·3(4)	40·6 ± 2·8(6)***	38·5 ± 2·0(4)	45·4 ± 2·7(6)*	32	17
Iprindole	24·4 ± 1·3(5)	31·2 ± 0·6(7)***	35·4 ± 1·7(5)	40·4 ± 1·4(7)**	17	13

The number of groups of 4 mice used in each case is shown in parentheses.
*** $P<0.01$, ** $P<0.05$, * $P=0.1$.

† Calculated using % reversible inhibition =

$$100\times\left[1-\frac{\log(100/\%\text{ residual activity after phenelzine + tricyclic})}{\log(100/\%\text{ residual activity after phenelzine alone})}\right]$$

MAO by the tricyclics, the finding that they are, if anything, more effective with 5-HT as substrate is unexpected, although Arora & Meltzer (1976) reported that repeated injection of imipramine into rats caused more inhibition of brain MAO when this was assayed directly with 5-HT than was observed with benzylamine. A possible alternative explanation is that they interfere in some way with access of the phenelzine to the brain neurones or glial cells. It is unlikely to be a consequence of the blockade by these compounds of biogenic amine uptake mechanisms since iprindole is far weaker than the other three compounds in this respect (Sulser & Mobley 1980).

The extrapolation of these results to the clinical situation remains problematical. Although the effect we have obtained is relatively small, this is after a single dose in a species in which the half-life of the antidepressants is probably short (Cassano et al 1965). In man, the rate of metabolism and excretion is much slower than it is in small rodents (Eschenhof & Rieder 1969), giving more opportunity for accumulation on repeated dosing. At least a week or two of treatment is required before any alleviation of the symptoms of depression occurs with either the tricyclic antidepressants or the established MAO inhibitors. The fact that there is an interaction between tricyclic antidepressants and irreversible MAO inhibitors which manifests itself at the enzyme level should also be borne in mind when depressed patients are treated with tricyclics and irreversible MAO inhibitors in combination (e.g. see Pare 1984), although how this interaction would affect the enzyme activity when these drugs are given repeatedly, as in normal clinical practice, is uncertain.

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