J. Pharm. Pharmacol. 1987, 39: 392–394
Communicated November 17, 1986

The inhibition of monoamine oxidase by tricyclic antidepressants: the influence of the nature of the substrate and the source of the enzyme

A. L. GREEN*, H. ADRIENNE MCGACHY, *Department of Bioscience and Biotechnology, University of Strathclyde, Glasgow G4 0NR, UK*

Five tricyclic antidepressants, amitriptyline, clomipramine, desipramine, imipramine and iprindole, have comparable potencies as inhibitors of monoamine oxidase in rodent brain and liver. With rodent brain, potency was always greater with phenethylamine as substrate than with benzylamine, and was generally least with 5-HT. With mouse liver, in which monoamine oxidase is mainly B type, potency with tyramine and dopamine as substrates was close to that found with phenethylamine. The kinetics of inhibition varied with both the substrate and the tissue, and were inconsistent with a simple ping-pong model for substrate oxidation. The relevance of these observations to clinical effectiveness is discussed.

Numerous workers have confirmed the original observation by Usdin & Usdin (1961) that tricyclic antidepressants inhibit monoamine oxidase (EC 1.4.3.4, MAO) in-vitro. However, there are many inconsistencies among the reported inhibitory potencies, and it is still unclear whether this inhibition is relevant to the clinical effectiveness of these compounds (Sulser et al 1978; Sulser & Mobley 1980). Depending on which tissue is used as the source of the enzyme both the A form (Achee & Gabay 1979) and the B form (Roth & Gillis 1975a,b) of MAO have been reported to be the more susceptible to inhibition, and even with tissues in which the enzyme is almost exclusively B type, such as human platelets, sensitivity to inhibition depends markedly on which substrate is used (Edwards & Burns 1974). In an attempt to clarify some of these discrepancies we have examined both the effect of the choice of tissue and the choice of substrate on the inhibition of MAO by five of the more commonly used tricyclic antidepressants.

Methods

Rat, mouse and guinea-pig brain homogenates were prepared in 0·1 M sodium phosphate buffer (pH 7·4). Rat brain and mouse liver mitochondria were isolated by differential centrifugation of brain or liver homogenized in 0·3 M sucrose (Green & El Hait 1980). MAO was assayed using minor modifications of the radioisotopic method of Otsuka & Kobayashi (1964). When [^{14}C]5-HT, [^{14}C]2-phenethylamine (PEA), [^{14}C]benzylamine or [^{14}C]tyramine were used as substrates, the non-basic reaction products were extracted from the acidified reaction mixture (total 2·4 mL) into diisopropyl ether (10 mL). The aqueous phase was then frozen and the organic solvent layer was decanted into 5 mL of 0·6% PPO in toluene for counting. With [^{14}C]dopamine as substrate, the reaction products were first extracted into ethyl acetate as described by Green & El Hait (1980). Aqueous solutions of the tricyclic antidepressants were normally added to the enzyme 5 min before the substrate, but this is not critical as the extent of inhibition did not depend on the preincubation time. Preliminary experiments showed that centrifugation of rat brain mitochondria in contact with amitriptyline followed by resuspension in fresh buffer resulted in total recovery of the enzyme activity towards both 5-HT and benzylamine, thus confirming previous reports (Edwards & Burns 1974; Roth & Gillis 1974; Achee & Gabay 1979) that inhibition of both the A and B forms of MAO by tricyclic antidepressants is fully reversible.

Results and discussion

The concentrations of the five antidepressants required to produce 50% inhibition of the oxidation of low concentrations of 5-HT, PEA or benzylamine by rat brain homogenates are shown in Table 1. These I50 values were obtained by interpolation from Dixon type plots of the reciprocal of the enzyme activity against the inhibitor concentration. However, these plots were generally not linear; with 5-HT, inhibition was always greater at higher inhibitor concentrations than would have been expected from the extent of inhibition seen at low concentrations, whereas the converse was usually found with benzylamine or PEA. Similar abnormalities in this type of plot were reported

Table 1. I50 values at 37 °C and pH 7·4 for the inhibition of MAO in rat brain homogenates by tricyclic antidepressants.

	I50 (μM) with substrate		
Inhibitor	5-HT (11·5 μM)	PEA (5 μM)	Benzylamine (18·5 μM)
Clomipramine	50	6	18
Imipramine	170	8	20
Amitriptyline	150	8	25
Desipramine	160	15	60
Iprindole	170	22	270

* Correspondence.

by Achee & Gabay (1979) for the inhibition by tricyclic antidepressants of 5-HT or PEA oxidation by bovine liver mitochondria. Nevertheless, with the single exception of iprindole with benzylamine as substrate, all the compounds were more potent against the type B MAO substrates (PEA and benzylamine) than against the type A substrate (5-HT). Amitriptyline was also tested using mouse or guinea-pig brain homogenates as the enzyme source with similar results, the B form being the more sensitive. The I50 values were 9, 30 and 100 μM for mouse brain and 20, 60 and 120 μM for guinea-pig brain with PEA, benzylamine and 5-HT as substrates, respectively. This preferential inhibition of type B MAO accords with previous studies on rabbit tissues (Roth & Gillis 1974, 1975b), but is at variance with studies using bovine brain (Achee & Gabay 1979). However, Achee & Gabay (1977) reported that inhibition of bovine brain mitochondrial MAO by selegiline ((−)-deprenyl) and pargyline, which are selective irreversible inhibitors of type B MAO, failed to show the expected double sigmoid plot of inhibition against the logarithm of the inhibitor concentration when tyramine was used as the substrate, so it may be unsound to apply the normal A/B distinction to this particular tissue.

Although both PEA (at low concentration) and benzylamine are predominantly substrates for type B MAO (Fowler & Tipton 1984), it is clear from Table 1 that all the tricyclic antidepressants are more potent inhibitors of PEA oxidation than of benzylamine oxidation. Similar observations have been made with human platelets (Edwards & Burns 1974) and human brain (Roth 1976). To assess further the importance of the choice of substrate on the inhibitory potency of these compounds against type B MAO, we have also tested them using mouse liver mitochondria as the enzyme source, as the B form appears to predominate in this tissue (Tong et al 1979), and have included tyramine and dopamine as substrates. In a preliminary experiment pre-incubation of mouse liver mitochondria with 0·1 μM selegiline produced 91, 88, 66 and 0% inhibition of the oxidation of benzylamine, tyramine, dopamine and 5-HT, respectively, confirming that the oxidation of tyramine and dopamine in this tissue is mainly due to MAO-B. The results, shown in Table 2, indicate

Table 2. I50 values at 25 °C and pH 7·4 for the inhibition of MAO in mouse liver mitochondria by tricyclic antidepressants.

	I50 (μM) with substrate			
Inhibitor	PEA (5 μM)	Tyramine (14·5 μM)	Dopamine (13·5 μM)	Benzylamine (18·5μM)
Clomipramine	9	13	25	120
Imipramine	20	27	25	250
Amitriptyline	12	15	17	40
Desipramine	40	60	45	300
Iprindole	25	50	55	1500

that, as found with rat brain, PEA oxidation is more susceptible to inhibition by these compounds than is benzylamine oxidation, particularly with iprindole as the inhibitor. They also show that tyramine and dopamine resemble PEA as substrates more closely than benzylamine. As noted with rat brain, plots of the reciprocal of the enzyme activity against the inhibitor concentration were frequently non-linear, the curvature being most marked with benzylamine and least with PEA. Since benzylamine is the most specific of these substrates for MAO-B, this non-linearity cannot be ascribed to partial oxidation of the substrate by the less susceptible type A enzyme.

A more detailed kinetic study was carried out over a range of substrate concentrations using amitriptyline as a representative inhibitor. With the rat brain enzyme and 5-HT (10–200 μM, K_m about 70 μM), amitriptyline at 40 or 100 μM was a competitive inhibitor with a K_i of 130 μM. Amitriptyline has been previously shown to act as a competitive inhibitor of 5-HT oxidation by bovine brain MAO (Achee & Gabay 1979). With rat brain and PEA (1·25–10 μM, K_m about 5 μM) inhibition by amitriptyline at 5, 10, or 20 μM was almost pure non-competitive with a K_i of 7–9 μM, whereas with mouse liver and PEA (2·5–20 μM, K_m about 11 μM), inhibition was close to pure non-competitive with 5 μM amitriptyline but became mixed (an increase in K_m as well as a further decrease in V_{max}) at 10 or 20 μM amitriptyline. With rat brain and benzylamine (20–120 μM, K_m about 60 μM) inhibition was close to competitive at 20 μM amitriptyline but mixed at 40 μM amitryptyline. The actual extent of inhibition found in these kinetic studies with MAO-B substrates accorded well with the I50 values in Tables 1 and 2, but the confusing kinetic patterns are typical of those described in previous literature. Thus, for the inhibition of PEA oxidation by amitriptyline, mixed inhibition was found with bovine brain (Achee & Gabay 1979), mixed (but close to competitive) inhibition with rabbit brain (Roth & Gillis 1975b), non-competitive inhibition with human brain (Roth 1976) and, with human platelets, inhibition varying from near uncompetitive at a low concentration of amitriptyline to pure non-competitive at a high concentration (Edwards & Burns 1974). Benzylamine oxidation by human platelets was inhibited competitively by amitriptyline, irrespective of concentration, but the secondary plot of slope against benzylamine concentration was markedly non-linear (Edwards & Burns 1974).

Roth (1976, 1978) suggested that these kinetic anomalies might arise if the tricyclic antidepressants varied in their affinity for the oxidized and reduced forms of type B MAO (i.e. those in which the flavin prosthetic group is in its oxidized or reduced form). Kinetic studies on purified type B MAO from various sources have yielded parallel double reciprocal plots from which it has been assumed that the enzyme acts by a ping-pong type mechanism. However, Husain et al (1982) concluded from a more detailed pre-steady state study using

purified bovine liver MAO that, depending on the substrate, the pathway could involve ternary complexes as well as binary ones, and that whereas the rate-determining step with benzylamine was the initial dehydrogenation of the substrate, the rate-determining step with PEA was the reoxidation of the reduced form of the enzyme. In any MAO-containing tissue for which this distinction holds, a tricyclic antidepressant binding more strongly to the reduced form of the enzyme than to the oxidized form might be expected to preferentially inhibit PEA oxidation and to inhibit it non-competitively, as observed in our experiments. The competitive inhibition found with amitriptyline and 5-HT suggests that the dehydrogenation step is also the rate limiting one for MAO-A. To explain the non-linearity in the Dixon type plots observed by ourselves and others it would appear necessary to invoke the existence of ternary complexes. The decrease in slope of the plot of reciprocal velocity against inhibitor concentration with increasing inhibitor concentration could arise if oxygen interacted to a limited extent with the reduced form of the enzyme even in the presence of bound inhibitor. The increase in slope of the reciprocal velocity against inhibitor concentration plot found with 5-HT could result if the pathway for the enzyme-catalysed reaction involved ternary complexes and if amitriptyline bound to more than one site on the enzyme surface in a manner that totally prevents the reoxidation of the reduced flavin.

The relevance of MAO inhibition to the clinical effectiveness of tricyclic antidepressants remains uncertain. The fact that iprindole, which is a much weaker inhibitor of amine uptake processes than the other four compounds (Sulser & Mobley 1980), shares with them the ability to inhibit MAO, especially the B form, at comparable concentrations, suggests that MAO inhibition may be a contributory factor (Edwards & Burns 1974). The clinical significance of PEA oxidation is obscure, but dopamine oxidation is also strongly inhibited by these antidepressants. Although MAO-B contributes little to dopamine oxidation in rodent brain (Green & El Hait 1980), this may not be so for human brain (O'Carroll et al 1983), and, in human brain, even noradrenaline, normally regarded as a type A substrate, may be oxidized to a considerable extent by MAO-B (Garrick & Murphy 1982). While much higher concentrations of tricyclic antidepressants are needed to inhibit MAO than are needed with established irreversible MAO inhibitors, they may accumulate sufficiently in brain tissue on repeated administration to reach the required levels (Cassano et al 1965; Edwards & Burns 1974). Sullivan et al (1977) reported that in depressed patients treated for three weeks with 200–300 mg daily of amitriptyline or imipramine, blood platelet MAO was significantly reduced. A clinical study by Rowan et al (1982) has shown that, despite earlier views to the contrary, there was little qualitative difference between the antidepressant effects exerted by amitriptyline and phenelzine. Both required administration for two weeks before any therapeutic effect appeared, and both showed similar efficacies in patients showing broadly the same patterns of symptoms.

REFERENCES

Achee, F. M., Gabay, S. (1977) Biochem. Pharmacol. 26: 1637–1644

Achee, F. M., Gabay, S. (1979) Ibid. 28: 1197–1203

Cassano, G. B., Sjöstrand, S. E., Hansson, E. (1965) Psychopharmacologia 8: 1–11

Edwards, D. J., Burns, M. O. (1974) Life Sci. 15: 2045–2058

Fowler, C. J., Tipton, K. F. (1984) J. Pharm. Pharmacol. 36: 111–115

Garrick, N. A., Murphy, D. L. (1982) Biochem. Pharmacol. 31: 4061–4066

Green, A. L., El Hait, M. A. S. (1980) J. Pharm. Pharmacol. 32: 262–266

Husain, M., Edmonson, D. E., Singer, T. P. (1982) Biochemistry 21: 595–600

O'Carroll, A. M., Fowler, C. J., Phillips, J. P., Tobbinz, I., Tipton, K. F. (1983) Naunyn Schmiedeberg's Arch Pharmacol. 322: 198–202

Otsuka, S., Kobayashi, Y. (1964) Biochem. Pharmacol. 13: 995–1006

Roth, J. A. (1976) J. Neurochem. 27: 1107–1112

Roth, J. A. (1978) Molecular Pharmacol. 14: 164–171

Roth, J. A., Gillis, C. N. (1974) Biochem. Pharmacol. 23: 2537–2545

Roth, J. A., Gillis, C. N. (1975a) Ibid. 24: 151–152

Roth, J. A., Gillis, C. N. (1975b) Molecular Pharmacol. 11: 28–35

Rowan, P. R., Paykel, E. S., Parker, R. R. (1982) Br. J. Psychiat. 140: 475–483

Sullivan, J. L., Dackis, M. D. C., Stanfield, C. (1977) Am. J. Psychiat. 134: 188–190

Sulser, F., Mobley, P. L. (1980) in: Hoffmeister, F., Stille, G. (eds) Psychotropic Agents. Handbook of Experimental Pharmacology, Vol 55/1. Springer-Verlag, Berlin, pp 471–490

Sulser, F., Vetulani, J., Mobley, P. L. (1978) Biochem. Pharmacol. 27: 257–261

Tong, J. H., Limson-Zamora, M., D'Iorio, A., Bégin-Heick, N. (1979) Biochem. J. 177: 943–949

Usdin, E., Usdin, V. R. (1961) Proc. Soc. Exp. Biol. Med. 108: 461–463