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Amethocaine-induced inhibition of mitochondrial monoamine oxidase activity

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Amethocaine (tetracaine) (1–10 μM) produces a concentration-dependent in-vitro inhibition of mitochondrial membrane-bound MAO activity towards tyramine (18–84% in brain and 19–84% in liver) and 5-hydroxytryptamine (5-HT) (23–94% in brain and 20–100% in liver). At relatively higher concentrations (25–300 μM) of amethocaine, benzylamine oxidation is inhibited in brain (24–91%) and liver (29–100%). The extent of MAO inhibition is appreciably reduced when preincubation time of the enzyme with a low concentration (7·5 μM) of amethocaine is increased from zero to 45 min. This inhibition is reversible. The K_m of MAO for tyramine is increased in brain (106–473%) and liver (121–352%) in the presence of amethocaine (2–7·5 μM) accompanied by a decrease in V_{max} (21–51% in brain and 18–57% in liver). Similarly the K_m of MAO for 5-HT is increased to the extent of 79–336% in brain and 51–225% in liver and the corresponding V_{max} is decreased by 35–55% and 39–74%, respectively, in the presence of 2–5 μM amethocaine. At relatively higher concentrations (25–100 μM), amethocaine increases the K_m of MAO for benzylamine in brain (25–101%) and liver (26–85%) and decreases the V_{max} by 28–64% and 32–63% in the respective tissues. Thus these results suggest that amethocaine preferentially inhibits MAO-A and the nature of inhibition is reversible and of mixed type.

Local anaesthetics alter various physicochemical properties of synthetic and biological membranes (Lee 1976; Gordon et al 1980; Haque et al 1983; Haque & Poddar 1985) as well as the activities of many membrane-bound enzymes (Gordon et al 1980; Vanderkooi et al 1981; Haque & Poddar 1985). Monoamine oxidase (MAO, EC.1.4.3.4), a mitochondrial membrane-bound enzyme, is known to exist in two functionally distinct forms, MAO-A and MAO-B (Johnston 1968; Achee et al 1977) and is inhibited by amethocaine and other amine based local anaesthetics (Fowler et al 1980; Haque et al 1983; Haque & Poddar 1984). Changes in the lipid microenvironment of various membrane-bound enzymes including MAO have been reflected in their kinetic and regulatory properties (Houslay & Tipton 1973: Fowler & Oreland 1980; Gordon et al 1980; Fowler et al 1982). However, little is known about the changes in the kinetic properties of MAO in the presence of amethocaine. We report the in-vitro effect of amethocaine on the kinetic parameters of mitochondrial MAO of rat brain and liver.

MATERIALS AND METHODS

Tyramine hydrochloride, 5-hydroxytryptamine creatinine sulphate, benzylamine bovine serum albumin and amethocaine (tetracaine) hydrochloride were purchased from Sigma Chemical Co., USA. All other reagents were of analytical grade.

Adult male albino rats of Charles Foster strain (125–150 g) maintained on standard laboratory diet with free access to water were used to prepare brain mitochondrial fraction according to Gray & Whittaker (1962), as modified by Bradford et al (1973), and liver mitochondria according to Schneider & Hogeboom (1950). MAO activity using tyramine and 5-HT was determined by the method of Green & Haughton (1961). Benzylamine oxidation by MAO was measured according to Popov et al (1971). All incubations were at 37 °C using air as gas phase in the absence and presence of varying concentrations (1–300 μM) of amethocaine which was added just before the substrate. For kinetic studies, tyramine or 5-HT concentrations were varied from 0·1 to 1·0 mM, while the benzylamine concentration varied from 0·02–0·2 mM. K_m and V_{max} were determined by Lineweaver-Burk plots. Preliminary experiments were made to ensure that the enzyme activity was linear with respect to incubation time and concentration of enzyme.

The protein content of the enzyme preparations was determined according to Lowry et al (1951) with bovine serum albumin as standard.

Statistical analysis was by two-tailed Student's *t*-test.

RESULTS

Fig. 1 shows that amethocaine (1–10 μM) produced a concentration-dependent significant ($P < 0{\cdot}02$) inhibition (in-vitro) of MAO activity of rat brain and liver mitochondria using tyramine and 5-HT as substrates. However, at relatively higher concentrations (≥ 25 μM) amethocaine inhibited benzylamine oxidation in both the tissues in-vitro. The IC50 values for amethocaine (calculated from Fig. 1A, B) were 4·4 and 4·0 μM for tyramine oxidation in brain and liver, respectively, and 2·6 and 2·3 μM for 5-HT oxidation in brain and liver, respectively, while for benzylamine the values were comparatively high in both brain (72·5 μM) and liver (62·0 μM).

The effect on MAO activity of varying the preincubation time (0–45 min at 37 °C) of rat brain and liver mitochondria with amethocaine is seen in Fig. 2. It appears that the extent of MAO inhibition towards tyramine and 5-HT was appreciably reduced in both the tissues when the preincubation time of the enzyme with

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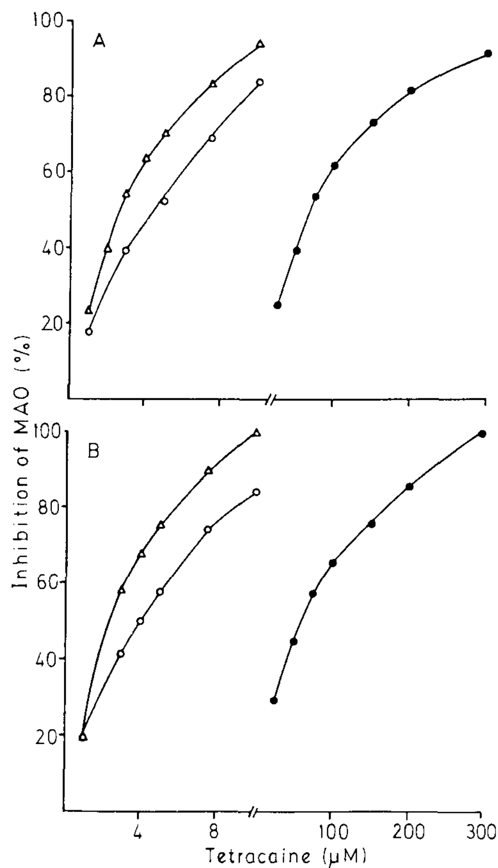

FIG. 1. In-vitro inhibitory effect of amethocaine on rat (A) brain and (B) liver mitochondrial MAO activity using tyramine (○), 5-HT (△) and benzylamine (●) as substrate. Each point represents the mean of four separate determinations. Other details are given in the text.

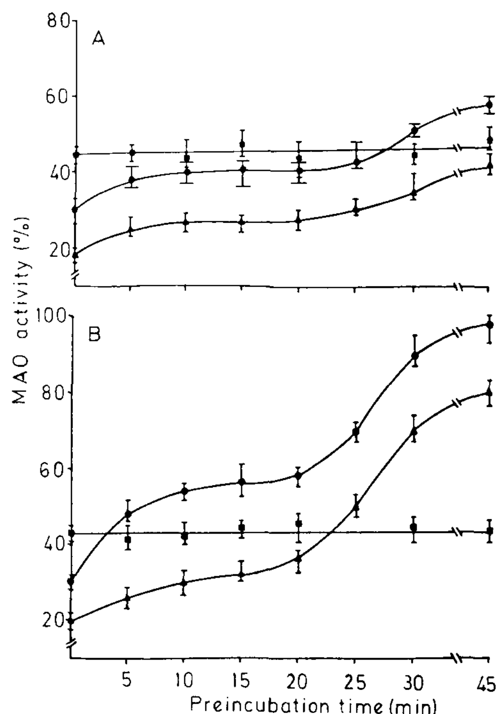

FIG. 2. Effect of preincubation time on rat (A) brain and (B) liver mitochondrial MAO activity in the presence of amethocaine (7·5 μM for (●) tyramine or (▲) 5-HT and 75 μM for (■) benzylamine). Each value represents the mean of four separate determinations; bars encompass all determinations. Other details are given in the text.

7·5 μM amethocaine was increased from zero to 45 min at 37 °C. However, no such reduction of MAO inhibition was observed with the increase of preincubation time (0–45 min) of the enzyme with 75 μM amethocaine when benzylamine was used as substrate. Dilution experiments were performed to test the reversibility of amethocaine-induced MAO inhibition on brain and liver mitochondria. Repeated washing of amethocaine-treated brain or liver mitochondria with 25 mM sodium phosphate buffer, pH 7·2 by centrifugation at 20 000*g* for 45 min at 4 °C restored about 90% of the MAO activity towards tyramine in both the tissues (Table 1).

Lineweaver-Burk plots showed that amethocaine produced a concentration-dependent increase in K_m and decrease in V_{max} of rat brain and liver mitochondrial MAO towards tyramine, 5-HT and benzylamine. K_m of MAO for tyramine increased in brain (106–473%) and liver (121–351·6%) and the corresponding V_{max} decreased by 21·0–50·8% and 18·3–56·5% in brain and liver, respectively, in the presence of amethocaine (2–7·5 μM). With 5-HT as substrate for MAO, amethocaine (2–5 μM) produced an increase in K_m in brain (78·5–335·7%) and liver (50·5–224·8%) and a decrease in V_{max} in brain (35–54·5%) and liver (38·5–74·3%). K_m of MAO for benzylamine, however, was found to increase in brain (25–101%) and liver (26–85%) accompanied by a decrease in V_{max} to the extents of 27·8–64·3% and 31·5–62·8% in brain and liver, respectively, in the presence of higher concentrations (25–100 μM) of amethocaine.

DISCUSSION

It is well known that MAO exists in two functionally distinct forms: MAO-A, which chiefly oxidizes 5-HT and noradrenaline, and MAO-B which preferentially oxidizes β-phenethylamine and benzylamine (Johnston 1968; Achee et al 1977). The present study confirms previous findings (Fowler et al 1980; Yasuhara et al 1982) that amethocaine is an MAO-A selective inhibitor. However, amethocaine being a diamine is not acted upon by mitochondrial diamine oxidase (DAO) due to the presence of semicarbazide, an inhibitor of DAO (Youdim 1975) in the MAO assay media. Hence the preincubation time-dependent reduction of amethocaine (low concentration)-induced MAO inhibition in

rat brain and liver mitochondria (Fig. 2) raises the possibility of either hydrolysis of the ester bond or the slow metabolism of amethocaine by MAO. These possibilities do not arise when high concentrations of amethocaine are present in the incubation mixture.

Almost complete recovery of MAO activity by repeated washing of amethocaine-treated mitochondria (Table 1) indicates that the inhibition is reversible. The results of Ackermann-Potter plots (not shown) also confirm the reversibility of the inhibition. The results of the kinetic study reveal that amethocaine produces a concentration-dependent increase in K_m and decrease in V_{max} of both brain and liver mitochondrial MAO towards tyramine, 5-HT and benzylamine. The gradual decrease in both substrate affinity (K_m^{-1}) and catalytic property (V_{max}) of both brain and liver mitochondrial membrane-bound MAO in the presence of increasing concentrations of amethocaine reveals that this drug-induced inhibition of MAO is neither entirely competitive nor non-competitive. Amethocaine as a substituted amine is expected to compete with the monoamine substrates and consequently would produce a competitive inhibition of MAO activity, as suggested by Fowler et al (1980) and Yasuhara et al (1982). It is reported that amine anaesthetics exist as both neutral and cationic species (Ritchie & Green 1980) and the former is more permeable to biomembranes than the latter (McLaughlin 1975). It may be assumed that amethocaine at the concentrations used in this study where it exists in both ionic and neutral forms, produces both electrostatic and non-polar interactions with the active site of MAO-A, buried in the hydrocarbon core of the membrane (Huang 1980) and produces a mixed-type inhibition of MAO-A. However, it is not clear how amethocaine at concentrations $\geqslant 50$ μM increases the lipid fluidity of biological membranes (Haque & Poddar 1985) and produces a mixed type inhibition of MAO-B which has previously been shown to be independent of the fluidity of bulk membrane lipid (Huang 1980). The possibility of direct interaction of the drug with MAO and other mitochondrial enzymes (Vanderkooi et al 1981) cannot be ruled out.

Table 1. Effect of washing on the in-vitro inhibition of MAO activity by amethocaine in rat brain and liver mitochondria.

Mitochondrial tissue	Treatment	Activity of MAO towards tyramine (ΔOD_{450nm}/h (mg protein)$^{-1}$) Before washing	After washing
Brain	Control	0·423 ± 0·012 (100)	0·440 ± 0·016 (100)
	Amethocaine (5 μM)	0·205 ± 0·010 (48·5)	0·397 ± 0·012 (90·2)
Liver	Control	0·470 ± 0·014 (100)	0·467 ± 0·045 (100)
	Amethocaine (5 μM)	0·192 ± 0·010 (40·9)	0·420 ± 0·009 (89·9)

Each value represents the mean ± s.e.m. of three separate determinations. Other details are described in the text. Values in parentheses represent the per cent activity of MAO.

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