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A method for stabilization of monoamine oxidases in homogenates of rat intestine epithelium

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Abstract—In a homogenate of epithelium isolated from the small intestine of male Wistar rats, the amine oxidase activity with 10^{-3}M tyramine was 9200 ± 200 nmol (g tissue)$^{-1}$ h^{-1} of which 91% was due to the A form of monoamine oxidase (MAO) and 9% to the B form. Semicarbazide-sensitive amine oxidase activity was not detected with either 10^{-3}M tyramine or 10^{-4}M benzylamine as substrate. However, it was detectable in the homogenate of the gut residue where the activity with 10^{-4}M benzylamine was 3600 ± 200 nmol (g tissue)$^{-1}$ h^{-1}. The MAO activity, in homogenates of epithelium prepared with 0·1 M sodium phosphate pH 7·4, was stable at 4°C for at least 6 h whilst at minus 20°C it decreased by 70% within 24 h. Incorporation of 10% (v/v) glycerol into the homogenization medium stabilized the enzymes. The total activity and proportions due to MAO-A and MAO-B and kinetic constants for tyramine and 5-hydroxytryptamine, did not alter during 5 weeks storage at −20°C. The ability to store tissue homogenates should facilitate studies of intestinal amine oxidases.

Mammalian small intestine contains the A and B forms of monoamine oxidase (MAO) (amine:oxygen oxidoreductase (deaminating) (flavin-containing) EC 1.4.3.4) (Squires 1972; Ilett et al 1980; Strolin Benedetti et al 1983) and semicarbazide-sensitive amine oxidase (SSAO) (Lewinsohn et al 1978). These amine oxidases occur in cells of the villi and crypts of the epithelium and the remainder of the gut tissue (Hasan & Tipton 1984; Callingham et al 1985). However, it is the epithelial enzymes which deaminate dietary amines during passage along the gut and in order to investigate their properties in-vitro the epithelium should be isolated. After the preparation of homogenates, it is convenient if they may be stored for subsequent investigations. The amine oxidases of many mammalian tissues are stable for several weeks at −20°C in homogenates prepared with phosphate buffer. However, in our experience, the intestinal enzymes rapidly lose activity under such conditions. The present study was carried out to determine conditions under which they may be stored without alteration to their catalytic activity.

Materials and methods

Isolation of intestinal epithelium. Male Wistar rats, 180–200 g, were used. The intestine, from the pyloric to caecal end, was removed and cut into 20 cm lengths which were washed through with isotonic saline. Epithelium was isolated by the method of Merchant & Heller (1977), using 1 h incubation in the "buffer B", described by those authors, so that cells from villi and crypts were isolated together. The cells were harvested by centrifugation at 5000*g* for 10 min and resuspended to 1 g per 3 mL with 0·1 M sodium phosphate pH 7·4 in either H_2O or H_2O–glycerol mixtures indicated in the results and homogenized with a Thomas homogenizer. Homogenates were prepared with tissue from 4 rats to assess enzyme stability and from 12 rats to determine MAO-A, MAO-B and SSAO activities and kinetic parameters for tyramine and 5-hydroxytryptamine (5-HT).

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Measurement of amine oxidase activity. Activity was measured at 37°C and pH 7·4 with a Clark-type oxygen electrode, using 0·5 mL homogenate in a final volume of 3 mL made up with 0·05 M sodium phosphate buffer saturated with oxygen from air. Combined activities of MAO and SSAO were assayed with 10^{-3}M tyramine and the proportion due to MAO-A and MAO-B was determined by titration with Lilly 51641 [(*N*-(2-*O*-chlorophenoxy)ethyl)cyclopropylamine HCl]. MAO-B and SSAO were assayed with 10^{-4}M benzylamine. The activity inhibited by 10^{-3}M semicarbazide was defined as SSAO. The activity which was resistant to 10^{-3}M semicarbazide and was not inhibited by a concentration of Lilly 51641 (10^{-6}M) which selectively inhibited all MAO-A activity, was defined as MAO-B. Lilly 51641 was preincubated with enzyme preparation for 15 min and semicarbazide for 5 min, at 37°C. Preliminary time courses of the inhibition of amine oxidase activity by Lilly 51641 indicated that the reaction between enzyme and inhibitor reached completion within 15 min. There was no loss of enzyme activity for samples preincubated with buffer for 30 min.

Analysis of data. Michaelis constants and maximum velocities were derived from Hanes plots (s/v against s) (Hanes 1932) constructed with five substrate concentrations ranging from approximately one-third to four times the Michaelis constant. Lines of best fit were obtained using unweighted linear regression. Statistical comparisons were made using Student's *t*-test with $P < 0.05$ being taken as significant.

Results

Stability of tyramine deaminating activity in homogenates of epithelium. The activity, in homogenates prepared with 0·1 M sodium phosphate pH 7·4, was stable for at least 6 h at 4°C. At minus 20°C it decreased by 70% within 24 h, after which it remained constant for several weeks. This indicated that the loss of activity could have been caused by freezing and thawing so the effect of incorporating glycerol into the homogenization medium was investigated. Addition of glycerol at 5%, 10% and 20% (v/v) had no significant effect (n = 4) upon the tyramine deaminating activity in fresh homogenates. Table 1 shows that

Table 1. Effect of glycerol on the stability of tyramine deaminating activity in homogenates of rat intestine epithelium. Isolated epithelium was homogenized with 0·1 M sodium phosphate pH 7·4 in either H_2O = without glycerol or H_2O:glycerol (90:10 by vol) = with glycerol. A, B, C represent homogenates prepared with epithelium from different groups of 4 rats. Enzyme activity was measured with 10^{-3}M tyramine at 37°C and pH 7·4.

Weeks at minus 20°C	Tyramine deaminating activity (nmol (g tissue)$^{-1}$ h^{-1}) Without glycerol	With glycerol A	B	C
0	4700 ± 200	6000 ± 200	5500 ± 100	9200 ± 200
5	1600 ± 100*	5800 ± 200	5300 ± 200	9200 ± 100
10	1700 ± 100*	5500 ± 200	5100 ± 200	8800 ± 200

Means ± s.e. are given (n = 4). Asterisks indicate differences ($P < 0.05$) between fresh homogenate = 0 weeks and stored samples.

the tyramine deaminating activity, of homogenates prepared with medium containing 10% (v/v) glycerol, was stable at minus 20°C for at least 10 weeks.

Some properties of amine oxidase activities in fresh and stored homogenates. With tyramine as substrate, Lilly 51641 at concentrations from 10^{-11} to 10^{-4}M gave similar biphasic inhibition curves for fresh homogenate, prepared with 10% (v/v) glycerol in the medium, and samples stored at minus 20°C for 5 weeks (Fig. 1). There was a plateau between 10^{-7} and 10^{-5}M and

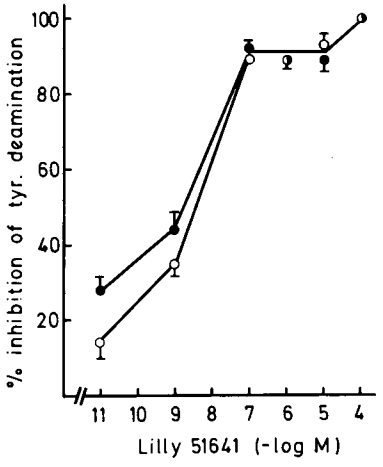

FIG. 1. In-vitro inhibition by Lilly 51641 of the deamination of tyramine by a homogenate of rat intestine epithelium when freshly prepared (●) and after storage at minus 20°C for five weeks (○). Each point represents the mean (±s.e., shown when error bars exceed the size of the symbol) of triplicate estimations. Epithelium, isolated from twelve rats, was homogenized with 0·1 M sodium phosphate pH 7·4 in H_2O-glycerol (90:10 by vol). Enzyme preparation was preincubated with Lilly 51641 at 37°C for 15 min after which activity was measured with 10^{-3}M tyramine at 37°C and pH 7·4 as described in Materials and methods. The plateau regions of the curves, for the inhibition of tyramine deamination, were not significantly different ($P = > 0{\cdot}05$).

complete inhibition occurred at 10^{-4}M inhibitor. In the plateau region, inhibition of amine oxidase activity, of fresh and stored homogenate, was not significantly different and the mean value indicated that 91% of the amine oxidase activity was due to MAO-A. The Michaelis constants and maximum velocities for tyramine and 5HT were not altered by the storage conditions (Table 2).

Table 2. Kinetic parameters for deamination of tyramine and 5-hydroxytryptamine (5HT) by the amine oxidase activity in fresh and stored homogenate of rat intestine epithelium. Epithelium was isolated from 12 rats and homogenized with 0·1 M sodium phosphate pH 7·4 in H_2O-glycerol (90:10 by vol). Michaelis constants and maximum velocities were determined at pH 7·4 and 37°C as described in Materials and methods with fresh homogenate and samples stored for 5 weeks at minus 20°C.

	Michaelis constant (μM)		Maximum velocity (nmol (g tissue)$^{-1}$ h^{-1})	
Homogenate	Tyramine	5-HT	Tyramine	5-HT
Fresh	63	109	9600	8200
Stored	68	104	9400	8800

With benzylamine as substrate MAO-B activity in the fresh homogenate of epithelium was 530±20 nmol (g tissue)$^{-1}$ h^{-1} whilst SSAO was not detectable. The MAO-B activity in the fresh homogenate of gut residue was 1100±50 nmol (g tissue)$^{-1}$ h^{-1} and SSAO 3600±200 nmol (g tissue)$^{-1}$ h^{-1}.

Discussion

In this investigation, Lilly 51641 was used to determine relative activities of the A and B forms of MAO where as clorgyline is usually employed (Fowler et al 1981). However, these selective irreversible inhibitors of MAO-A (Fowler & Ross 1984) have been shown to give the same values for the enzymes from tissues of several species including the rat (Squires 1972). Therefore, our results may be compared with those of others who used clorgyline. We found that MAO-A constituted 91% of the amine oxidase activity in homogenate of isolated epithelium whilst Strolin Benedetti et al (1983) found that it accounted for 76% of the activity in homogenate of whole intestine. Thus, it appears that the proportion of MAO-A is higher in the epithelium than the gut residue. This is in agreement with Callingham et al (1985) who reported that MAO-A was the predominant amine oxidase of both epithelium and gut residue and occurred with the highest proportion in epithelium. In this investigation, the activity of MAO-B was found to be highest in the gut residue which also agrees with results of Callingham et al (1985). With regard to SSAO, we found that it occurred with highest activity in the gut residue.

Comparison of our results, with fresh and stored homogenates, shows that monoamine oxidases of rat intestine epithelium can be stabilized by including glycerol in the homogenization medium. When homogenates were prepared with 10% (v/v) glycerol, the total enzyme activity was unaltered during 10 weeks at minus 20°C. The values in Table 1 show that a two-fold variation can occur in the tyramine deaminating activity of the epithelium. The animals used were bred and maintained under the same conditions within our animal breeding unit and the reason for the variation is not known. However, in our experience such variation is not uncommon and the data were selected to illustrate it. The activity of MAO-A relative to MAO-B and kinetic constants for tyramine and 5-HT were unaltered after 5 weeks at minus 20°C. The ability to store preparations of intestinal monoamine oxidases for subsequent investigation, should facilitate the study of their properties and function.

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