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COMMUNICATIONS

The effects of homogenization of rat liver in different buffer solutions on the yield and kinetic properties of monoamine oxidase

BARBARA J. BROWNE*, C. J. FOWLER, B. A. CALLINGHAM, *Department of Pharmacology, University of Cambridge, Hills Road, Cambridge CB2 2QD, U.K.*

The enzyme monoamine oxidase (MAO, EC 1.4.3.4) appears to exist in more than one catalytically active form (see Houslay, Tipton & Youdim, 1976; Severina, 1976). The substrate selective inhibitor clorgyline and a variety of substrates have been used to classify the enzyme into two forms, MAO-A and MAO-B (Johnston, 1968). According to this classification, in the rat liver, 5-hydroxytryptamine (5-HT) is metabolized by MAO-A alone, benzylamine and β-phenethylamine by MAO-B, and tyramine by both forms of the enzyme (Hall, Logan & Parsons, 1969; Houslay & Tipton, 1974). It is usually assumed that the ratio of the two forms and their catalytic properties are unchanged during the preparation of tissue homogenates. The addition of certain buffers, such as tris, to preparations of MAO can influence its specific activity (Browne, Laverty & Callingham, 1973). For example, tris buffers are found to be non-competitive inhibitors of 5-hydroxytryptamine, tyramine and β-phenethylamine but not benzylamine oxidation by MAO of rat liver (Fowler, Callingham & Houslay, 1977). As an extension to this work, it was decided to see if homogenization of rat liver in different buffers could affect the properties of the enzyme.

Eight rats, 175–200 g, were killed by a blow to the head, and their livers rapidly removed, blotted and cooled in ice. The livers, in pairs, were finely chopped and mixed together to make four samples of tissue pulp. Aliquots of each sample were homogenized (1 : 10 w/v) in a conical glass homogenizer, in one of the four following buffers: 1. 1 mM potassium phosphate, pH 7·8; 2. 154 mM potassium phosphate, pH 7·8; 3. 0·25 M sucrose, buffered with 10 mM potassium phosphate, pH 7·8 ('sucrose buffer'); 4. 100 mM hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid), brought to pH 7·8 by potassium hydroxide.

Preliminary experiments indicated that these four buffers did not cause any inhibition or activation of the MAO, when added to the homogenates in 1 mM phosphate in order to produce the molarities of the buffers shown above. Moreover, no inhibition was observed even when these buffers were preincubated with the homogenate for 30 min before assay. This would suggest that any changes in activity are due to effects of homogenization in the buffer, and not to any inhibitory effects of the buffer itself.

The homogenates were centrifuged for 15 min at 600 *g* to remove nuclei and cell debris. The supernatant fractions were then used for assay.

The protein contents of the homogenates in 1 and 154 mM phosphate and in sucrose buffer were determined by the method of Lowry, Rosebrough & others (1951) with bovine serum albumin as standard. Since hepes appeared to interfere with this assay to give artificially high values, protein contents of homogenates in this buffer were determined by the microbiuret method of Goa (1953) with the corresponding homogenate in 1 mM phosphate for comparison. Protein contents were assayed in this manner, since discrepancies can occur between the various methods when bovine serum albumin is used as standard (Bradford, 1976). The use of the 1 mM phosphate homogenates for comparison when the method of Goa (1953) was used, would minimize these discrepancies. The presence of sucrose interferes with the method of Goa, making it essential to use both methods in these experiments.

MAO activity was measured radiochemically by the method of McCaman, McCaman & others (1965) as modified by Callingham & Laverty (1973). [^{3}H]-Tyramine, [^{3}H]5-HT, [^{14}C]benzylamine and [^{14}C]β-phenethylamine were used as substrates. Unless otherwise stated, all assays were carried out at 37° in an atmosphere of oxygen. Enzyme activities are expressed either as yields (nmol (of substrate deaminated) ml^{-1} h^{-1}), or as specific activities (nmol (of substrate deaminated) (mg protein)$^{-1}$ h^{-1}), calculated as mean values ± standard error of the mean. No corrections were made for the efficiencies of extraction of the deaminated metabolites of the different substrates. The buffers used did not change the efficiencies of extraction. In experiments where clorgyline was used to inhibit the MAO, the homogenates were preincubated for 20 min to allow the irreversible inhibition of MAO-A (Egashira, Ekstedt & Oreland, 1976; Fowler & Callingham, 1978a).

Homogenization of rat liver in the four buffers affected the amount of enzyme activity recovered in the manner shown in Table 1. The highest yield of MAO activity was found in 1 mM phosphate and the lowest in 154 mM phosphate and 100 mM hepes. When the

* Correspondence.

Table 1. *The effect of homogenization of rat liver in four buffer solutions on the yield and specific activity of MAO.* Aliquots of the same liver sample were homogenized in each of the four buffers and the MAO activity measured with the substrates in the concentrations shown. MAO activities (in nmol of substrate consumed $ml^{-1}\ h^{-1}$ or $(mg\ protein)^{-1}\ h^{-1}$) are expressed as the means ±s.e.m. of 4 experimental groups assayed in duplicate (i.e. n = 4). The figures in parentheses represent the MAO activity as % of the activity in 1 mM phosphate.

Substrate and buffer	MAO yield (n mol $ml^{-1}\ h^{-1}$)	MAO spec. act. (n mol $(mg\ protein)^{-1}\ h^{-1}$)
5-HT (0·25 mM)		
Phosphate 1 mM	2103 ± 60 (100)	148·6 ± 10·4 (100)
Phosphate 154 mM	704 ± 47** (33·5)	64·8 ± 5·6* (43·6)
Sucrose	1439 ± 73** (68·4)	129·3 ± 4·2^{NS} (87·0)
Hepes	757 ± 20** (36·0)	65·9 ± 3·3* (44·3)
Tyramine (0·25 mM)		
Phosphate 1 mM	4064 ± 44 (100)	286·0 ± 10·4 (100)
Phosphate 154 mM	1288 ± 78** (31·7)	118·6 ± 10·2** (41·5)
Sucrose	2630 ± 118* (64·7)	236·7 ± 8·7* (82·8)
Hepes	1371 ± 29** (33·7)	119·6 ± 7·3** (41·8)
β-Phenethylamine (0·05 mM)		
Phosphate 1 mM	3991 ± 43 (100)	281·0 ± 11·9 (100)
Phosphate 154 mM	1336 ± 59** (33·5)	122·6 ± 6·7** (43·6)
Sucrose	2605 ± 67** (65·3)	234·5 ± 4·2† (83·5)
Hepes	1449 ± 24** (36·3)	126·4 ± 7·4** (45·0)
Benzylamine (0·25 mM)		
Phosphate 1 mM	3519 ± 75 (100)	247·6 ± 9·9 (100)
Phosphate 154 mM	1268 ± 67** (36·0)	116·1 ± 6·1** (46·9)
Sucrose	2434 ± 115** (69·2)	218·8 ± 5·0† (88·4)
Hepes	1491 ± 32** (42·4)	131·0 ± 10·8** (52·9)

**$P<0·001$; *$P<0·01$; †$P<0·05$; t-test on actual values
NS, not significant.

MAO activity was expressed in terms of protein content (i.e., specific activity), the homogenates fell into two groups, with the low ionic strength homogenates having about twice the specific activity of those of high ionic strength. This agrees with some preliminary results from the rat heart (Della Corte, 1975). When

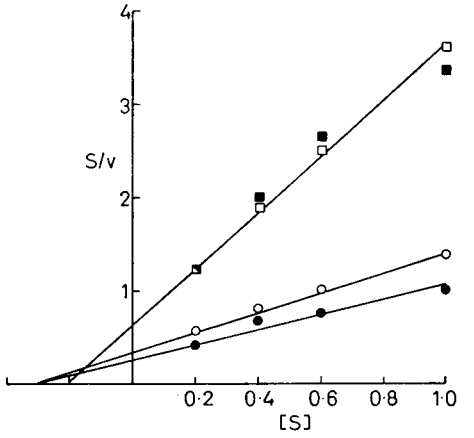

FIG. 1. S *vs* S/v plots of the specific activity of MAO in rat livers homogenized in 4 buffer solutions. For each buffer, initial velocities of the reaction were calculated from progress curves drawn from duplicate assays at 4 time intervals for 4 separate experiments. Tyramine was used as substrate. ●——●, 1 mM phosphate; ○——○, 0·25 M sucrose + 10 mM phosphate (sucrose buffer); ■——■, 154 mM phosphate; □——□, 100 mM hepes. The mean Km value (calculated by the method of Wilkinson, 1961) for the MAO of homogenates in sucrose buffer is significantly higher than those in either 154 mM phosphate or 100 mM hepes buffer, but is not significantly different from that in 1 mM phosphate (95% confidence limits of a ratio, Goldstein, 1967). Ordinate: S/v (arbitrary units). Abscissa: S (mM).

tyramine was used as substrate, this difference was seen in both apparent Km and Vmax values (Fig. 1). However, the substrate specificity of MAO in the different buffers was unchanged (Table 1). These experiments would also suggest that the osmotic strength of the buffers is not important, as homogenization in 1 mM phosphate and 'sucrose' buffers appear to produce similar specific activities of enzyme.

Further experiments showed that the dependence of the MAO activity on the oxygen tension was not affected by the particular buffer used, although, as has been previously shown, it was dependent on the particular substrate (Fowler & Callingham, 1978b). Moreover, the substrate-selective inhibition of MAO by tris-HCl buffer was unchanged.

When the MAO activity in each of the buffers was subjected to increasing concentrations of clorgyline (which selectively inhibits MAO-A), the expected double-sigmoid inhibition curves were seen when tyramine was used as the substrate (Fig. 2). Although only small changes in the position of the plateau region were found, indicating that there was little change in the ratio of MAO-A to MAO-B, the enzyme activities of homogenates in buffers of high ionic strength were inhibited by significantly lower concentrations of

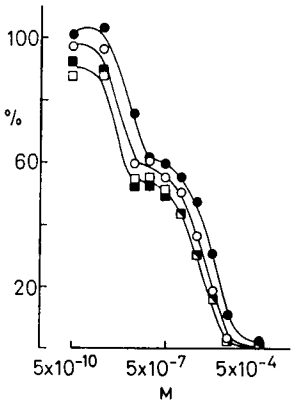

FIG. 2. The effect of homogenization of rat liver in 4 buffer solutions on the inhibition of MAO activity by clorgyline. Tyramine (0·25 mM) was used as substrate. Each point represents the mean of the MAO activity of duplicate determinations in 4 separate experiments, expressed as a percentage of control value. The s.e.m. of these mean values did not exceed ±3% at any time. ●——●, 1 mM phosphate; ○——○, 0·25 M sucrose +10 mM phosphate; ■——■, 154 mM phosphate; □——□, 100 mM hepes. The concentration of clorgyline required to produce the same degree of inhibition, was significantly greater for homogenates in buffers of low ionic strength (95% confidence limits of a ratio, Goldstein, 1967). Ordinate: MAO activity (%). Abscissa: Clorgyline (M).

clorgyline. This would be expected from the observations that clorgyline is sufficiently potent to be used as a means of estimating the number of MAO active centres (Egashira, Ekstedt & others, 1976; Fowler & Callingham, 1978a).

In those situations where maximum MAO activity is required together with preservation of subcellular structures (for example, in the preparation of mitochondria uncontaminated with other subcellular fragments), the most suitable medium appears to be one of low ionic strength made isotonic with a non-ionic agent such as sucrose. Buffers of high ionic strength are unsuitable since they appear to change the kinetic properties of the enzyme. A crude homogenate would give maximum yields when homogenized in 1 mM phosphate buffer. It is inadvisable to add a non-ionic detergent, such as Triton X-100, as this has been shown irreversibly to inhibit rat liver MAO (C. J. Fowler, B. A. Callingham, T. J. Mantle & K. F. Tipton, unpublished).

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