Post-mortem stability of monoamine oxidase in rat tissues

D. PARKINSON*, B. A. CALLINGHAM, *Department of Pharmacology, University of Cambridge, Hills Road, Cambridge, CB2 2QD, U.K.*

The interpretation of data from biochemical studies of human tissues obtained at post-mortem, is complicated if the stability of the system to be examined is unknown, since delay is inevitable. This could apply especially to monoamine oxidase (MAO, EC 1.4.3.4), which appears to exist in more than one catalytically separable form (see Fowler, Callingham & others, 1978). Changes in the relative proportions of the different forms of the enzyme in the hours after death would make interpretation of experimental results impossible. The post-mortem stability of MAO seems only to have been examined in detail in the brains of rabbits (Grote, Moses & others, 1974) and rats (Vogel, Orfei & Century, 1969; Fahn & Côté, 1976) and man (Mackay, Davies & others, 1978). However, in all these studies, only one substrate for MAO was used, so there is no information on the relative stability of the different enzyme forms.

Thus before any study of MAO in the human heart was made, it was felt that the post-mortem stability of the enzyme in the heart and some other tissues of the rat should be assessed. The use of several substrates and the irreversible selective MAO inhibitor, clorgyline (Johnston, 1968) should reveal any relative changes in the different forms of MAO that could occur after death.

Twenty-one male Wistar rats, 250 g, were killed by cervical dislocation. The brains, hearts and livers were rapidly removed from 9 of the rats, and the other rats placed in a refrigerator at 4° to simulate conditions in the mortuary. The organs were removed from these animals 8 and 24 h later. The various organs were homogenized in ice-cold 1 mM potassium phosphate buffer, pH 7·8 (1:10 w/v). The homogenates were centrifuged at 600 *g* for 15 min to remove nuclei and cell debris. The supernatant fractions were deep-frozen until assayed. MAO activity was measured radiochemically by the method of McCaman, McCaman & others (1965) as modified by Callingham & Laverty (1973). [^{3}H]5-Hydroxytryptamine (5-HT), [^{3}H]tyramine, [^{14}C]β-phenethylamine and [^{14}C]benzylamine were used as substrates. All assays were at 37° in an atmosphere of oxygen. MAO 'specific activities' were expressed as nmol (of substrate metabolized) (mg protein)$^{-1}$ min^{-1}, calculated as means together with 95% confidence limits of a ratio (Goldstein, 1967). MAO 'activities' were expressed as nmol (of substrate metabolized) ml^{-1} (of homogenate) min^{-1}, calculated as means ± s.e.m. The values are not corrected for the efficiencies of extraction into the organic phase of the deaminated metabolites of the different substrates (see below).

* Correspondence.

Protein contents of the homogenates were measured by the micro-biuret method of Goa (1953). When clorgyline was used as an irreversible inhibitor of MAO, it was pre-incubated for 20 min with the homogenates before the addition of substrate.

There was a reduction in the protein contents of all homogenates prepared from the organs of animals stored at 4° after death, compared with homogenates from freshly-killed animals, although these reductions were not always significant. The largest differences were seen in homogenates of liver, which might be expected from the presence in this organ of a large pool of enzymes capable of degrading protein.

Table 1 summarizes the changes that occurred in the MAO activity in homogenates of rat brain, heart and liver during post-mortem storage of the whole animal at 4°. In the brain, there was a significant reduction in MAO activity with tyramine, 5-HT and benzylamine, while with β-phenethylamine as substrate, the reduction was not significant. There appeared to be little further reduction in activity between 8 and 24 h.

In the heart, only the activity towards benzylamine as substrate fell significantly after death. In the liver, while the MAO activity towards all four substrates fell

Table 1. *The effect of post-mortem storage at 4° on the MAO activity of rat brain, heart, and liver.* The organs were removed from the animals at the times shown after death. Tyramine (Tyr), 5-hydroxytryptamine (5-HT), β-phenethylamine (β-PEA) and benzylamine (Bz) were used at a concentration of 0·5 mM as substrates. Levels of significance compared with initial activity by Student's *t*-test: *$P<0.05$; **$P<0.02$; ***$P<0.01$. (MAO activity is expressed as nmol of substrate metabolized ml^{-1} of homogenate min^{-1}).

Time (h)	Protein (mg ml^{-1})	MAO activity Tyr	5-HT	β-PEA	Bz
1. Brain					
0	20·7 ±1·13	53·2 ±5·28	43·4 ±2·90	38·9 ±6·66	38·0 ±2·88
8	14·6** ±1·74	32·0** ±2·79	27·4*** ±2·21	31·0 ±5·09	25·6*** ±1·64
24	16·0 ±2·16	41·8 ±2·73	28·2*** ±1·58	30·8 ±2·72	27·8* ±2·36
2. Heart					
0	10·8 ±0·5	67·5 ±9·50	60·0 ±1·85	20·7 ±3·70	3·90 ±0·465
8	8·7 ±0·86	49·8 ±10·4	40·7 ±6·87	12·95 ±3·48	1·84** ±0·318
24	8·2* ±0·73	43·0 ±5·3	44·6 ±5·23	13·3 ±1·75	1·73*** ±0·212
3. Liver					
0	29·8 ±1·96	112·5 ±10·5	60·8 ±5·09	163·0 ±14·7	147·0 ±10·9
8	22·0*** ±1·07	89·5 ±17·2	45·0 ±9·10	93·5** ±17·3	86·0*** ±13·10
24	18·3*** ±1·07	55·6*** ±11·5	32·4*** ±5·57	73·0*** ±11·1	74·0*** ±9·15

Table 2. *The effect of post-mortem storage at 4° on the MAO specific activity of rat brain, heart and liver.* (Results shown in Table 1 expressed in terms of the protein content of the homogenates). No significant differences were seen between the MAO specific activities in the organs after storage when compared with the initial specific activity (95% confidence limits of a ratio). (MAO specific activity is expressed as nmol of substrate metabolized mg of protein^{-1} min^{-1}).

	MAO specific activity			
Time (h)	Tyr	5-HT	β-PEA	Bz
1. Brain				
0	2·62 (1·99–3·25)	2·24 (1·84–2·64)	1·94 (1·39–3·49)	1·86 (1·51–2·21)
8	2·38 (1·58–3·18)	2·03 (1·38–2·68)	2·16 (1·52–2·80)	1·89 (1·37–2·41)
24	2·88 (2·03–3·73)	1·94 (1·40–2·48)	2·15 (1·38–2·92)	1·93 (1·27–2·59)
2. Heart				
0	6·13 (4·22–6·94)	5·51 (4·10–6·92)	1·89 (1·20–2·58)	0·36 (0·26–0·46)
8	5·35 (3·13–7·57)	4·54 (3·11–5·97)	1·36 (0·49–2·23)	0·21 (0·11–0·29)
24	5·35 (4·81–6·89)	5·49 (4·21–6·77)	1·65 (1·16–2·14)	0·21 (0·16–0·26)
3. Liver				
0	3·79 (2·98–4·56)	2·06 (1·73–2·39)	5·48 (4·61–6·35)	4·81 (3·82–5·80)
8	3·96 (2·25–5·67)	1·99 (1·07–2·91)	4·18 (2·39–5·97)	3·84 (2·56–5·12)
24	2·99 (1·51–4·47)	1·76 (1·02–2·50)	3·95 (2·54–5·36)	4·02 (2·86–5·18)

significantly by 24 h to about 50% of initial activity, only the activity designated as MAO-B showed a significant reduction at 8 h, i.e. the MAO activity that attacks both benzylamine and β-phenethylamine in the rat liver (see Fowler & others, 1978). MAO-A deaminates 5-HT in this organ, while tyramine is a substrate for both forms of the enzyme in the rat liver.

It is more usual to measure the MAO activity of a tissue in terms of its protein content, i.e. 'specific activity'. Table 2 shows the MAO activities from Table 1 expressed in this way. With all substrates tested, no significant reduction in specific activity of the MAO in rat brain, heart or liver could be seen, although, often, there was a significant loss of protein. Thus it seems likely that the MAO protein was degraded at a similar rate to the other proteins in the tissues. The observation that a fall in specific activity of 42% against benzylamine in the heart fails to reach significance, is probably because the MAO activity against this substrate in the rat heart is so poor that the errors are inevitably large.

Confirmation that there was no significant change in the relative proportions of MAO-A and -B in the heart was obtained from the profiles of the curves describing the inhibition of the MAO activities by clorgyline, with benzylamine as substrate (Fig. 1). Double-sigmoid inhibition curves, indicating the presence of both MAO-A and -B (see Youdim, 1975) were obtained with hearts from all three groups of rats. Although the inhibition curves were similar in

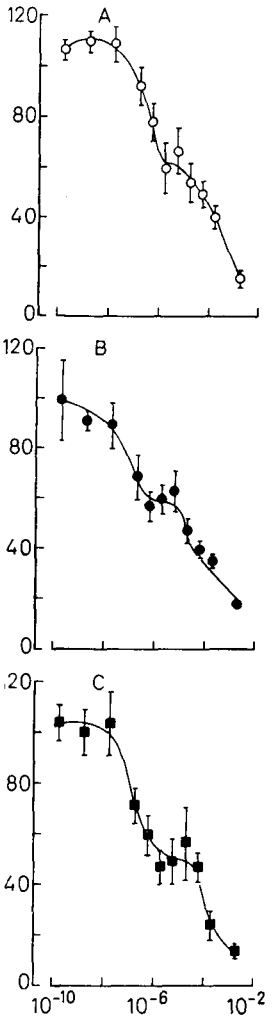

FIG. 1. The effect of clorgyline on the *in vitro* activity of MAO of homogenates of rat hearts after post-mortem storage of the animals at 4°, with 0·5 mM benzylamine as substrate: ○——○, groups assayed immediately after death, n = 9; ●——●, after 8 h storage, n = 5; ■——■, after 24 h storage, n = 6. Each point represents the mean MAO activity (± s.e. of a ratio) of the homogenates, assayed in duplicate, expressed as a percentage of the activity in the absence of inhibitor (ordinate), plotted against the molar concentration of clorgyline (abscissa).

overall shape, there was a small shift to the left of the first sigmoid component of the inhibition curve, corresponding to inhibition of MAO-A activity, obtained from the hearts of rats killed 24 h earlier (Fig. 1C), when compared with the same part of the curve from hearts of freshly-killed animals (Fig. 1A). (Significant differences in inhibition were seen at clorgyline concentrations of 1 and 5 × 10^{-7} M.) This difference can be explained by the fall in MAO protein content

during post-mortem storage, which leads to an increase in the apparent sensitivity of the MAO activity to inhibition by clorgyline (Lyles & Greenawalt, 1977; Fowler & Callingham, 1978).

Vogel & others (1969) reported that the activity (measured in terms of mg of organ) of the MAO in the rat brain towards tryptamine as substrate, did not change after storing the animal corpses for 16 h at either 4° or 20°. Although the MAO activity in the heart and liver also remained stable over the same period at 4°, falls of 50 to 70% were seen at 20°. Grote & others (1974) found that the MAO activity of two rabbit brains towards kynuramine as substrate did not fall significantly after 24 h at 22°. Here again, the activity was measured in terms of the weight of the organ. In neither paper was the protein content measured. On the other hand, Fahn & Côté (1976) measured the specific activity of the MAO in the decapitated heads of rats stored at 21° and showed that it steadily declined to 78% of its initial value when 5-HT was used as substrate. This decay in specific activity towards 5-HT is presumably due to the relatively high storage temperature used by these authors, since we have found no loss even after 24 h at 4°.

As man takes longer than rat to cool to 4°, and loss of MAO activity appears to be greater at the higher storage temperatures, it will be virtually impossible to estimate the amount of enzyme activity in a human organ at the point of death. However, provided two important conditions are satisfied, the present results and those reported by Fahn & Côté (1976) indicate that a fairly reliable estimate can be made of the specific activity. The first condition demands that there is little delay between death and cooling the corpse to 4°. The second demands that the particular assay method used for the measurement of MAO specific activity be capable of providing absolute values, i.e. values corrected for losses incurred in the extraction of the tissue and in the assay procedure itself. Assay methods, such as that used here, in which deaminated products of various radioactively labelled substrates are extracted into organic solvents, are particularly suspect, since the efficiency of extraction into the solvent varies greatly with the particular substrate and solvent system used (see Tipton & Youdim, 1976). For example, 5-HT metabolites are extracted by benzene–ethyl acetate (1 : 1 v/v) at an efficiency of about 90%, while dopamine metabolites are extracted at about 18%. A similar ratio of extraction efficiencies is seen when the less toxic mixture of toluene–ethyl acetate (1 : 1 v/v) is used. It is also possible that the extraction efficiency can be changed by the actual conditions of the assay. While in many animal experiments, changes in specific activity compared with control values measured at the same time, are usually adequate, absolute values are required for comparison of results between different laboratories and between different species.

Absolute values of the specific activity of the MAO content of an organ may also be required if an accurate measure is to be made of the ratio of MAO-A to -B, by the use of different substrates for the two forms. Recently, Cross, Glover & others (1978) have thrown some doubt on the validity of the exclusive use of clorgyline to estimate this ratio. They have shown that, in the human brain, the ratios of MAO-A to -B in the occipital cortex and caudate nucleus appear to be different when the specific activities towards 5-HT and benzylamine are compared, although no such difference was revealed when clorgyline was used to inhibit the MAO activity towards tyramine.

In conclusion, we suggest that even given the limitations of the otherwise simple and reliable assay method used, our results indicate that the measurement of the specific activity of MAO in post-mortem material should give a reliable guide to that activity at the point of death.

This work was supported by a grant from the British Heart Foundation. D.P. is a Vandervell Foundation Scholar. We thank May & Baker, Ltd., for a gift of clorgyline (M & B 9302).

April 30, 1978

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