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MONOAMINE OXIDASE ACTIVITIES OF DISSOCIATED CELL FRACTIONS FROM RAT VENTRICULAR MUSCLE

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Abstract—Cell fractions enriched in cardiac muscle cells (myocytes), on the one hand, and in non-myocytes, on the other, were prepared by dissociation of rat ventricular tissue with collagenase. Amine oxidase activities in homogenates of these cell fractions and also in homogenates of the corresponding undissociated ventricular tissue were compared. In addition, the activity of alkaline phosphatase (AP), an enzyme found predominantly associated in the heart with non-myocytes, particularly capillary endothelial cells, was also measured. No significant difference in the activity of MAO-A (assayed with 1 mM 5-hydroxytryptamine) was found between myocyte and non-myocyte fractions. In contrast, the activities of alkaline phosphatase (AP) and also the semicarbazide-sensitive amine oxidase (SSAO), assayed with 1 μM benzylamine (BZ), were both significantly higher in non-myocytes, by several-fold, than in myocyte fractions. Studies of the inhibition by clorgyline of 1 mM BZ metabolism confirmed that both MAO-A and MAO-B can also contribute to BZ oxidation in the rat heart. These experiments indicated different ratios of MAO-A: MAO-B in the various cell fractions. The ratios of the percentage contributions of MAO-A and MAO-B, respectively, to the total metabolism of 1 mM BZ were 78:20 (myocytes), 43:52 (non-myocytes) and 57:32 (undissociated tissue). These results suggest that MAO-B, in addition to AP and SSAO, may be associated preferentially with non-myocyte constituents of the rat heart. Although cardiac myocytes appear to contain predominantly MAO-A, this enzyme form is also localized, with high activity, to the non-myocyte fraction. However, since the non-myocyte fraction is heterogeneous in its cell content, containing vascular components of the coronary microcirculation, as well as other cells of connective tissue origin, the exact cellular localization of the enzyme activities within this fraction has not yet been defined.

Monoamine oxidase (monoamine O_2: oxidoreductase, EC 1.4.3.4, MAO) is an enzyme found predominantly on the outer membrane of the mitochondrion. Two forms of the enzyme, called MAO-A and MAO-B, can be distinguished in many animal tissues on the basis of their different relative sensitivities towards inhibition by the acetylenic drugs, clorgyline and deprenyl. In a variety of tissues containing both forms of the enzyme, 5-hydroxytryptamine (5-HT) and benzylamine (BZ) are generally found to be substrates for MAO-A and MAO-B, respectively, whereas tyramine (TYR) can be deaminated by both MAO activities (see [1] for review).

In the rat heart, some differences in the usual apparent substrate specificities of the two forms occur. Here, 5-HT and TYR are substrates for MAO-A alone [2, 3] whereas BZ can be deaminated by both enzyme forms, particularly in the adult rat [2, 4, 5]. Recent studies have explained these apparently anomalous findings on the basis of a considerable excess of MAO-A active sites over those of MAO-B in this tissue. Thus, although MAO-A has a very poor ability to deaminate BZ, nevertheless when MAO-A is by far the predominant enzyme form present, then a significant contribution of this enzyme to the total BZ metabolism can be observed [6]. This becomes increasingly evident with age, since the ontogenic increase in specific activity of rat heart MAO [3, 7] appears to be due to a selective increase in the MAO-A component [5, 8].

BZ is also deaminated in the rat heart (and other tissues such as blood vessels) by an amine oxidase distinct from MAO-A and MAO-B (see [9] for review). This enzyme is resistant to inhibition by millimolar concentrations of the acetylenic MAO inhibitors, but in contrast is inhibited completely by 10^{-3} M semicarbazide, which has little or no activity against MAO. The K_m of around 5 μM for deamination of BZ by the semicarbazide-sensitive amine oxidase (SSAO) is about 20–50 times lower than that for BZ metabolism by MAO activities, such that at low micromolar concentrations, the substrate is metabolized preferentially by SSAO in heart homogenates [4, 10]. In contrast, as BZ concentrations are increased, metabolism by MAO activities becomes more important and this change is accentuated by the high substrate inhibition of SSAO which reduces its activity at higher BZ concentrations [4]. At the present time the physiological importance of this enzyme is unknown, although its sensitivity towards semicarbazide and some other carbonyl reagents may suggest that the enzyme belongs to the group of amine oxidases believed to require pyridoxal phosphate or some other carbonyl function for catalysis (see [11] for review).

Although the properties of MAO and SSAO activities have been characterized in some detail in

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homogenates of the rat heart, this approach gives little information about possible differences in the cellular localization of these enzymes. The heart is a heterogeneous tissue containing neurones, cardiac muscle, connective tissue cells and vascular components from the coronary circulation. By the use of the proteolytic enzyme collagenase to dissociate heart ventricular tissue into various cellular constituents [12, 13], we have prepared fractions enriched in cardiac muscle cells (myocytes) and others containing mainly vascular and connective tissue cells (non-myocytes), in order to compare the amine oxidase activities in the different fractions. Alkaline phosphatase activity was also assayed in these samples, since this enzyme is believed to be associated predominantly with vascular (especially endothelial) components of the heart, with little or none found on the cardiac muscle cells [14]. Consequently, this enzyme was used to provide a marker for assessing the degree to which the separation of myocyte from non-myocyte constituents had been achieved. The results presented here suggest that MAO-A may be associated with both myocyte and non-myocyte constituents of the rat heart, whereas MAO-B, SSAO and alkaline phosphatase activities appear to be more concentrated within non-myocyte fractions. Some preliminary data from these and similar studies have previously been communicated [15, 16].

MATERIALS AND METHODS

Animals and chemicals. Adult male Wistar rats weighing 250–500 g were obtained from our Departmental rat breeding colony in the Animal Services Unit, University of Dundee. Radioactive substrates used for amine oxidase assays were (G-^{3}H)-5-hydroxytryptamine (5-HT) creatinine sulphate and (methylene-^{14}C)-benzylamine (BZ) hydrochloride from the Radiochemical Centre (Amersham, U.K.). Collagenase (type 1A), bovine serum albumin (Fraction V), diethanolamine, *p*-nitrophenol and *p*-nitrophenyl phosphate (ditris salt) were purchased from Sigma (London) Chemical Co. (Poole, U.K.). Clorgyline hydrochloride was a gift from May & Baker Ltd, Dagenham, U.K.

Preparation of cell fractions and homogenates. Pooled ventricular tissue (approx. 1.5 g total weight) from two rats at a time was chopped into small pieces (approx. 2 mm^3) with scissors. Most of the chopped tissue was used for dissociation into cells, but a small sample (approx. 200 mg) was stored at −20° for eventual homogenization as undissociated tissue.

The isolation of cell fractions enriched in myocytes and non-myocytes was modified from other published methods [12, 13] to provide a simple, relatively quick means of obtaining good yields of the cells, upon which enzyme assays could be performed. Briefly, chopped tissue was washed and then incubated at 37°, with shaking, in a disaggregation buffer (D.B.) containing 0.5 mg/ml collagenase [12]. Dissociated cells were harvested at approx. 30 min intervals from the tissue by filtration through nylon mesh (200 μm pore size). The undissociated tissue was continually recycled by reincubation in fresh collagenase solutions. Those filtrates (generally, the second, third and fourth collected) which contained a high proportion of intact myocytes among the cells, were pooled for cell purification. Later fractions and remaining undissociated tissue were discarded. Since the myocytes are larger than the non-myocyte constituents of the heart, the former were isolated and collected by centrifugation (10 *g* for 10 min). The resulting supernatants were later used as a source of non-myocytes (see below).

The myocyte pellets were resuspended in 2 ml D.B. (without collagenase) and layered carefully over 8 ml bovine serum albumin (B.S.A., 4% w/v in D.B.) in conical glass centrifuge tubes (capacity 12 ml). Intact myocytes, being denser than broken myocytes and residual non-myocyte cells, were enriched by sedimentation through the BSA medium (10 *g* for 10 min).

Fractions enriched in non-myocyte cells were obtained by centrifugation (800 *g* for 10 min) of the filtrate supernatant above. The cells were resuspended and recentrifuged in 4 ml D.B., and the resulting cell pellets were finally resuspended in 2 ml D.B. After layering on B.S.A. as above, the cells were separated from subcellular debris by centrifugation at 100 *g* for 10 min.

In preliminary experiments, light microscopy was used to confirm that the cell fractions were enriched in myocytes on the one hand, and with non-myocyte cells (such as endothelial and smooth muscle cells, as well as connective tissue constituents such as fibroblasts) on the other. Neuronal structures could not be identified, as these presumably are destroyed during collagenase treatment. On some occasions, samples of these fractions were dried on glass slides, fixed and then stained with haematoxylin and eosin. These studies also revealed that separation of myocytes from non-myocyte cells had been effectively achieved, although a few larger fragments of coronary blood vessels were found to co-purify with the myocytes, and in addition some dissociated myocytes occasionally retained a small adhering strip of capillary. Thus, more quantitative assessments of the purity of the fractions relied upon the distribution of alkaline phosphatase activity between them (see Introduction and Discussion). Also at this stage, the non-myocyte fraction contained a small quantity of red blood cells which had originally been trapped within the chopped tissue. These cells lysed very easily and did not appear to be recovered in the subsequent washing procedure (see below). Also, in separate experiments when 1 ml of rat blood alone was subjected to the same purification and washing procedure as the non-myocyte fractions, no detectable amine oxidase or alkaline phosphatase activities could be found in the small residual red blood cell fraction. Thus, it seems unlikely that the initial presence of a small amount of trapped blood in the chopped and washed tissue contributes in any significant way to the enzymatic activities of the final heart cell fractions.

Before performing enzyme assay on the purified heart cells, it was necessary to wash the cell pellets to remove contaminating B.S.A., which would interfere with protein estimations of cell homogenates. Consequently, cell pellets were resuspended in 1 mM potassium phosphate buffer, pH 7.8 (the eventual homogenization medium), centrifuged at 100 *g* for

Table 1. Amine oxidase activity towards 1 mM 5-HT and 1 μM BZ, and corresponding alkaline phosphatase activity of dissociated cell and undissociated tissue homogenates

Deaminating activity (nmole/hr/mg protein)	Myocyte	Non-myocyte	Undissociated tissue
1 mM 5-HT	279 ± 26 (92)	598 ± 172 (197)	303 ± 43 (100)
1 μM BZ (no clorgyline)	0.37 ± 0.12* (43)	1.63 ± 0.36 (191)	0.85 ± 0.10 (100)
1 μM BZ ($+10^{-3}$ M clorgyline)	0.24 ± 0.10† (32)	1.58 ± 0.40 (208)	0.76 ± 0.10 (100)
Alkaline phosphatase (μmole/5 min/mg protein)	0.18 ± 0.06† (38)	1.01 ± 0.16 (210)	0.48 ± 0.07 (100)

Enzyme activities are mean values ± S.E. of 5 preparations each from 2 rats (mean ± S.E. body weight of animals 356 ± 28 g). Figures in parentheses express values relative to corresponding undissociated tissue homogenates (= 100). Statistical comparisons between non-myocytes and myocytes: *$P < 0.05$, †$P < 0.01$.

10 min and then this washing and centrifugation step was repeated. The cells begin to swell in this hypotonic medium but can be recovered from these washing steps. Finally the washed cell pellets were resuspended in a small volume (usually 1.5 ml) of homogenization buffer and stored at −20°.

Thawed cell suspensions and undissociated tissue samples were further disrupted in a ground-glass hand-held homogenizer (tissue (g): homogenization buffer (ml) ratio of 1:10 for the undissociated tissue samples). In the latter case the supernatants were decanted from any larger undisrupted particulate matter remaining in the homogenizer after tissue disruption. Owing to the very low deaminating activity of the rat heart towards 1 mM BZ as substrate, and the relatively limited amount of material available in the cell fractions, all homogenates were used without the use of low-speed centrifugation to remove any unbroken cells or smaller debris, since this was found to result in the removal of a significant amount of the total enzymatic activity from the homogenates, with a consequent reduction in the sensitivity and reliability of the assays.

Enzyme assays. Amine oxidase activities were assayed in triplicate by the radiochemical method of Callingham and Laverty [17] as described fully in [18]. The substrates used were 5-HT (sp. act. 2 μCi/μmole) at 1 mM, and BZ at 1 μM (10 μCi/μmole) and 1 mM (0.5 μCi/μmole) final concentrations. For inhibitor studies, appropriate aqueous solutions of clorgyline were preincubated with homogenate samples for 20 min at 37°, before addition of radioactive substrate. Assay times were 20 min (1 mM 5-HT and 1 μM BZ) and 30 min (1 mM BZ).

Alkaline phosphatase activities in homogenates were estimated spectrophotometrically by the method of Roth [19] which follows the production at 405 nm of *p*-nitrophenol from *p*-nitrophenyl phosphate (10 mM solution in 2.5 ml 1M diethanolamine buffer, pH 9.8, containing 0.5 mM $MgCl_2$). The rate of *p*-nitrophenol production at room temperature was linear for at least 5 min and was estimated from a standard curve relating absorbance units to *p*-nitrophenol concentration, constructed by adding 50–250 μl aliquots of a standard aqueous *p*-nitrophenol solution (0.5 mM) to 2.5 ml of diethanolamine assay buffer.

Statistical significance was tested by the non-parametric Wilcoxon rank-sum method (two-tailed).

Protein concentrations of homogenates were estimated by the method of Lowry *et al.* [20] with bovine serum albumin as standard. Experiments on a total of nine different preparations were performed to obtain the specific enzyme activities shown subsequently in Tables 1 and 2. Mean protein contents of these homogenates were (mg/ml): 3.9 ± 0.7 (myocytes), 1.27 ± 0.32 (non-myocytes) and 7.6 ± 1.4 (undissociated tissue). These values represented mean total protein recoveries of around 5.9 and 1.9 mg for myocyte and non-myocyte homogenates

Table 2. Deamination of 1 mM BZ and corresponding alkaline phosphatase activity of dissociated cell and undissociated tissue homogenates

Deaminating activity (nmole/hr/mg protein)	Myocyte	Non-myocyte	Undissociated tissue
1 mM BZ	10.0 ± 1.2* (95)	32.6 ± 12.3 (310)	10.5 ± 2.5 (100)
Alkaline phosphatase (μmole/5 min/mg protein)	0.16 ± 0.02* (37)	0.62 ± 0.15 (144)	0.43 ± 0.04 (100)

Enzyme activities are mean values ± S.E. of 4 preparations each from 2 rats (mean ± S.E. body weight of animals 335 ± 14 g). Figures in parentheses express values relative to corresponding undissociated tissue homogenates (= 100). Statistical comparisons between non-myocytes and myocytes: *$P < 0.05$.

respectively (from around 1.5 g initial chopped tissue) compared with around 15.2 mg in the homogenates prepared from 200 mg of undissociated tissue.

RESULTS

In a first series of experiments, corresponding homogenates of myocytes, non-myocytes and undissociated ventricular tissue were used for determinations of alkaline phosphatase activity, as well as deaminating activity towards 1 mM 5-HT and 1 μM BZ. These assays on fractions from a given preparation were all performed on the same day and the specific enzyme activities obtained are shown in Table 1.

The metabolism of 1 mM 5-HT was always assayed after preincubation of homogenates in the presence and absence of clorgyline (10^{-6} M). This concentration of clorgyline completely inhibited 5-HT metabolism (data not shown) indicating that deamination of this substrate was brought about by MAO-A alone in the different fractions. In the absence of clorgyline, 5-HT metabolism in non-myocytes was approximately twice the activity in myocytes, although this difference was not statistically significant.

Deamination of 1 μM BZ was also measured after preincubation with and without clorgyline (10^{-3} M). This inhibitor concentration would be sufficient to inhibit any contribution of MAO activities to the metabolism of 1 μM BZ, and consequently the residual activity corresponds to that of the semicarbazide-sensitive amine oxidase [4, 9, 10]. Although clorgyline produced little inhibition (3% and 11%) of the activities in non-myocytes and undissociated tissue homogenates, respectively, there was a much larger inhibitory effect (35%) in myocyte fractions, suggesting that MAO activities may be proportionally more important for 1 μM BZ metabolism in these fractions.

It was a consistent finding in these studies that metabolism of 1 μM BZ was several-fold higher in non-myocyte than myocyte fractions, with intermediate activities being found in the undissociated tissue homogenates. This difference between non-myocytes and myocytes was greatest in samples assayed in the presence of 10^{-3} M clorgyline, where the results suggested a 6.6-fold higher SSAO activity in non-myocytes. A similar distribution of alkaline phosphatase activity was found, with 5.6-fold higher values in non-myocytes compared with myocytes, and intermediate activity being measured in the undissociated tissue homogenates.

The data of Table 2 represent a second series of experiments in which the deamination of 1 mM BZ by the different cell and tissue fractions was examined. Here, the activity of non-myocyte fractions was significantly higher (3.3-fold) than myocyte fractions. On the other hand, the activity in undissociated tissue was slightly, but not significantly, higher than that in the myocytes. Again, the activity of alkaline phosphatase was significantly higher (3.9-fold) in non-myocyte fractions, although the difference in these experiments was not quite as great as in those of Table 1.

The sensitivity of 1 mM BZ metabolism to inhibition by varying clorgyline concentrations was also examined with the homogenates described in Table 2. Figure 1 shows the mean inhibition plots obtained after experiments on each individual homogenate. In each case, the inhibition curve was double-sigmoid in shape, with a plateau region at 10^{-6} to 10^{-7} M clorgyline. These results indicated that MAO-A (inhibited below 10^{-7} M) and MAO-B (inhibited above 10^{-6} M) contribute to the total metabolism of 1 mM BZ. In addition, a small contribution ($\leq$10%) by the "clorgyline-resistant" component was also evident at 10^{-3} M clorgyline. The position of the plateau regions was significantly different when comparing myocyte with non-myocyte fractions. From these curves, it was estimated that the percentage contributions of MAO-A and MAO-B, respectively, to 1 mM BZ metabolism were 78 and 20% (myocytes), 43 and 52% (non-myocytes) and 57 and 32% (undissociated tissue). These results indicated that after dissociation of the original tissue into different cell fractions, MAO-A is the predominant enzyme form associated with the myocytes, whereas MAO-B occurs to a proportionally greater extent within the non-myocyte fraction.

DISCUSSION

In the present paper, we have attempted to extend the relatively limited information available about the cellular distribution of different amine oxidase enzymes in the rat heart. In a previous study, Lowe *et al.* [3] reported that after chemical sympathectomy of the heart, produced by administration of 6-hydroxydopamine to rats, there was no change in the deamination of 5-HT, kynuramine or tryptamine in homogenates of whole heart or of various selected heart regions. These amines are all predominantly MAO-A substrates in this tissue [3, 4, 21], and the

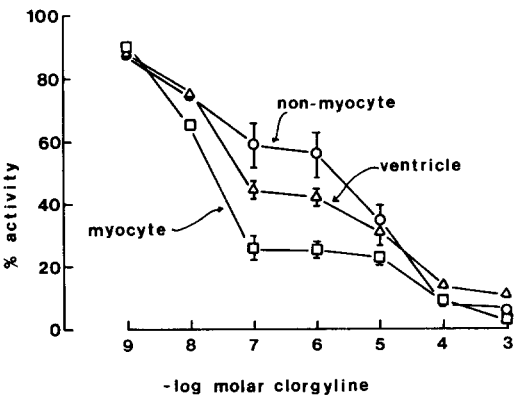

Fig. 1. Inhibition of 1 mM BZ metabolism by clorgyline. Homogenates of myocytes (□), non-myocytes (○) and undissociated ventricular tissue (△) were preincubated for 20 min with clorgyline. Remaining deaminating activities were assayed in triplicate with 1 mM BZ as substrate, and are expressed as a percentage of the corresponding control samples preincubated without inhibitor. Each point is the mean ± S.E. of the ratio for four different preparations. Points at 10^{-6} and 10^{-7} M on the myocyte inhibition curve are significantly different ($P < 0.05$) from those on the non-myocyte curve.

absence of a decrease in activity after sympathectomy indicates that almost all the MAO-A activity is extraneuronal, with at most an insignificant fraction being present within sympathetic nerves. This conclusion was supported by histochemical evidence, again using a substrate for MAO-A (tryptamine), which demonstrated enzymatic staining on mitochondria of myocardial cells, capillary endothelial cells, pericytes and various interstitial cells. Clearly, therefore, this enzyme form has a widespread cellular distribution within the tissue.

On the other hand, the distribution of MAO-B and the semicarbazide-sensitive amine oxidase (SSAO) has not previously been examined. Investigating this problem is not aided by the very low activity of these particular enzymes in the rat heart. However, the use of sensitive radiochemical methods to examine the deamination of benzylamine (BZ) at appropriate concentrations, and its inhibition by selective inhibitor drugs, has been of great value in determining the activities and properties of MAO-B and SSAO in the rat heart [4-6, 10]. Of course, as previously described (see Introduction), the preponderance of MAO-A in this tissue results in significant metabolism of BZ by this enzyme form also at certain BZ concentrations.

The current approach has involved the use of the proteolytic enzyme collagenase in order to dissociate ventricular tissue into its constituent cells. Amine oxidase activities were then studied in the resultant fractions enriched in cardiac myocytes, on the one hand, and in non-myocyte constituents, on the other. In addition, these activities were compared with those of samples of the original undissociated tissue from which the cell fractions were derived.

The major criterion used for assessing the separation of myocytes from non-myocytes, apart from visual inspection by light microscopy, was the assay of alkaline phosphatase (AP) in the cell fractions. The use of this as an enzyme marker is based upon histochemical evidence that AP is associated with capillaries and larger blood vessels of the cardiac microcirculation [14, 22, 23]. The enzyme appears to be located predominantly on endothelial cells, although its presence on fibroblasts and vascular smooth muscle cells in rat aorta has also been reported [24]. These were all cell types identifiable to variable extents in our non-myocyte fractions prepared from rat ventricular tissue, and these may all have contributed to the AP activity measured. On the other hand, cardiac muscle cells are believed to contain little or no AP activity. Some histochemical staining due to AP activity of the intercalated discs of myocytes has been described [22, 25] but this required longer incubation times than were necessary for demonstration of capillary staining and therefore seems to be a relatively minor source of AP in the heart [14].

In the experiments described here, AP activity was found to be about four to six-fold higher in non-myocyte fractions, with intermediate activities in the "parent" undissociated tissue. It was impossible to prepare myocyte fractions without some small contamination by a few segments of capillary and larger blood vessels which were of a sufficient size to co-purify with the myocytes. Consequently, these vascular elements may be the source of some or all of the AP activity of the myocyte fractions.

The activity of SSAO showed a similar distribution to that of AP, in the cell fractions, suggesting that SSAO may also be associated predominantly with some cellular component(s) of the non-myocyte fraction. It is therefore a strong possibility that at least part of this activity is localized in the coronary microvasculature, since other major blood vessels of the rat are known to possess a high activity of this enzyme [10, 26]. Whether or not it is present in the various types of connective tissue cell remains to be determined. In relation to a possible vascular localization, we have recently found SSAO activity in smooth muscle cells, isolated by enzymatic dissociation of the rat aorta and, in addition, have used histochemical techniques to localize this amine oxidase to the medial (smooth muscle-containing) layers of the vessel wall. In these studies, little staining of adventitial layers was found (Singh and Lyles, in preparation). SSAO has now been described in some detail in a variety of rat tissues (see [9] for review) in some of which it appears to be associated at least partly with cell plasma membrane fractions [27, 28]. The *in vitro* substrates of this enigmatic enzyme include (besides BZ) the biogenic amines β-phenylethylamine, dopamine, tyramine and tryptamine, but its possible role in amine metabolism *in vivo* remains unknown. It is likely that the association of this enzyme with vascular tissue, at least, may suggest future strategies for elucidating the physiological importance of SSAO in intact tissues.

The present studies also suggest some differences in the cellular localization of MAO-A and MAO-B. MAO-A activity, assayed with 5-HT, was present in both myocyte and non-myocyte cell fractions, a result consistent with earlier histochemical conclusions [3]. There was a suggestion from our data that the specific enzyme activity of MAO-A may be higher in non-myocyte than myocyte fractions, although the differences were not statistically significant. Clear evidence for the predominance of MAO-A within the myocytes was obtained from the experiments examining the inhibition by clorgyline of 1 mM BZ metabolism. Here, only a small proportional contribution (20%) of MAO-B to total BZ metabolism was observed. On the other hand, a much higher proportion of MAO-B (52%) and a correspondingly lower proportion of MAO-A (43%) was indicated by the inhibition curves for the non-myocyte fractions. These differences between the plateau regions of the inhibition curves for myocytes and non-myocytes may not appear to be particularly large. However, it should be appreciated that these curves would be consistent with fairly large differences in the distribution of MAO-B activity. As an illustration of this point it is possible to estimate the actual contributions of MAO-A and MAO-B to the total metabolism of 1 mM BZ shown in Table 2, by using the proportional contributions of MAO-A and MAO-B indicated by the inhibition curves (Fig. 1). Thus, the calculated specific enzyme activities (nmoles BZ metabolized/h/mg protein) would be for myocytes: 7.8 (MAO-A) and 2.0 (MAO-B); and for non-myocytes: 14.0 (MAO-A) and 17.0 (MAO-B). This type of indirect method, with its reliance upon

the accuracy of measuring both the total BZ metabolism and also its inhibition by clorgyline, is necessary for assessing MAO-B activity in the different fractions, since there is no amine substrate currently available which is specific for MAO-B alone in the rat heart. Thus it can be seen that when calculated in this form, the data are consistent with MAO-B activity which is 8.5-fold higher in non-myocyte than myocyte fractions, and thus it seems likely that this enzyme form is also associated predominantly with some cellular component(s) of non-myocyte origin.

As discussed earlier for AP activity, the presence of relatively low activities of SSAO and MAO-B in the myocyte fractions may also be due to a real association of small amounts of these enzymes with the myocytes, or alternatively may be due to a contamination of the fractions with vascular elements. Further evidence that our cell separation technique was responsible for the different MAO-A:MAO-B ratios was obtained from the corresponding inhibition curve for the undissociated tissue. This should be representative of both myocyte and non-myocyte constituents together, and did indeed produce inhibition patterns showing intermediate ratios of A:B compared with those of the myocyte and non-myocyte fractions alone.

In conclusion, from the results presented in this paper, it appears that the use of proteolytic enzymes to dissociate the rat heart into constituent cell fractions can provide useful information about possible differences in the distribution of the deaminating activities present within this organ. The applicability of these, and similar methods, for the study of amine oxidase enzymes in other animal tissues, of varying cellular heterogeneity, is currently being investigated in this laboratory.

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