Properties of Monoamine Oxidase in Monkey Heart

Masako KATAYAMA, Shinichi KOBAYASHI, Katsuji OGUCHI
and Hajime YASUHARA
Department of Pharmacology, School of Medicine, Showa University, Tokyo 142, Japan
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Abstract—The activities of monoamine oxidase (MAO) in a homogenate and the mitochondrial fraction of monkey (Macaca facicularis) heart were measured with labelled benzylamine, serotonin (5-HT), tyramine and β-phenylethylamine (PEA) as substrates. The effects of clorgyline, deprenyl and semicarbazide on MAO were also investigated. Benzylamine deamination was more sensitive to deprenyl than to clorgyline, although it was not completely inhibited by a high concentration of either, and the remaining activity was completely inhibited by pretreatment with semicarbazide. The activities of 5-HT and tyramine were both more sensitive to clorgyline than to deprenyl, and both inhibitors gave single-sigmoidal inhibition curves. Experiments with increasing concentrations of PEA as substrate and clorgyline and deprenyl as inhibitors indicated that at higher concentrations, PEA was a substrate for MAO-A as well as MAO-B in monkey heart. These results suggest that monkey heart mitochondria contain not only MAO-A and MAO-B, but also clorgyline resistant amine oxidase (CRAO). The ratio of MAO-A, MAO-B and CRAO was determined from plots of inhibitions with each substrate. The kinetic constants of MAO in monkey heart were compared with those of MAO in hearts of other animals. The enzymic properties of MAO in monkey heart were discussed on the basis of these results.

Monoamine oxidase (MAO) (monoamine oxidoreductase deaminating EC 1.4.3.4.) has been found to be present in many animal tissues (1–5), where it is located predominantly in the outer membrane of mitochondria (6–8) and catalyzes the oxidation of biogenic amines such as serotonin (5-HT) and catecholamines. The site of action of MAO is consistent with the distributions of 5-HT and noradrenaline in the body. Thus, a close relationship between MAO and sympathetic nerves has been recognized (9).

In 1968, Johnston (10) proposed that there are two forms of the enzyme: MAO-A, which is sensitive to clorgyline at low concentration, and MAO-B, which is less sensitive to clorgyline, but highly sensitive to deprenyl.

Recently, another amine oxidase activity has been found that is sensitive to semicarbazide, but resistant to concentrations of clorgyline that completely inhibit MAO-A and MAO-B activities (11–17). This new amine oxidase was named “clorgyline resistant amine oxidase” (CRAO), or “benzylamine oxidase” (18), since it deaminates benzylamine selectively.

Many reports have been published on MAO and CRAO of hearts of various species such as rats (19–23), humans (16, 18, 24), cattle (4, 25), and chicks (3, 14). In this work, we studied the properties of MAO and CRAO in a homogenate and the mitochondrial fraction of monkey heart using clorgyline, deprenyl and semicarbazide as inhibitors and discussed monkey heart MAO as an experimental model of human heart MAO.

Materials and Methods

1. Preparation of samples: Adult monkeys (Macaca facicularis) were sacrificed by bleeding from the common carotid artery. The heart was rapidly removed, freed of fat, chopped up and homogenized in a Waring
blender with 7 volumes of 0.01M phosphate buffer (pH 7.4) containing 0.25M sucrose. The homogenate was centrifuged at 600×g for 10 min at 4°C, and the supernatant was used as the homogenate preparation. Aliquots of this supernatant were further centrifuged at 10,000×g for 20 min, and the resulting pellet was suspended in the buffer and used as the mitochondrial preparation. These preparations were divided into aliquots and kept frozen until used for studies. Portions were thawed for experiments on the day of use.

2. Assay of MAO activity: MAO activity was determined by radiochemical procedures based on the method of Wurtman and Axelrod (26). The incubation medium contained radioactive substrates (1.6–20 μCi/μmol) and unlabelled substrates in a total volume of 100 μl of phosphate buffer (0.01 M, pH 7.4). After incubation at 37°C under an atmosphere of air, the reaction was stopped by cooling the tubes on ice and acidifying the mixture with 20 μl of 3N HCl. The medium was extracted by shaking it with 1 ml of toluene:ethylacetate (1:1) saturated with water. A 0.5 ml aliquot was taken for measurement of radioactivity in a Packard Tri-Carb Liquid Scintillation Spectrometer. In inhibition studies, the enzyme preparation was preincubated at 37°C for 30 min with clorgyline, deprenyl or semicarbazide before addition of substrate. Then the remaining activity was determined by the assay method described above. In this assay, the activity was linearly proportional to the amount of protein and to the time. Specific activities were in all cases corrected for the efficiencies of extraction of metabolites and were expressed in nmol/mg protein/hr.

3. Km determination: Km values were calculated from Lineweaver-Burk plots.

4. Protein assay: Protein concentration was determined by the biuret method as modified by Yonetani (27) with bovine serum albumin as a standard.

Results

1. Effects of inhibitors on MAO activity in monkey heart: The homogenate and mitochondrial fraction were incubated with benzylamine as substrate and serial dilutions of clorgyline or deprenyl as inhibitor. As shown in Fig. 1, single-sigmoidal inhibition curves were obtained, and benzylamine deamination was not completely inhibited by 1 mM clorgyline or deprenyl. This residual activity towards clorgyline amounted to about 35% of that in the mitochondrial fraction. Figure 2 shows that pretreatment of the enzyme sample with 1 mM semicarbazide completely eliminated this residual MAO activity, indicating that it was that of CRAO. The observation of higher sensitivity of the activity to clorgyline than to deprenyl indicated the presence of MAO-B.

Results with 5-HT (0.1 mM) and tyramine (0.1 mM) as substrates are shown in Fig. 3. The deaminations of both substrates were inhibited by clorgyline, which gave single-sigmoidal inhibition curves. There was no remaining activity, and the curves were more highly sensitive to clorgyline than to...
deprenyl, indicating that both 5-HT and tyramine were deaminated solely by MAO-A.

The sensitivities of PEA deamination to clorgyline and deprenyl were examined at four concentrations of PEA, as shown in Fig. 4. The inhibition curves were all single-sigmoidal, and the deamination was highly sensitive to deprenyl, irrespective of the PEA concentration: the deamination was inhibited completely by 1 mM clorgyline or 0.001 mM deprenyl. At higher concentrations of PEA, the sensitivity to clorgyline increased and that to deprenyl decreased. Thus PEA was deaminated by MAO-B.

2. Specific activities towards various substrates: Table 1 shows the specific activities of MAO (treated with 1 mM semicarbazide), CRAO (treated with 1 mM clorgyline), and an untreated preparation of the homogenate,
measured with tyramine, 5-HT, benzylamine and PEA (0.1 and 0.01 mM). The activity of the untreated preparation was nearly equal to the sum of the MAO and CRAO activities. CRAO activity was scarcely detectable with either concentration of tyramine, 5-HT or PEA, but accounted for a significant proportion of the total activity with both concentrations of benzylamine.

3. Kinetic analysis of monkey heart MAO: The Michaelis-Menten kinetic constants for MAO in monkey heart were determined with tyramine, 5-HT, benzylamine and PEA as substrates. The results are summarized with those reported for human (28), rat (11, 29–31) and frog (32) heart in Table 2.

Table 2. K_m values of MAO from monkey heart and other animal hearts (µM)

| Substrate | Monkey | Human\(^{28}\) | Rat \(^{29}\) | Frog \(^{32}\) |
|-----------|--------|----------------|-------------|-------------|
| Tyramine  | 95     | n.d.           | 80\(^{28}\)  | 114         |
| 5-HT      | 125    | 132            | 206\(^{30}\) | 204         |
| Benzylamine| 100    | 151            | a) 21\(^{11}\) | 9           |
|           |        |                | b) 310      |             |
| PEA       | 2      | 197            | 26\(^{31}\)  | 21          |

For measurement of MAO and CRAO activities, enzyme preparations were pretreated with 1 mM semicarbazide and clorgyline, respectively, for 30 min.

Discussion

Neff and Yang (33) reported that the two forms of MAO showed distinct differences in specificities for certain amines: 5-HT and norepinephrine were described as specific substrates for MAO-A, benzylamine and PEA for MAO-B, and tyramine, dopamine and tryptamine for both forms. However, subsequent results (34–39) do not support this simple scheme, and it is now generally agreed that the relative sensitivity towards clorgyline should be used as the primary criterion for defining MAO-A and MAO-B activities (34). We applied this criterion to the results obtained in this work to determine the relative proportions of MAO-A, MAO-B and CRAO in monkey heart (see Table 3). Reported results on the selective inhibition of monoamine oxidase in heart tissue of different species are listed in Table 3 with our results on monkey heart. We found that monkey heart mitochondria contained MAO-A, MAO-B and CRAO; and we found that tyramine and 5-HT were oxidized only by MAO-A, PEA was oxidized only by MAO-B, and benzylamine was oxidized by both MAO-B and CRAO. Table 3 shows that our results for tyramine and benzylamine are different from those obtained with human heart. Moreover, the activity ratios with various substrates are different from those for rat and frog hearts,
although there is some correspondence. Furthermore, the \( K_m \) values for 5-HT and benzylamine of monkey heart MAO are similar to those of human heart MAO, but that for PEA is much smaller than the value in human heart (28). In addition, the \( K_m \) values of rat (11, 29–31) and frog (32) hearts for these substrates are different from those of monkey heart (see Table 2). These differences in the characters of the enzymes in monkey heart and human heart lead to the conclusion that the enzymes in monkey heart are unsuitable for use as experimental models of those in human heart.

Concentration-dependent changes in the substrate specificity of rat brain mitochondrial MAO with PEA as substrate were reported previously (40, 41). In this work, a double-sigmoidal curve towards PEA was not obtained, but at higher concentrations of PEA, the activity became more sensitive to clorgyline and less sensitive to deprenyl. These results suggest that a small amount of MAO-A activity contributes to deamination of PEA at higher concentrations, although according to the Johnston’s criteria (10), PEA appears to be metabolized exclusively by MAO-B in monkey heart.

It is frequently assumed that the substrate specificities and sensitivities to inhibitors of MAO-A and MAO-B are similar in various organs and tissues and in different animal species. This, however, seems to be an oversimplification and does not even apply to the same organ, since monkey heart MAO differs from that of human heart even though monkey heart bears a close resemblance to human heart.

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Table 3. Comparison of activity ratios based on inhibition curves of MAO and CRAO activities in hearts of various animals

| Substrate           | MAO-A: MAO-B: CRAO (% of total activity) |
|---------------------|------------------------------------------|
| Monkey heart        |                                          |
| Tyramine (0.1, 0.5 mM) | 100:0:0                                  |
| 5-HT (0.1, 0.5 mM)  | 100:0:0                                  |
| Bz (0.1 mM)         | 0:64:36                                  |
| Bz (0.5 mM)         | 0:72:28                                  |
| PEA (0.01 mM)       | 0:100:0                                  |
| Human heart 28)     |                                          |
| Tyramine (0.5 mM)   | 20:80:0                                  |
| 5-HT (0.5 mM)       | 100:0:0                                  |
| Bz (0.5 mM)         | 0:100:0                                  |
| PEA (0.5 mM)        | 0:100:0                                  |
| Dopamine (0.5 mM)   | 20:80:0                                  |
| Rat heart            |                                          |
| Tyramine (0.01–5.0 mM) 42) | 100:0:0                                  |
| Bz (0.001–0.01 mM) 42) | 0:0:100                                  |
| Bz (0.1 mM) 42)      | 26:22:52                                 |
| Bz (1 mM) 42)        | 35:33:32                                 |
| PEA, 5-HT and dopamine 11) | 100:0:0                                  |
| Frog heart 32)       |                                          |
| 5-HT (0.1 mM)       | 87:0:13                                  |

5-HT, serotonin; Bz, benzylamine; PEA, \( \beta \)-phenylethylamine. 28) D. Parkinson and B.A. Callingham, 1979. 22) M. Mikami, 1983. 42) D.E. Clarke, G.A. Lyles and B.A. Callingham, 1982.
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