Identification of type A monoamine oxidase in mouse and rabbit liver mitochondria

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Summary. Type A monoamine oxidase was identified in liver mitochondria of mouse and rabbit. 5-Hydroxytryptamine was a common substrate for type A and type B monoamine oxidase in these enzyme preparations where its concentration was 1.0 mM.

Mitochondrial monoamine oxidase [amine:oxygen oxidoreductase (deaminating, flavin-containing); EC 1.4.3.4] (MAO) can be classified in 2 functional forms, viz. type A and type B[1]. Some tissues were reported to contain a single enzyme form; human platelet[2] and the livers of mice[3,4], rabbits[3,4] and cows[5] contain only type B MAO, while human placenta only type A MAO[6]. However, in the present communication, we could identify type A MAO in mouse and rabbit livers.

Materials and methods. Mitochondrial fractions were prepared from the pooled livers of 10 male mice, weighing about 20 g, and 3 male rabbits, weighing about 3 kg, as described previously[7]. Clorgyline, a selective inhibitor of type A MAO[1], was generously supplied by May & Baker Ltd., Dagenham, England. Deprenyl, a selective inhibitor of type B MAO[8], was kindly donated by Prof. J. Knoll, Department of Pharmacology, Semmelweis University of Medicine, Budapest, Hungary.

MAO activities toward 5-hydroxytryptamine (5-HT) were determined by a slight modification of a new photometric assay[9]. The details of the procedure are given in our previous paper[10]. MAO activities toward tyramine were measured fluorometrically by a slight modification[7] of the method of Guilbault et al.[11] and Snyder and Hendley[12]. For each assay (final volume, 3.0 ml), 0.026-0.520 mg of mitochondrial protein was used. The assays were carried out at 37 °C for 10 or 30 min. Care was taken not to convert more than 10% of the substrates to reaction products to obtain activities which approximate their initial velocity. It was confirmed that each inhibitor did not interfere with the photometry or the fluorometry when hydrogen peroxide was added directly.

Protein was measured by a slight modification[13] of the conventional biuret method.

Results and discussion. Figure 1 shows the inhibition of MAO in mouse liver mitochondria by clorgyline and deprenyl, using different concentrations of 5-HT and tyramine as substrates. With tyramine as substrate, all the curves were almost single sigmoidal; clorgyline, when present in the assay mixture at a concentration of 10^{-7} M, showed only a slight inhibition, but deprenyl, at the same concentration, almost completely blocked tyramine deamination. These data show that tyramine is deaminated mainly by type B MAO in mouse liver mitochondria, which agrees well with the previous reports[3,4].

When 5-HT, a well-known type A substrate[3], was used as substrate, inhibition patterns became quite different; with 20 μM 5-HT as substrate, its deamination was completely

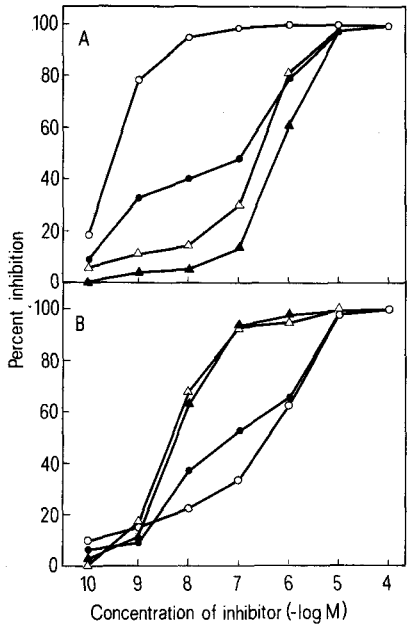

Fig. 1. Inhibition of MAO in mouse liver mitochondria by clorgyline (A) and deprenyl (B) using different concentrations of 5-HT and tyramine as substrates. The activities in the absence of inhibitors with 20 μM 5-HT (○—○), 1.0 mM 5-HT (●—●), 20 μM tyramine (△—△), and 1.0 mM tyramine (▲—▲) were 18.1, 416, 43.5 and 311 nmoles/mg protein/30 min, respectively. Each point represents the mean obtained from duplicate determinations upon a single enzyme source prepared from pooled livers of 10 mice.

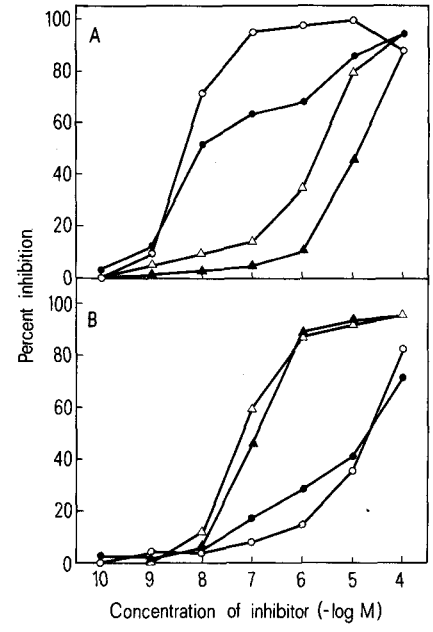

Fig. 2. Inhibition of MAO in rabbit liver mitochondria by clorgyline (A) and deprenyl (B) using different concentrations of 5-HT and tyramine as substrates. The activities in the absence of inhibitors with 20 μM 5-HT (○—○), 1.0 mM 5-HT (●—●), 20 μM tyramine (△—△) and 1.0 mM tyramine (▲—▲) were 10.9, 220, 39.8 and 437 nmoles/mg protein/30 min, respectively. Each point represents the mean obtained from duplicate determinations upon a single enzyme source prepared from pooled livers of 3 rabbits.

inhibited by 10^{-7} M clorgyline, while the inhibition by 10^{-7} M deprenyl was about 30%. In addition, the inhibition patterns were almost single sigmoidal. These results indicate that 5-HT is deaminated mainly by type A MAO, demonstrating the presence of type A enzyme in mouse liver. When 1.0 mM 5-HT was used, the patterns were dramatically changed; a clear plateau appeared at 10^{-9}–10^{-7} M clorgyline. Consistent with the change in inhibition by clorgyline, the susceptibility to deprenyl increased at 1.0 mM of 5-HT. These data show that 5-HT is deaminated by both type of MAO in mouse mitochondria at high concentrations.

Figure 2 shows the inhibition of MAO in rabbit liver mitochondria, using different concentrations of 5-HT and tyramine as substrates. The results obtained with rabbit liver were generally similar to those for mouse liver; tyramine was deaminated mainly by type B MAO, while 5-HT at 20.0 µM mainly by type A and at 1.0 mM by both types. These data, therefore, demonstrate the presence of type A MAO in rabbit liver mitochondria.

In the literature[3,4], mouse and rabbit livers have been claimed to contain a single MAO form, type B, using tyramine and kynuramine as substrates. However, inhibition curves with non-specific substrates such as tyramine and kynuramine are not sensitive enough to detect 2 forms of MAO, if the activity of 1 form accounts for less than 10% of the total activity[14]. In fact, we also could not identify type A MAO in mouse and rabbit livers with only the aid of tyramine as substrate (figures 1 and 2). To solve this problem, we have used 5-HT as a type A substrate, and could successfully identify type A enzyme in both mouse and rabbit liver mitochondria.

In our previous papers[7,15,16] we have demonstrated that inhibition patterns are drastically changed, as regards different substrate concentrations, when β-phenylethylamine, phenylethanolamine and N-methylphenylethanolamine are used as substrates. This was also the case for 5-HT with mouse and rabbit liver mitochondria (figures 1 and 2). Thus, our present results lend further support to our warning[7,15] that substrate specificities of the 2 types should be evaluated over a wide substrate concentration range.

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Effect of prostaglandins and dibutyryl cyclic AMP on the morphology of cells in primary astroglial cultures and on metabolic enzymes of GABA and glutamate metabolism

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Summary. Prostaglandins (PGE_1) and dibutyryl cyclic AMP (dBc AMP) induce similar morphological changes in astrocytes obtained in primary cultures. PGE_1 and dBc AMP increased 2 enzymes of GABA and glutamate metabolism, GABA-T and AAT, but did not modify GDH and GLN-S. Prostaglandins probably affect the cAMP content of glial cells and act in the same way as dBc AMP on glial cell differentiation.

It is now generally accepted that glial cells play a role in synaptic activity regulation. Primary cultures of astrocytes are of major importance for the study of astrocytic development and function. Such cultures, obtained from newborn mice or rats, do not contain neuronal cells[1].

We have previously shown, using primary cultures of dissociated newborn mice brain hemispheres that astrocytes: a) contain a high level of GFA (2–3 µg/mg P), an astrocytic marker[2], and have a very low GAD activity (a neuronal marker); b) contain GABA-T, the GABA metabolizing enzyme, the activity of which increased during cell growth and was inducible by high extracellular GABA concentrations[3], c) show morphological changes when dBc AMP was added to the medium, which were associated with an increasing GABA-T activity[4].

Prostaglandins and particularly PGE_1 are known to affect neuroblastoma cells[5]. The sensitivity of glial cells to dibutyryl cyclic AMP (dBc AMP) and to PGE_1 are compared here, considering the morphological changes and some biochemical modifications concerning GABA and Glu metabolism induced in the presence of these 2 agents. The procedure for culturing glial cells has been described previously[3]. In brief, for the preparation of 10 cultures, cerebral hemispheres from 3 newborn Swiss mice were dissociated mechanically and passed through a sterile nylon sieve (80 µm pore size) into 10 ml of a modified Eagle's