Multiplicity of monoamine oxidase in chick brain

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Summary. The substrate- and inhibitor-related characteristics of monoamine oxidase (MAO) were studied on chick brain mitochondria. It was found that neither 5-hydroxytryptamine nor β-phenylethylamine is the specific substrate for type A and type B MAO in chick brain.

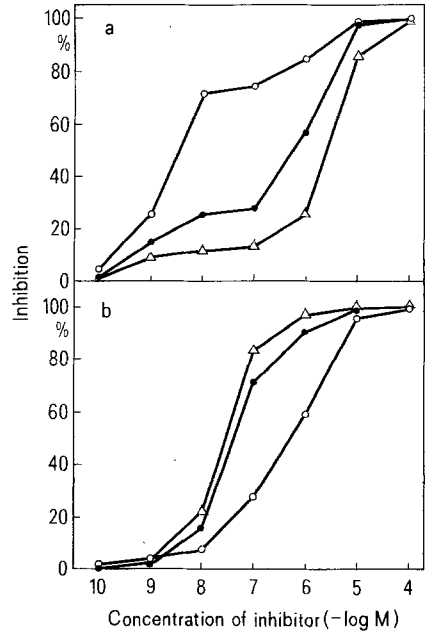
Recently, there has been considerable interest in the multiple forms of monoamine oxidase [amine: oxygen oxidoreductase (deaminating, flavin-containing); EC 1.4.3.4.] (MAO). In particular, the existence of 2 types of MAO, which are designated as type A and type B enzyme, has been well-documented[1-3]. Even though it is not clear if each type of MAO has its own enzyme protein, it is unequivocally accepted that both types of MAO are demonstrable in vitro and in vivo in their substrate specificity and inhibitor sensitivity. Type A MAO is active with 5-hydroxytryptamine (5-HT) and norepinephrine as substrates, and is sensitive to the inhibition by low concentration of clorgyline. Type B MAO is active with β-phenylethylamine (PEA) and benzylamine, and is sensitive to the inhibition by low concentration of deprenyl. Some substrates, such as kynuramine, tyramine, tryptamine and dopamine, are oxidized by either type of MAO. It should be noted that the concept of the 2 types of MAO is primarily based on the sensitivity of MAO to clorgyline[1].

In recent years, however, a few reports have shown that 5-HT is oxidized to some extent by type B MAO in some tissues[4-6]. The present study deals with the substrate- and inhibitor-related characteristics of type A and type B MAO in chick brain mitochondria, since there are few such studies for avian tissues. Our result showed that MAO from chick brain reveals are even more atypical nature in substrate specificity than any other MAO preparation so far reported.

Materials and methods. Young adult chicks of the F_1 hybrid between the White Leghorn and the Rhode Island Red breed weighing about 1 kg were used. The animal was killed by decapitation and the brain removed rapidly. It was homogenized with 9 vol. of 0.25 M sucrose in a Potter-Elvehjem homogenizer fitted with a Teflon pestle allowing cooling in an ice bath and centrifuged at 1500 × g for 5 min to remove cellular debris. The resulting supernatant was centrifuged at 18,000 × g for 20 min and the crude mitochondrial pellet was suspended in the sucrose solution. The suspension was recentrifuged at 18,000 × g for 30 min and the pellet was resuspended in a small amount of water. This was used as an enzyme source.

MAO activity towards 5-HT was assayed by a radiochemical procedure[7]. The concentration of the substrate was 125 µM. The determination of MAO activity with kynuramine as substrate was carried out fluorometrically by the method of Kraml[8] with a slight modification[9] with the substrate concentration of 82 µM. MAO activity towards PEA was measured fluorometrically by our method[10], and the concentration of the substrate was 125 µM. 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[11], was kindly donated by Prof. J. Knoll, Department of Pharmacology, Semmelweis University of Medicine, Budapest, Hungary. It was confirmed that clorgyline and deprenyl neither interfered with the formation of fluorescent compounds nor quenched their fluorescence when kynuramine and PEA were used as substrates. For each assay, 0.07–0.43 mg of mitochondrial protein was used. The assay mixture was preincubated at 37 °C for 10 min to ensure the maximal enzyme inhibition. Protein was determined by the biuret method[12].

Results and discussion. The inhibition of chick brain MAO by clorgyline, using 3 substrates, is shown in the figure (a). The deamination of 5-HT, which is said to be a preferred substrate for type A MAO, was much more susceptible to clorgyline than that of PEA, which is said to be a preferred substrate for type B MAO. The deamination of kynuramine, which is said to be commonly oxidized by both types of MAO, was found to be intermediate in sensitivity to clorgyline. Thus, the substrate susceptibility was in the order that might be expected under the classification of MAO into types A and B. However, plateaus were observed for all the substrates: in the inhibition curves with 5-HT and kynuramine as substrates, plateaus appeared in the range of 10^{-8} to 10^{-7} M of clorgyline. In the curves with PEA, a plateau was also clear in the same concentration range of clorgyline, although the percent inhibition at which the plateau appeared, was relatively low. Therefore, according to the postulation that type A MAO is inhibited by low concentration of clorgyline[1], the conclusion can be drawn that both 5-HT and PEA are oxidized by either type of MAO in chick brain mitochondria. It should be recalled that, in beef heart mitochondria[4] and in pig liver and brain[5], 5-HT is oxidized by either type of MAO, while PEA is oxidized exclusively by type B MAO. Our result with chick brain seems unique, since the tissue in which both 5-



Inhibition of MAO in chick brain mitochondria by clorgyline (a) and by deprenyl (b). The concentrations of 5-HT (○——○), kynuramine (●——●) and PEA (△——△) were 125, 82 and 125 µM, respectively. Each point represents the mean obtained from duplicate determinations. The assay mixture was preincubated with the inhibitor at 37 °C for 10 min.

HT and PEA are oxidized by either type of MAO has never been reported.

The differentiation observed in MAO inhibition by deprenyl was not so marked as that by clorgyline: neither plateaus nor shoulders were observed for all the substrates (figure, b). This situation is similar to that of rabbit tissues with deprenyl[12]. However, the substrate susceptibility was in the order consistent with the type A and type B classification. It seems likely that percent inhibition of MAO by 10^{-7} M deprenyl reflects the percentage of type B MAO in chick brain mitochondria, since the approximate reverse relationship in MAO inhibition between the 2 inhibitors was obtained at the concentration of 10^{-7} M.

In the present communication, we demonstrated that neither 5-HT nor PEA is the specific substrate for the 2 types of MAO with chick brain mitochondria. This result lends further support to the idea that the classification of MAO into type A and type B is of limited value[6].

1 J.P. Johnston, Biochem. Pharmac. *17*, 1285 (1968).
2 H.-Y.T. Yang and N.H. Neff, J. Pharmac. exp. Ther. *187*, 365 (1973).
3 H.-Y.T. Yang and N.H. Neff, J. Pharmac. exp. Ther. *189*, 733 (1974).
4 T.J. Mantle, M.D. Houslay, N.J. Garrett and K.F. Tipton, J. Pharm. Pharmac. *28*, 667 (1976).
5 B. Ekstedt and L. Oreland, Arch. int. Pharmacodyn. Thér. *222*, 15 (1976).
6 C.J. Fowler, B.A. Callingham, T.J. Mantle and K.F. Tipton, Biochem. Pharmac. *27*, 97 (1978).
7 R.E. McCaman, M.W. McCaman, J.M. Hunt and M.S. Smith, J. Neurochem. *12*, 15 (1965).
8 M. Kraml, Biochem. Pharmac. *14*, 1684 (1965).
9 B. Century and K.L. Rupp, Biochem. Pharmac. *17*, 2012 (1968).
10 O. Suzuki, E. Noguchi and K. Yagi, Biochem. Pharmac. *25*, 2759 (1976).
11 J. Knoll and K. Magyar, in: Advances in Biochemical Psychopharmacology, vol. 5, p. 393. Ed. E. Costa and M. Sandler. Raven Press, New York 1972.
12 E. Layne, in: Methods in Enzymology, vol. III, p. 447. Ed. S.P. Colowick and N.O. Kaplan. Academic Press, New York 1957.
13 R.F. Squires, in: Advances in Biochemical Psychopharmacology, vol. 5, p. 335. Ed. E. Costa and M. Sandler. Raven Press, New York 1972.

Tetrapyrrole biosynthesis from 4,5-dioxovaleric acid in *Rhodopseudomonas spheroides*

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Summary. Porphyrin biosynthesis from 4,5-dioxovaleric acid was studied in cell suspensions of *R. spheroides.* The experiments show that 4,5-dioxovaleric acid is a far precursor of porphyrins through δ amino laevulinic acid formation in a transamination reaction involving also l-alanine. It differs radically from the classical δ aminolaevulinic acid synthesis using glycine and succinyl CoA as substrates.

δ Aminolaevulinic acid (δ ALA), a classical porphyrin precursor, is synthetized from succinyl CoA and glycine through a reaction catalyzed by δ ALA synthetase. However, Tait[3] has suggested that δ ALA transamination might be involved in δ ALA synthesis, particularly in some systems in which no δ ALA synthetase has been detected. The substrate in this transamination is 4,5 dioxovaleric acid (DVA). Recently, Lohr and Friedmann[3] working with *Zea mays* leaf extracts found a novel NADH-dependent reduction of the 1-carboxyl group of α ketoglutarate yielding 4,5-dioxovalerate acid, followed by a transmission between this product and l-alanine to yield δ ALA. In addition, Neuberger and Turner[5], working with *R. spheroides,* pointed out that the transamination reaction favours δ ALA formation from DVA.

In this work we synthetise pure DVA using a new method described by Kissel and Heilmeyer, Jr[6], and we show that DVA is an earlier precursor of tetrapyrrole biosynthesis than δ ALA in *R. spheroides.*

Material and methods. 4,5-DVA was synthetized according to Kissel and Heimeyer, Jr[6]. Its purity was checked preparing the 2,4-dinitrophenyl osazone (m.p. 245–248 °C). Culture, growth and harvesting were performed according to Lascelles[7].

Cells were grown in anaerobiosis under light at 36–37 °C and incubations were carried out as indicated in the legends of the figures. After different times of incubation, the cell suspensions were centrifuged at 40,000 × g and the porphyrin content of supernatants was determined spectrophotometrically using the extinction coefficient for coproporphyrin III[8].

Results and discussion. As is shown in figure 1, a small amount of porphyrins can be synthetized without l-alanine being present. When this amino acid was added, porphyrin synthesis increased, indicating that DVA is a precursor of porphyrins through a transamination involving δ ALA. l-Glutamic acid cannot replace l-alanine as a transamination substrate. In figure 2 it is shown porphyrin formation

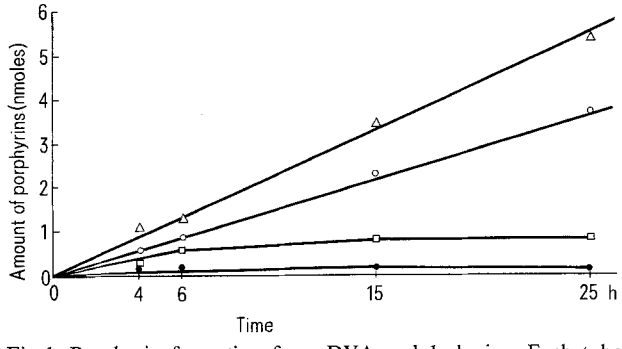

Fig. 1. Porphyrin formation from DVA and l-alanine. Each tube contained 3 mg/ml cell suspensions; 0.1 M phosphate buffer pH: 6.9; 8×10^{-3} M DVA; 8×10^{-4} M $MgSO_4$ and the following l-alanine concentrations: □, none; ○, 8×10^{-3} M; △, 1.6×10^{-2} M; ●, blank without DVA and 1.6×10^{-2} M l-alanine. Incubations were carried out in anaerobiosis under light at 36–37 °C.