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Monoamine oxidase A and B activities in liver of riboflavin-deficient rats

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The nutritional requirement of riboflavin and iron for the normal functioning of monoamine oxidase [MAO; monoamine: oxygen-oxidoreductase (deaminating); EC 1.4.3.4] is well known [1, 2]. Recently, we briefly reported that a nutritional deficiency of riboflavin (B_2) affects the oxidation of phenethylamine (PEA) by liver MAO to a somewhat greater extent than it affects the oxidation of serotonin (5-HT) [3]. The discrepancy in rates, though small, might be attributable to the differences between two functional forms of MAO that are widely postulated in order to explain the substrate and inhibitor specificities of this enzyme. Thus, MAO A has 5-HT and norepinephrine as the preferred substrates and is inhibited by low concentrations of clorgyline [4], whereas MAO B has PEA and benzylamine as preferred substrates and is inhibited by low concentrations of deprenyl [5]. The nature of the two forms of MAO is not clear (cf. Ref. 6), but there is evidence that lipids play a role in the expression of MAO multiplicity [7, 8]. In the present context, it is of interest that riboflavin deficiency affects the lipid content of rat liver mitochondria and results in discontinuities of the outer membrane, as detected by electron microscopy [9]. The present investigation reports the effect of riboflavin deficiency on the two forms of MAO in rat liver, as measured with the respective preferred substrates.

Young male Sprague–Dawley rats inititally weighing 40–45 g were used. They were fed solid diets formulated as for iron deficiency [10], except that in this case iron (310 mg/kg) was included, and riboflavin omitted. Three different experiments were conducted under similar conditions during the course of a 9-month period. In the first, animals were provided with the riboflavin-deficient diet for 25 days; in the second experiment for 10 and 19 days; and in the third for 8 and 20 days. The last experiment also included a group that had received the deficient diet for 22 days followed by the riboflavin-supplemented diet for a further 8 days. The supplement of riboflavin was included in the food, as in the case of the control rats, at a level of 25 mg/kg. For each period of riboflavin deficiency there were four experimental animals and four controls. At the end of the experimental period the rats were decapitated and their livers were removed, weighed, and frozen (−70°) until assayed for MAO activity.

In the MAO assay, tissue was prepared as a homogenate in 0.25 M sucrose, 2 g of liver/100 ml. Incubation was carried out at 37° and pH 7.4, with ^{14}C-labeled 5-HT and PEA as substrates. Other conditions of the assays were as described previously [11], except that the deaminated metabolites of 5-HT were extracted with benzene–ethyl acetate (1:1) in this work. The final concentrations and specific activities of these substrates were: 5-HT, 10^{-4} M, 10 Ci/mole; PEA, 2×10^{-5} M, 10 Ci/mole. In experiments with clorgyline and deprenyl, these inhibitors were preincubated with the enzyme preparation for 25-30 min at 37° before the addition of substrate. MAO activity has been expressed as nmoles of product formed per mg protein per hour.

5-Hydroxytryptamine binoxalate, 5-[2-^{14}C] (sp. act. 57.0 Ci/mole), and phenylethylamine hydrochloride, β-[ethyl-1-^{14}C] (sp. act. 48.25 Ci/mole), were purchased from the New England Nuclear Corp., Boston, MA. Deprenyl was donated by Dr. J. Knoll, Department of Pharmacology, Semmelweis University of Medicine, Budapest, Hungary. Clorgyline was supplied by May & Baker Ltd., Dagenham, U.K.

The effects of riboflavin deficiency on liver MAO A (activity with 5-HT as substrate) and on MAO B (activity with PEA) are depicted in Fig. 1. The activity decreased rapidly during the first 10 days of deficiency, and then more slowly, until at 20 days there was a levelling off. Furthermore, at each stage of the deficiency, the activity with PEA (MAO B) was more affected than with 5-HT (MAO A). This differential effect of riboflavin deficiency on the oxidation of two characteristic substrates was also reflected

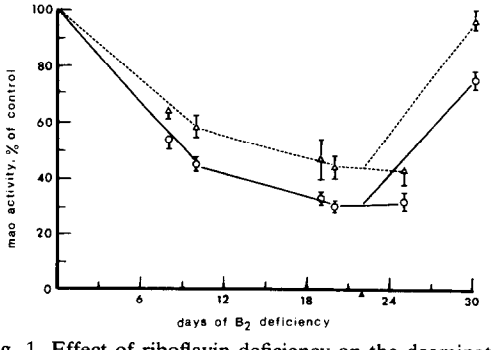

Fig. 1. Effect of riboflavin deficiency on the deamination of 5-HT (△ - - - - - △) and PEA (○ ——— ○) activity by rat liver. Some deficient rats were given a riboflavin supplement beginning on day 22 (see arrowhead on abscissa). Each point is the mean of four rats. Standard error bars are shown.

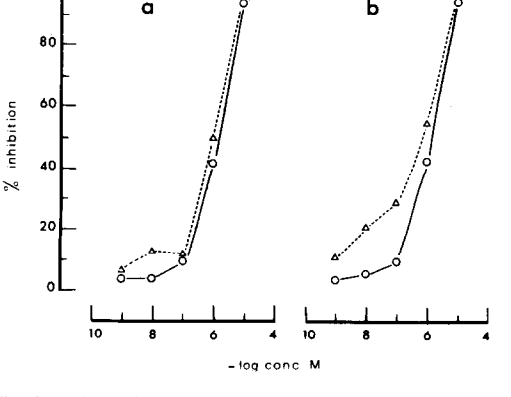

Fig. 2. Effect of clorgyline on the deamination of PEA by control (○ ——— ○) and B_2-deficient (△ - - - △) rat liver MAO. Panel a: 20 days of B_2 deficiency; panel b: 25 days of B_2 deficiency. Assays were done in duplicate. Duplicates differed by less than 10 per cent.

in the rates during recovery of the rats given a riboflavin supplement from day 23 to day 30, inclusive: the MAO activity with 5-HT reached control level by day 8 whereas MAO activity with PEA had attained only 75 per cent of the control value at that time.

The effect of clorgyline on the deamination of PEA by MAO of control and riboflavin-deficient livers is illustrated in Fig. 2. After 20 days of deficiency there was a tendency toward greater sensitivity of the oxidation of PEA to clorgyline. This tendency was even clearer in a second experiment, with liver MAO of rats that were fed a riboflavin-deficient diet for 25 days (Fig. 2b). No differences from the control were seen in the effects of clorgyline on the deamination of 5-HT or in the effect of deprenyl on the deamination of 5-HT and PEA in riboflavin deficiency.

The present study demonstrates that nutritional deficiency of riboflavin in the rat has a greater effect on the deamination of PEA than of 5-HT. In attempting to interpret these small, but consistent, differences with two substrates associated, respectively, with MAO B and MAO A, we have been led to reflect upon the various proposals concerning these two MAO activities and the differences between them. Three possibilities have been suggested. First, that there are two different monoamine oxidases of mitochondria [8, 12]. As simple and attractive as this hypothesis may be, it has thus far not been possible to separate physically two such species from liver or any other organ. A second possibility is that there are two different substrate binding sites on a single protein. Severina [13] has extended this hypothesis by designating the two sites as a hydrophobic region and a polar site, respectively, on the peptide chain. However, recent evidence by Edwards and Pak [14] rules out this possibility. The third suggestion is that different lipids associated with a single protein may determine the type of enzymic activity. This idea, originating from the finding of Tipton *et al.* [7] that the various MAO-active fractions detected electrophoretically by Collins *et al.* [15] were due to the association of lipid material with the fractions, has received the most attention in the recent literature [7, 8]. Lipids seem to be associated with both types of MAO, but it is not clear which form of the enzyme functions in the more lipophilic environment. Data on substrate specificity, thermostability, and sensitivity to trypsin suggest that it is MAO B [16]. However, delipidation of MAO preparations results in the loss of MAO A type of activity [7, 8, 17], and the observation that the loss of this activity incurred during purification is partially restored with diphosphatidylglycerol [18] suggests that MAO A is the more lipid-dependent. In fact, Sawyer and Greenawalt [17] have adduced evidence for association of lipid with both types of MAO activity: MAO A activity, lost by delipidation, was partially restored by mitochondrial lipids, and only MAO B activity was found to be sensitive to the lipid phase transition of the outer mitochondrial membrane.

In relation to the experiments with riboflavin-deficient rats, the greater influence of the deficiency on MAO B activity may reside in changes effected during the deficiency on the lipid composition of the mitochondria. Thus, arachidonic acid, a major component of the phospholipids of those organelles, is reduced in amount in riboflavin deficiency [9]. It is conceivable that the greater sensitivity of MAO B during the course of the vitamin deprivation indicates a critical change in the enzyme–lipid association, as the lipid composition of the bilayer changes. The increased sensitivity of PEA oxidation to clorgyline (Fig. 2) may also be the result of changes in the known interaction of the inhibitor with the membrane [19] taking place in the course of vitamin deprivation.

In summary, the decrease of MAO activity of rat liver as a result of nutritional deficiency of riboflavin affects MAO B type of activity more than A type. This has been ascribed to the effect of the deficiency on the lipid composition of the mitochondria. The present results, together with the data of Sawyer and Greenawalt [17], suggest that MAO B activity is associated with lipids of the outer mitochondrial membrane.

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