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SHORT COMMUNICATIONS

Monoamine oxidase activity in liver of rats given 3′-methyl-4-dimethylamino azobenzene (3′-Me-DAB) in the diet

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The properties of monoamine oxidase (MAO) [monoamine: O_2 oxidoreductase (deaminating); EC 1.4.3.4] have recently been reported in detail [1]. Johnston [2] demonstrated that clorgyline was an irreversible inhibitor of MAO, but that the sensitivity of the enzyme to this inhibitor depended upon the substrate used to assay the activity. He suggested the presence of two forms of enzyme; one, which termed the A-form MAO, sensitive to inhibition by clorgyline and capable of deaminating serotonin [2] and noradrenaline [3] and another, B-form, which was relatively insensitive to inhibition by clorgyline and active toward benzylamine [4] and β-phenylethylamine [5]. Tyramine, dopamine and kynuramine are substrates for both forms of the enzyme [6].

In addition, the properties of the two forms of MAO have been shown to vary widely between animal species and between organs from the same species. For instance, in rat brain, both A and B forms of MAO appear to be uniformly distributed and A-form MAO comprised approx. 60–70 per cent of total brain MAO activity [2]. Rat organs such as spleen and testis [7], contain predominantly only a single species of the A-form MAO. In contrast, B-form MAO represents approx. 80% of the MAO activity in rat liver [8] and essentially all of the MAO activity find in the mouse, rabbit liver [9] or human platelets [10].

Variation in the relative activities of forms A and B MAO have recently been studied from the standpoint of subcellular localization of multiple forms of MAO [11, 12]. The developmental changes in MAO activities in several organs, on the basis of their MAO-A/MAO-B ratio have also been reported [13, 14].

In the present studies, effects of a potent carcinogen, 3′-Me-DAB (3′-methyl-4-dimethylamino azobenzene), which acts specifically on the liver [15], on MAO activity in rat liver mitochondria were investigated. We acquired evidence for changes in the ratio of MAO-A/MAO-B in rat liver mitochondria while the animals were fed a diet containing 3′-Me-DAB.

Male Donryu rats weighing 80–100 g were fed on Oriental diet containing 3′-Me-DAB at a level of 0.06 per cent for several definite periods. Matched controls were fed the Oriental diet only. The rats were anesthetized with sodium pentobarbital given i.p. and the livers were quickly removed and homogenized in 10 volumes of 0.25 M sucrose (previously adjusted to pH 7.0 with 0.5 M $NaHCO_3$). The mitochondrial fractions were prepared by the differential centrifugation method described earlier [8]. The mitochondria were washed once by resuspension in 0.25 M sucrose solution, pH 7.0 and used as the enzyme preparation. All operations were carried out at 4°C. The protein content of the enzyme preparation was measured by the method of Lowry *et al.* [16] with bovine albumin as standard and previously adjusted to 10 mg/ml. MAO activity was estimated with kynuramine as substrate, as described earlier [17].

The MAO activity in liver mitochondria of rats maintained on an Oriental diet containing 0.06% 3′-Me-DAB during a 9 week period was determined. As shown in Fig. 1, MAO activity decreased rapidly and was about 50 per cent that of the control value in the first week. After that, the decrease was proportionate to the periods of 3′-Me-DAB ingestion. The level of MAO activity was maintained at about 30 per cent from the 3rd to the 9th week. To determine the mechanism of inhibition of MAO activity by 3′-Me-DAB, the effects of various concentrations of 3′-Me-DAB on MAO in rat liver mitochondria were studied *in vitro* using kynuramine as the substrate.

3′-Me-DAB at a concentration of 10^{-5} M inhibited MAO activity about 30 per cent, the inhibition being proportional to the concentration of 3′-Me-DAB, and MAO activity was

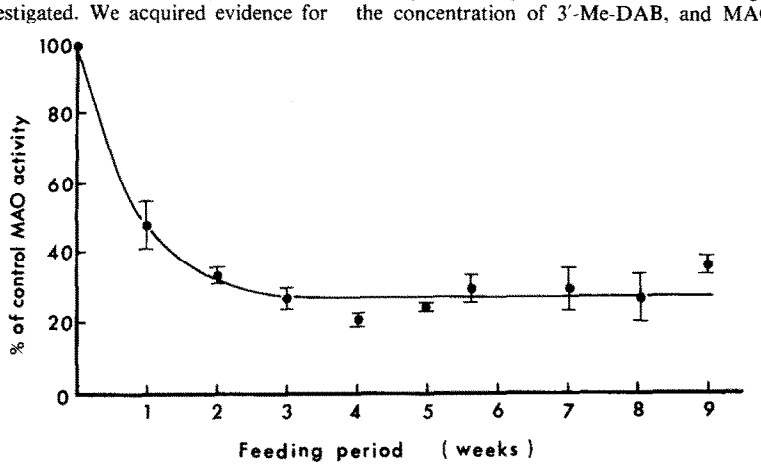

Fig. 1. Changes of MAO activity in liver mitochondria of rats ingesting 3′-Me-DAB in the diet (0.06 per cent) for several definite periods. MAO activity was assayed using kynuramine as substrate at 37° for 40 min. The protein concentration of the mitochondrial preparation was adjusted to 10 mg/ml. Each point represents the mean value obtained from triplicate experiments.

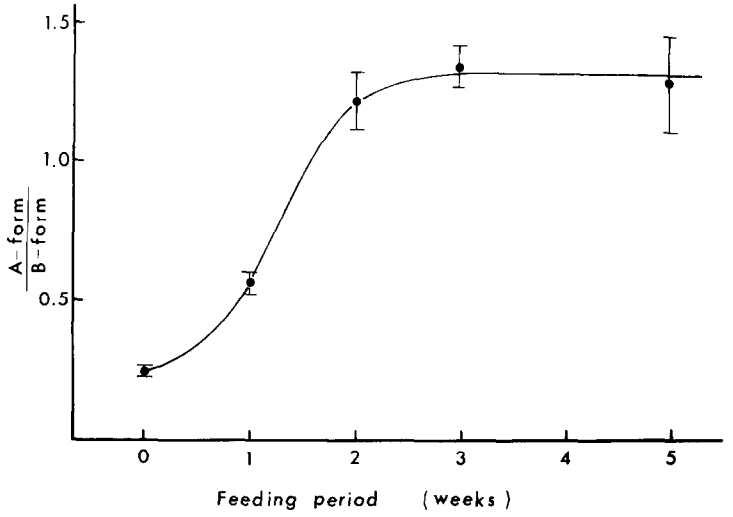

Fig. 2. Proportion of the A-form to B-form MAO in rat liver mitochondria. The plateau inhibition values were each obtained from dose–response curves using at least 10 concentrations of clorgyline. The proportion of the A-form to B-form MAO was estimated from the relationship between clorgyline sensitive and insensitive fraction of liver mitochondria were preincubated (in 10 mM phosphate buffer, pH 7.5) with clorgyline at 25° for 20 min before starting the reaction by adding kynuramine. Each point represents the mean value obtained from triplicate determinations.

inhibited in an apparent competitive fashion by 3′-Me-DAB as determined by Lineweaver–Burk double reciprocal plots (results not shown). The K_i value was calculated from apparent Michaelis constant, 63 μM, and was 1.57×10^{-6} M at the concentration of 1×10^{-6} M 3′-Me-DAB. Similar results were obtained when benzylamine was used as the substrate (unpublished observation).

Clorgyline inhibition curves distinct differences in percentage inhibition at their plateau, indicating relatively different activities of A-form and B-form MAO [9]. Using the mitochondrial preparations in liver of rats ingesting 3′-Me-DAB, the inhibition on MAO activity by various concentrations of clorgyline was then studied. After incubation at 25°C for 20 min with various concentrations of clorgyline using the mitochondria from livers of rats fed a basal diet, a typical plateau-shaped curve was obtained with kynuramine as substrate (inhibited about 20 per cent to total MAO activity with a low concentration of clorgyline), in agreement with the findings previously reported by different groups [8, 9]. In the mitochondrial preparations in liver of rats ingesting 3′-Me-DAB, the inhibition of a plateau-shaped curve obtained with clorgyline increased from 20 to 40 per cent and this phenomenon was proportional to the period of the 3′-Me-DAB ingestion.

The proportion of the A-form MAO was calculated from the above mentioned results from the plateau inhibition values and such is shown in Fig. 2. On the basis of clorgyline sensitivity [2], the A/B ratio was 0.2 using the control liver mitochondria. This proportion rose rapidly until over the 2nd week and approached the value of 1.3. That is, these enzyme preparations contained predominantly the A-form MAO, thus reversing the content of A-form to B-form MAO. This phenomenon indicates the possibility of an increase in the content of A-form or a decrease in the B-form MAO in these mitochondrial preparations. However, a high ratio of MAO-A/MAO-B could be mainly due to the specific inhibition of B-form MAO by 3′-Me-DAB of the metabolites [18], as the MAO activity showed 30 per cent of the control activity (see Fig. 1) and the A/B ratio was 1.3 in these mitochondrial preparations during the periods of 3′-Me-DAB ingestion.

Murphy *et al.* [19] reported that cultured neuroblastoma and glioma (immature cells) in rat brain contain predominantly A-form MAO and Mantle et al. [13] also suggested the predominance of the A-form MAO in foetal rat brain (6 days preparatum). Thus the tendency toward a predominance of the A-form MAO in the tissue as induced by the carcinogen, 3′-Me-DAB, should be given further attention along with the proportion to the rate of multiplicity of MAO enzyme in tumor cells.

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Hepatic drug metabolism in bushbabies (*Galago crassicaudatus* and *Galago senegalensis*) and tree shrews (*Tupaia glis*)

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The rates and substrate specificities of hepatic drug-metabolizing enzymes vary considerably from species to species. Wide species variations in both qualitative and quantitative aspects of drug metabolism have been described [see Refs. 1–3]. The examination of hepatic microsomal metabolic pathways in various species has been motivated, in part, by the need for experimental models applicable to the evaluation of drug metabolism in man. Accordingly, much attention has been focused on hepatic drug metabolism in primates [see Ref. 3], especially those most closely related to man. However, relatively little is known about hepatic drug-metabolizing enzyme systems in the more primitive primates, the prosimians. In this communication, some of the characteristics of hepatic microsomal mixed function oxidases in the prosimian species, lesser and greater bushbabies (*Galago senegalensis* and *Galago crassicaudatus*) and tree shrews (*Tupaia glis*), are described. For the sake of comparison, similar data obtained from rats are also presented.

Male Sprague–Dawley rats (225–250 g) were obtained from Zivic–Miller Laboratories, Pittsburgh, PA. Sexually mature tree shrews (130–175 g), lesser bushbabies (160–240 g) and greater bushbabies (800–1100 g) were purchased from Primate Imports of New York and allowed several weeks of acclimation prior to being killed. Primates were housed individually on 0.3 m^3 steel cages and fed monkey chow mixed with bananas. All animals received food and tap water *ad lib.*

Animals were anesthetized with ether between 9.00 and 10.00 a.m. and the livers were removed immediately. Acute exposure to ether had no effects on hepatic drug metabolism. Livers were homogenized in cold 1.15% potassium chloride and centrifuged at 9000 *g* for 20 min in a Sorvall refrigerated centrifuge. Aliquots of the supernatant fraction were removed for enzyme assays, and the rest was centrifuged at 105,000 *g* for 60 min in a Beckman preparative ultrocentrifuge. Microsomal pellets were resuspended in 1.15% potassium chloride containing 0.05 M Tris–HCl buffer (pH 7.4) at a concentration of 3–4 mg protein/ml. All steps in the preparation of microsomes were carried out with the tissue kept at 0–4°. Microsomal cytochrome P-450 was measured as described by Omura and Sato [4]. NADPH-cytochrome *c* reductase activity was assayed by the method of Phillips and Langdon [5] and microsomal protein was determined as described by Lowry *et al.* [6].

Ethylmorphine or aminopyrine demethylation and aniline hydroxylation were assayed as the amounts of formaldehyde [7] or para-aminophenol [8] formed, respectively, by the 0.5 ml liver 9000 *g* supernatant fraction (equivalent to 200 mg/ml) incubated with glucose-6-phosphate (9.0 μmoles), NADP (2.08 μmoles), $MgSO_4$ (24.2 μmoles), Tris–HCl buffer (0.05 M; pH 7.4) and either ethylmorphine–HCl (10 μmoles), aminopyrine (18 μmoles) or aniline (5 μmoles) in a final volume of 3.0 ml. Semicarbazide–HCl (25 μmoles) served as a trapping agent for formaldehyde produced from ethylmorphine and aminopyrine. Samples were incubated for 15 min at 37° under air. Benzo(a)pyrene hydroxylase activity was assayed as described by Nebert and Gelboin [9], using authentic 3-hydroxybenzo(a)pyrene as standard. Samples were incubated at 37° under air for 8 min. Samples were read against appropriate tissue blanks and standards. All enzyme assays were optimized in each species with respect to protein concentration and time of incubation. Group means were compared statistically using Duncan's multiple range test.

Hepatic microsomal protein concentrations were similar in lesser bushbabies (male and female), greater bushbabies (male) and rats (male) but somewhat lower in tree shrews (male and female) (Table 1). Cytochrome P-450 levels in the greater bushbaby also approximated those in the rat and were significantly greater than concentrations in lesser bushbabies and tree shrews. No species differences in NADPH-cytochrome *c* reductase activity were demonstrable. Similarly, neither cytochrome P-450 content nor NADPH-cytochrome *c* reductase activity was sex-dependent in the lesser bushbaby or tree shrew.

The rates of metabolism of ethylmorphine and aminopyrine were far lower in lesser bushbabies and tree shrews than in rats and greater bushbabies, paralleling species differences in cytochrome P-450 concentrations. In contrast, aniline hydroxylase activity was similar in the lesser bushbaby, greater bushbaby and rat, but significantly lower in the tree shrew. Species differences in benzo(a)pyrene metabolism presented yet another pattern. Activity was lower in male and female lesser bushbabies than in the other species studied.

In the lesser bushbaby, no sex differences in drug-metabolizing activity were demonstrable for any of the substrates employed. The rates of ethylmorphine and aminopyrine demethylation were also similar in male and female tree shrews. However, both aniline and benzo(a)pyrene were metabolized more rapidly by livers obtained from female tree shrews than from males. Thus, sex differences in xenobiotic metabolism in the tree shrew differ considerably from those in the rat. In rats, ethylmorphine, aminopyrine and benzo(a)pyrene are metabolized far more rapidly by males than by females and aniline metabolism is not sex-dependent [10, 11]. Our observations are consistent with those of Litterst *et al.* [12] who also found that the rates of hepatic drug metabolism in tree shrews were greater in females than in males for some substrates, including benzo(a)pyrene.

These observations indicate that divergent patterns of hepatic drug metabolism exist among even closely related lower primate species. Both qualitative and quantitative differences have been demonstrated. At least some of the species differences in drug-metabolizing activity may be related to varying cytochrome P-450 concentrations. Hepatic microsomes from