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Microsomal monoamine oxidase in sympathetically innervated tissues*

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MOST OF the monoamine oxidase (MAO) from rat liver homogenates appears to be predominantly associated with the mitochondrial fraction,[1–4] although a small portion of the enzyme activity has also been found in the "microsomal" fraction.[2] However, in rat,[5] human,[6] dog,[6] cat[7] and bovine[6] brain as well as in the bovine adrenal medulla[8,9] MAO has been reported to occur exclusively in the mitochondrial fraction. In fact, Rodriquez de Lores Arnaiz and de Robertis concluded that MAO is not present in synaptic vesicles or intact nerve endings.[5] In addition, no MAO has been found in the microsomal fraction of brain homogenates.[6] Recently, Roth and Stjärne[10] have reported that a large portion of the MAO in bovine splenic nerve appears to sediment not with the mitochondria, but rather in the fraction containing the amine storage granules. Further investigation revealed that during density gradient centrifugation the MAO in this preparation did not sediment any further than the amine storage granules in a linear sucrose gradient. In fact, the MAO activity peaked in a fraction less dense than that of the major amine peak.[11] The significance of this finding was unclear, but could be indicative of any of the following possibilities: (a) that the microsomal-like MAO may be contained in fragments of mitochondrial membrane sheared off during homogenization; (b) that splenic nerve tissue contains a specialized type of mitochondria with different sedimentation properties and electron microscopic morphology; (c) that some MAO in this tissue is located in a site other than mitochondria, perhaps in the amine storage granules. Therefore, an investigation was conducted to compare the distribution of MAO activity in rat liver with that in sympathetically innervated tissue such as the rat heart, vas deferens, salivary gland and also the bovine splenic nerve by means of techniques previously applied for the isolation of amine storage particles from bovine splenic nerve and rat heart.[12]

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Bovine splenic nerves were obtained from the slaughterhouse 20–30 min post mortem and were immediately chilled on ice. The nerves were then carefully dissected free from contaminating tissue, desheathed, minced and homogenized in 0·25 M sucrose by means of an Ultra-Turrax apparatus. The coarse tissue particles were removed by centrifugation at 9000 *g* for 10 min; the supernatant fraction obtained was diluted to 10 ml with 0·25 M sucrose and the suspension was centrifuged at 105,000 *g* for 1 hr. The supernatant fraction was discarded and the pellet was resuspended in 1·8 ml of 0·25 M sucrose. Then, 0·5 to 0·7 ml of this suspension was layered on 4·5-ml linear gradients, 0·25 to 2 M, or on modified linear gradients prepared as described previously.[12]

Sprague–Dawley rats weighing 150–250 g were injected via the tail vein with 100 μc ^{3}H-*dl*-NE (New England Nuclear Corp., *dl*-norepinephrine-7-^{3}H-hydrochloride; sp. act., 8·8 c/m-mole) maintained frozen in 1% sodium metabisulfite and diluted to 1 ml with saline immediately prior to injection. All further steps were carried out at 0–4°. The animals were killed by decapitation, the hearts were quickly dissected out, washed in 0·25 M sucrose, minced and homogenized in 2 ml of 0·25 M sucrose by means of a conical glass homogenizer (Kontes, size B) chilled in a salted ice bath. The duration of the homogenization was about 30 sec (20 strokes). Six rats were used for the preparation of two gradients (3 injected in each group). After homogenization, the coarse tissue particles were removed by low speed centrifugation at 9000 *g* for 10 min. The low speed supernatant fractions were pooled and diluted to 20 ml with 0·25 M sucrose and centrifuged at 105,000 *g* for 60 min. The supernatant fractions were discarded, the tubes were carefully wiped dry and the 2 pellets were resuspended in 0·7 ml of 0·25 M sucrose and then pooled. Then 0·5 ml was layered onto the gradients prepared as described previously.

Spleen and salivary gland tissues were prepared in the same manner as the heart with the exception that the rats were not injected with ^{3}H-NE as a marker for the endogenous amine.

All gradients were prepared in $\frac{1}{2} \times 2$ in. cellulose nitrate tubes and centrifuged in the SW-39 rotor at 169,000 *g* for 60 min. The gradients were fractionated by a constant infusion of heavy sucrose through a needle inserted into the bottom of the tube lifting up the gradient into a glass funnel tightly fitted onto the gradient tube and connected to a polyethylene cannula which drained into the test tubes used for collection. The heavy sucrose was colored with toluidine blue to mark the end of the fractionation. Each gradient was fractionated into 6-drop fractions. The fractions were collected into 0·2 ml of 0·5 M phosphate buffer, pH 7·0.

A portion (0·025 to 0·3 ml) from each fraction of the gradient was taken for assay of MAO activity according to the radiometric method of Wurtman and Axelrod.[13] Tryptamine-2-^{14}C (tryptamine-2-^{14}C-bisuccinate; sp. act., 2·73 mc/m-mole), obtained from New England Nuclear Corp., diluted with water to give 25–60 $\times$ 10^3 cpm/0·025 ml, was added to a portion of each density gradient fraction and made up to a final volume of 0·3 ml with 0·5 M potassium phosphate buffer, pH 7·0. These samples were incubated for 20 min and the reaction was terminated by addition of 2 N HCl. Six ml toluene was added and the tubes were shaken for 20 min. The tubes were centrifuged at 5000 rpm for 10 min and 4 ml of the toluene phase was transferred to scintillation vials containing 4 g of 2,5-diphenyloxazole and 100 mg of 1,4 *bis*-2(5-phenyloxazolyl)-benzene per liter of toluene. All results are corrected for a counting efficiency of 70 per cent.

In rat liver, where MAO has been conclusively demonstrated to be of mitochondrial origin, analysis by sucrose density centrifugation reveals that in both types of gradients employed the MAO gives a single peak (Figs. 1 and 2). On the other hand, analyses of rat heart and bovine splenic nerve under similar conditions demonstrates that both these tissues have a multiphasic distribution of MAO Figs. 1–3). The results obtained in the rat spleen and salivary gland seem to resemble more closely those obtained in the rat liver (Fig. 4).

Under the conditions of these experiments, MAO in rat heart and in bovine splenic nerve occurs in a microsomal-like fraction. The monolithic peak of MAO in the liver, homogenized in the same manner, indicates that there may exist in some tissues, subcellular particles that contain MAO other than "normal" mitochondria. The possibility that these microsomal particles exist in sympathetic nerve tissue should be considered. This possibility is supported by the demonstration of microsomal-like MAO in the purest of the preparations studied, the bovine splenic nerve, in which the relative content of sympathetic nerve tissue is probably 1000–10,000 times that in organs like the heart. While the NA-storing particles do overlap with MAO-containing particles in density gradient analysis, the peaks can occasionally be resolved when special techniques are employed.[11] Therefore, there is no firm basis to conclude that NA-storing particles do contain MAO. The alternative, that mitochondria

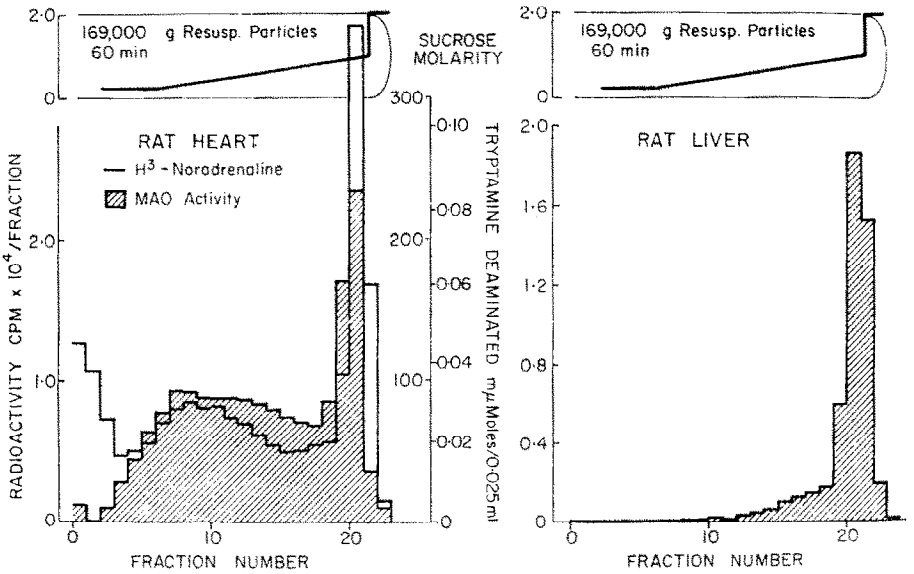

FIG. 1. Comparison of the distribution of rat liver MAO and rat heart MAO on a modified linear sucrose density gradient.

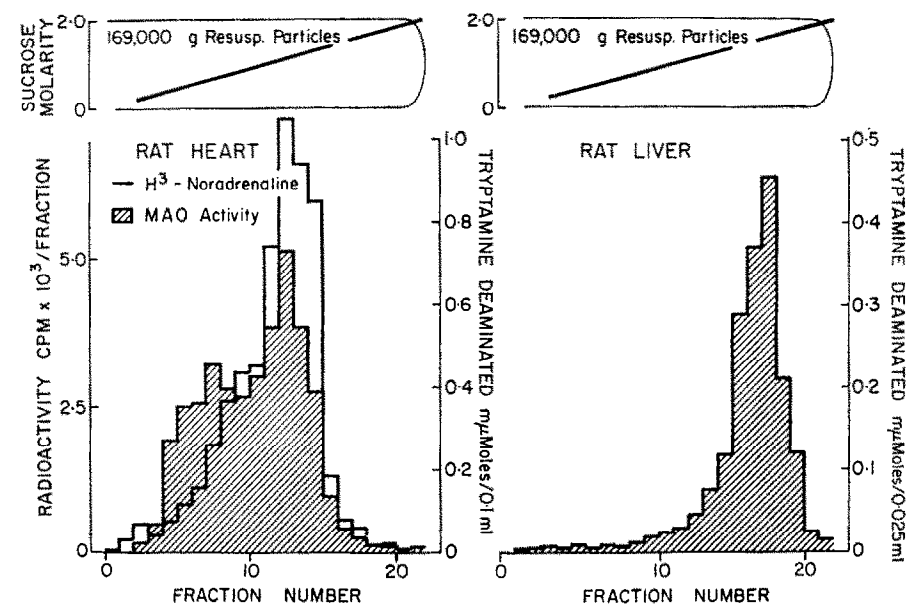

FIG. 2. Comparison of the distribution of rat heart MAO and rat liver MAO on a linear sucrose density gradient (0·25 to 2·0 M).

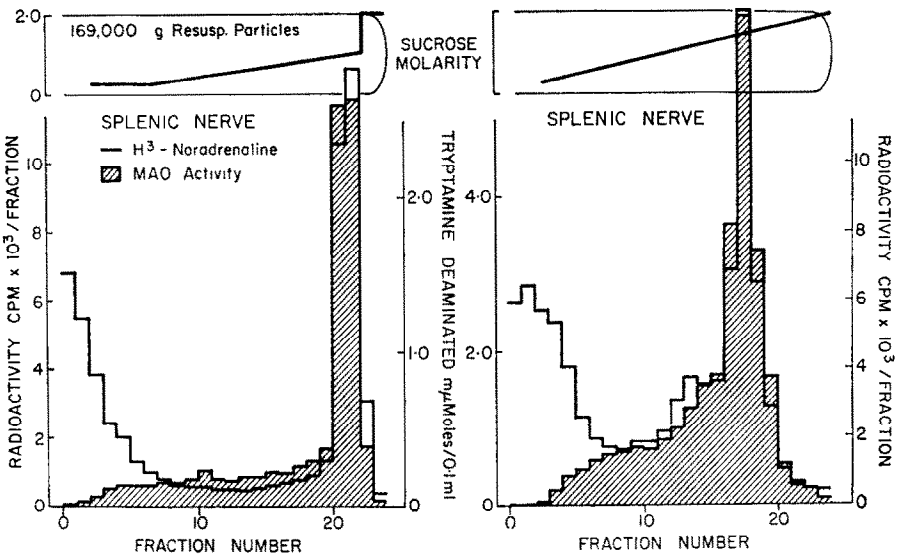

FIG. 3. Distribution of ^{3}H-noradrenaline and MAO in bovine splenic nerve obtained on a modified linear sucrose gradient and on a linear sucrose gradient.

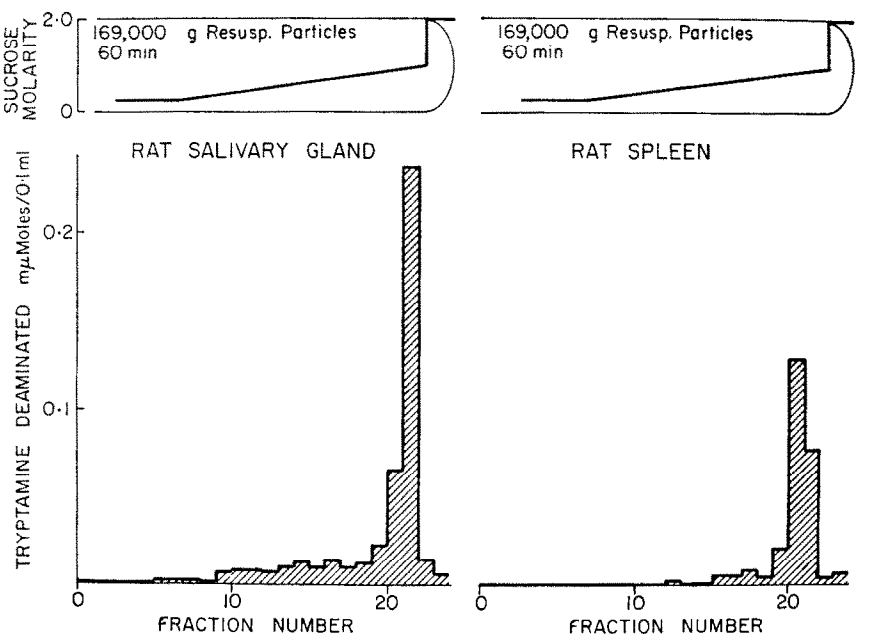

FIG. 4. Distribution of MAO activity of rat spleen and rat salivary gland on a modified linear sucrose density gradient.

in certain tissues differ physically from those in the liver and thus tend to fragment more easily, should also be considered. A recent investigation has in fact demonstrated that liver mitochondria can be fragmented by osmotic lysis, resulting in a mixed population of small vesicles derived from the outer mitochondrial membrane and larger bodies derived from the inner membrane.[13] It is of interest that the MAO activity could be recovered in the small vesicle fraction, indicating that this enzyme is probably associated with the outer membrane of the mitochondrial.

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Comment on microfluorometric determination of monoamine oxidase

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A MONOAMINE oxidase (MAO) assay was recently described by Kraml, in which kynuramine is converted to 4-hydroxyquinoline and the end product is assayed fluorometrically.[1] In our laboratory, the use of trichloroacetic acid (TCA) resulted in considerable quenching of the fluorescence, in agreement with Kraml. Some downward drifting of the readings was also observed. The problem of quenching and fluorescence stability can be entirely avoided by stopping the reaction with 2·0 ml of 0·6 M perchloric acid (PCA) instead of with 10% TCA. Examples of the relative fluorescence of standard samples of 4-hydroxyquinoline (4-HOQ) are shown in Table 1. Repeated determinations