Monoamine Oxidase Type B is Localized to Mitochondrial Outer Membranes in Mast Cells, Schwann Cells, Endothelial Cells and Fibroblasts of the Rat Tongue

Yang Xu¹, Kazusada Yoshitake², Akio Ito³ and Ryohachi Arai¹

¹Department of Anatomy and ²Department of Oral and Maxillofacial Surgery, Shiga University of Medical Science, Otsu, Shiga 520–2192 and ³Department of Chemistry, Faculty of Science, Kyushu University, Fukuoka, Fukuoka 812–8581

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I. Introduction

Monoamine oxidases are enzymes that degrade biogenic monoamines [1, 30]. There are two types of monoamine oxidase enzymes. Monoamine oxidase type A (MAOA) has a higher affinity for the substrates, serotonin and noradrenaline, and the inhibitor clorgyline, whereas monoamine oxidase type B (MAOB) has a higher affinity for the substrate, β-phenylethylamine, and the inhibitor deprenyl [17, 18]. Dopamine is a common substrate of both MAOA and MAOB [8]. MAOA and MAOB are comprised of distinct amino acid sequences in humans [3] and rats [16, 19], and their genes are located at adjacent sites on the X-chromosome in both humans [20, 21] and mice [7]. MAOA knock-out mice have elevated brain levels of serotonin, noradrenaline and dopamine; and show aggressive behavior [4]. In contrast, MAOB knock-out mice do not exhibit aggression, and only levels of β-phenylethylamine in the brain are increased [12]. Both MAOA- and MAOB-deficient mice demonstrate an increased response to stress [4, 12].

The subcellular localization of MAOA and MAOB has been examined in the central nervous system. A biochemical study demonstrated that MAOA and MAOB activities are found predominantly in the mitochondrial fraction of rat brain homogenates [31]. Immunohistochemical studies using electron microscopy have shown that MAOA immunoreactivity is associated with the outer membrane of mitochondria in catecholaminergic neurons of the brain and spinal cord of rat and monkey [33]; and that MAOB immunoreactivity is found on the mitochondrial outer membrane in serotonergic neurons and astrocytes of the rat brain [2]. In peripheral organs, MAOB activity has been shown to be far more abundant in the outer membrane fraction of rat liver mitochondria than in the inner membrane fraction [28]. Using an enzyme histochemical method employing a substrate common to both MAOA and MAOB, subcellular localization of MAO activity has been examined in the heart, liver, adrenal cortex and medulla, and kidney of rats [9, 24]. However, to our knowledge, no previous study has examined, by immunohistochemistry, the individual subcellular localization of MAOA or MAOB enzyme in peripheral tissues. Recently, we raised and characterized a rabbit antiserum against bovine MAOB, which crossreacts with rat MAOB [2, 27]. Using this antiserum, we have now performed immunohistochemistry at the electron microscopic level, to examine the subcellular localization of MAOB in the rat tongue. This organ of humans is known to contain MAOB activity [22].

II. Materials and Methods

Animals

Male Sprague-Dawley rats (n=3, 180–200 g) were obtained from Japan SLC (Hamamatsu, Japan). All experiments were carried out according to the Guidelines for Animal Experimentation at Shiga University of Medical Science. All efforts were made to minimize both the number...
of animals used and their suffering.

**Primary antiserum**

MAOB purified from bovine liver mitochondria was used to generate a rabbit anti-MAOB antiserum [27]. This anti-MAOB antiserum has been shown to immunoprecipitate MAOB from rat liver lysate [27]. Immunohistochemical analysis has shown that this antiserum stains MAOB-containing cells but not MAOA-containing cells in the rat brain, indicating that the antiserum specifically recognizes rat MAOB [2].

**Electron microscopic immunohistochemistry**

Three rats were anesthetized with sodium pentobarbital (60 mg/kg body weight, i.p.) and perfused through the ascending aorta with 50 ml of 0.01 M phosphate-buffered saline (pH 7.4, room temperature), followed by 300 ml of a fixative containing 4% paraformaldehyde, 0.3% glutaraldehyde and 0.2% picric acid in 0.1 M phosphate buffer (pH 7.4, 4°C). The tongues were dissected and placed in another fixative containing 4% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer for 24 hr at 4°C. Frontal, 50 μm thick sections were cut using a microslicer (Dosaka, Kyoto, Japan) and collected in 0.1 M phosphate-buffered saline (PBS).

For MAOB staining, we employed an immunoperoxidase technique using an avidin-biotin-peroxidase complex [15]. Sections were incubated in the following solutions: (1) 0.001% trypsin (type III, Sigma, St. Louis, MO, USA) in PBS for 5 min at room temperature; (2) 5% normal goat serum in PBS for 1 hr at room temperature; (3) rabbit anti-MAOB antiserum (1: 60,000 in PBS) with 1% normal goat serum for 48 hr at 4°C; (4) biotinylated goat anti-rabbit immunoglobulin G (BA-1000, Vector, Burlingame, USA, 1:1,000 in PBS) for 2 hr at room temperature; (5) avidin-biotin-peroxidase complex (PK-4000, Vector, 1:1,000 in PBS) for 2 hr at room temperature; and (6) 0.025% 3,3’-diaminobenzidine (Dojindo, Kumamoto, Japan), 0.6% nickel ammonium sulfate (Nacalai Tesque, Kyoto, Japan), and 0.006% hydrogen peroxide in 0.05 M Tris-HCl buffer (pH 7.6) for 10 min at room temperature. The stained sections were fixed with 1% osmium tetroxide (Nacalai Tesque) in 0.1 M phosphate buffer for 1 hr at 4°C, dehydrated and flat-embedded in epoxy resin (Luveak-812, Nacalai Tesque). The embedded sections were examined under a light microscope (BX51, Olympus, Tokyo, Japan). Small areas containing the epithelium, connective tissue and muscle fibers were trimmed from the embedded section and cut on an ultramicrotome. Ultrathin sections were collected on grids, stained with uranyl acetate (Nacalai Tesque) and lead stain solution (Sigma-Aldrich Japan, Tokyo, Japan), and examined in a transmission electron microscope (5000, Hitachi, Tokyo, Japan). As a control for the immunohistochemical staining, sections were processed as described above, omitting the primary antiserum. No immunoreactivity was found in the control sections.

### III. Results

Light microscopy showed that MAOB immunoreactivity was present in connective tissues, but not in the epithelium or muscle fibers (Fig. 1). Electron microscopy showed that MAOB immunoreactivity was present in mast cells (Fig. 2A), Schwann cells (Fig. 2B), capillary endothelial cells (Fig. 3A) and fibroblasts (Fig. 3B). In these cells, mitochondria showed MAOB immunohistochemical reaction products on and around their outer membranes (Figs. 2, 3). Some mitochondria in capillary endothelial cells were very weakly stained for MAOB (Fig. 3A).

### IV. Discussion

In summary, we have shown that MAOB is localized to the mitochondrial outer membranes in mast cells, capillary endothelial cells, Schwann cells and fibroblasts of the rat tongue.
Fig. 3
tongue connective tissue. The present study is the first to show the presence of MAOB in mast cells and capillary endothelial cells. We are also the first to immunohistochemically reveal the intracellular localization of MAOB at electron microscopic levels in peripheral tissues.

By electron microscopy, we found MAOB immunohistochemical reaction products on and around the mitochondrial outer membranes. Our question is now concerned with whether MAOB itself is localized not only on but also around the mitochondrial outer membranes. The immunohistochemical signal in our assay derives from 3,3′-diaminobenzidine oxidation products formed by peroxidase activity in the avidin-biotin-peroxidase complex [15]. In immunohistochemistry using peroxidase for electron microscopy, 3,3′-diaminobenzidine oxidation products can spread out from the site of the antigen [6, 23]. It therefore seems likely that the primary site of MAOB localization is on the mitochondrial outer membrane.

**Tele**-methylhistamine is the most likely substrate of MAOB in mast cells. Mast cells synthesize histamine, which is stored in granules that are discharged upon antigenic challenge or tissue damage [5]. Histamine released from mast cells acts mainly through H1-receptors [13]. Histamine metabolism occurs by two distinct pathways: oxidative deamination by diamine oxidase, and methylation by histamine methyltransferase (HMT) [11]. The histamine metabolite, *tele*-methylhistamine, is further processed for oxidative deamination by MAOB [14]. *Tele*-methylhistamine is detected in histamine-containing granules but not in the cytoplasm of mast cells of the rat peritoneum [10]. Although mast cells of the rat peritoneum have no detectable HMT activity [10], the epithelium of the guinea pig tongue was immunohistochemically stained for HMT [32]. Therefore, it is likely that histamine released from mast cells of the tongue is metabolized by HMT in the epithelium to *tele*-methylhistamine, which is taken into the cytoplasm of mast cells and then transported from the cytoplasm into granules. *Tele*-methylhistamine in the cytoplasm of mast cells is probably metabolized by MAOB on mitochondrial outer membranes, but *tele*-methylhistamine in granules is sequestered from the enzyme.

We have shown that MAOB is localized to mitochondrial outer membranes of capillary endothelial cells of the rat tongue. A previous study using immunohistochemistry at light microscopic levels showed the absence of MAOB in endothelial cells of human blood vessels [26]. This discrepancy could be due to differences in species (rats and humans) or experimental methods (electron and light microscopy). Our finding of MAOB in capillary endothelial cells of the rat supports an idea that the enzyme activity in microvessels plays a protective role for the brain by degradation of extraneous monoamines, such as a neurotoxin 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine, in the blood [25]. Similar roles are possibly played by MAOB in capillary endothelial cells of peripheral organs.

In the present study, MAOB immunohistochemical reaction products were found on and around the mitochondrial outer membranes of Shwann cells. A previous study using enzyme histochemistry showed that MAOB reaction products were localized throughout the whole extent of the cytoplasm of Shwann cells in cerebral arteries of the rat [29]. However, taking into consideration the possible diffusion of the reaction products, it is still likely that the primary site of MAOB localization is on the mitochondrial outer membrane of Shwann cells. We have also shown that MAOB is localized to the mitochondrial outer membranes in fibroblasts of the rat tongue. This is consistent with findings of a previous immunohistochemical study at light microscopic levels that showed fibroblasts of an artery of the human kidney contain MAOB [26]. Exact roles of MAOB in Schwann cells and fibroblasts are not known, but the enzyme may be involved in the metabolism of biogenic and extraneous monoamines.

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**VI. References**


![Fig. 2. A: Subcellular localization of MAOB in mast cells. Immunohistochemical reaction products (arrows) are observed on and around mitochondrial outer membranes. G, granule; Nu, nucleus. Bar=1 μm. B: Subcellular localization of MAOB in Schwann cells. Immunohistochemical reaction products (arrows) are observed on and around mitochondrial outer membranes. Ax, Axon; My, myelin sheath; Nu, nucleus. Bar=0.5 μm.](image1)

![Fig. 3. A: Subcellular localization of MAOB in capillary endothelial cells. Immunohistochemical reaction products (arrows) are observed on and around mitochondrial outer membranes. Some mitochondria are very weakly stained for MAOB (arrowhead). Nu, nucleus. Bar=1 μm. B: Subcellular localization of MAOB in fibroblasts. Immunohistochemical reaction products (arrows) are observed on and around mitochondrial outer membranes. Col, collagen fibers; Nu, nucleus. Bar=1 μm.](image2)
nephrine in mice lacking MAOA. Science 268; 1763–1766.


