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Immunocytochemical Localization of Catechol-*O*-Methyltransferase in Rat Parotid Gland (41478)

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Abstract. Catechol-*O*-methyltransferase (COMT) (EC 2.1.1.6) was localized in rat parotid gland using immunocytochemical methods. Immunoreactive deposits were found in the cytoplasm of myoepithelial cells, striated duct cells, myoepithelial-like cells of the small excretory duct, and small basal cells of the large excretory duct. The results confirm that the predominant localization of COMT is extraneuronal. After ligation of the main excretory duct, a marked reduction of COMT immunoreactivity was demonstrated in the striated duct. Chronic postganglionic sympathectomy did not produce a diminution of the COMT immunoreactivity in the parotid gland. The pattern of localization observed in the extraneuronal elements suggests that enzyme may function in extraneuronal inactivation of catechols in the parotid gland.

Catechol-*O*-methyltransferase (COMT) (EC 2.1.1.6) catalyzes the transfer of a methyl group from *S*-adenosylmethionine to one of the phenolic hydroxyls of a variety of catechols (1). This *O*-methylation reaction is important in the enzymatic inactivation of circulating catecholamines (2), the enzymatic inactivation of 2- and 4-hydroxyestrogens (3), the detoxification of xenobiotic catechols (4), and the local inactivation of catecholamines released as transmitters from the terminals of both central and peripheral catecholamine-containing neurons (5, 6).

Several biochemical studies have demonstrated the presence of COMT in salivary glands (7–9), and indirect evidence has suggested that COMT is localized both in extraneuronal and intraneuronal locations in the salivary gland (7, 10).

The localization of COMT in acinar and intercalated duct myoepithelial cells of the rat parotid gland has already been reported using immunocytochemical methods (11). In the present study we have shown, using the peroxidase-antiperoxidase method (12), that COMT is also present in striated duct cells, myoepithelial cells of the small excretory ducts, and in small basal cells of the excretory ducts. We have also examined the effects of sympathectomy and ligation of the excretory duct on the localization of COMT in the rat parotid gland.

Materials and Methods. Adult Wistar rats (200–400 g) of both sexes were used. Rats were anesthetized with Nembutal and perfused through the heart with 100 ml of periodate–lysine–paraformaldehyde (PLP) (13) 14 days after the surgical procedures. After perfusion the parotid glands were rapidly removed, cut into small pieces, and fixed with PLP for 3 hr at 4°. Tissues were dehydrated through graded alcohols and embedded in paraffin. Sections 8 μm in thickness were cut, mounted on glass slides with egg albumin and glycerine, and dried overnight at 37°. For immunocytochemical staining paraffin sections were deparaffinized with xylene and brought to water through graded alcohols.

Slides were washed with 0.1 *M* phosphate-buffered saline (PBS) and then treated with 0.005 *M* unbuffered periodic acid for 5 min and incubated with a solution of

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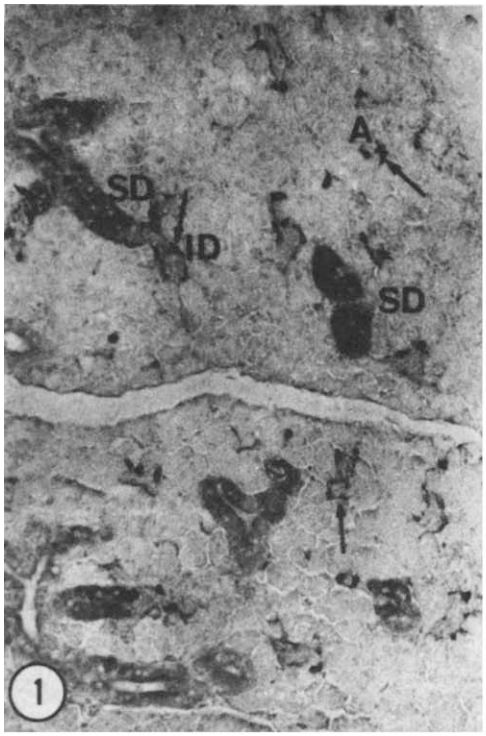
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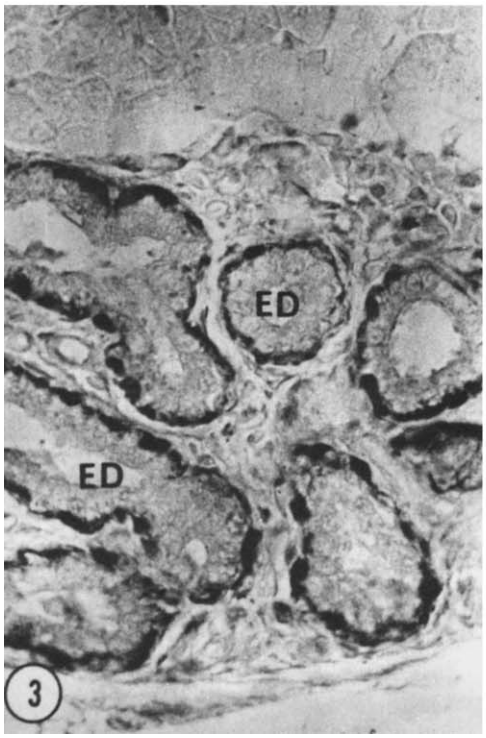
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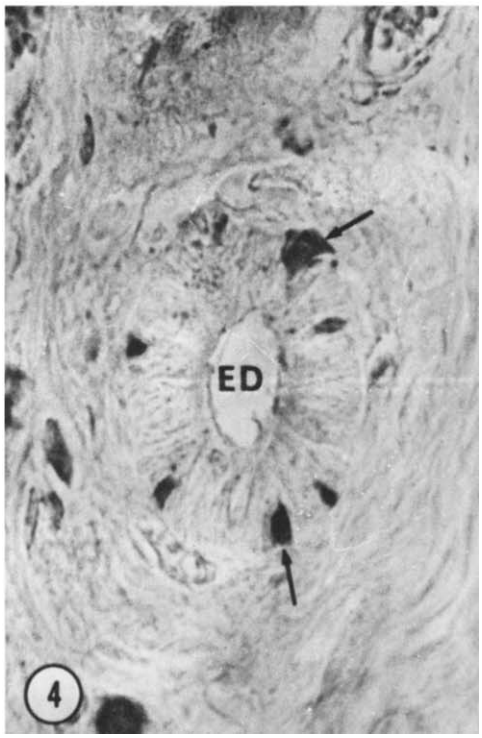
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$NaBH_4$ (10 mg/ml PBS) to inhibit endogeneous peroxidase activity (14). Sections were rinsed with two changes of PBS for 10 min and then incubated with a 1:50 dilution (in PBS) of normal goat serum for 30 min at room temperature.

Slides were incubated for 24 hr at 4° in a 1:1000 dilution (in PBS) of either rabbit antiserum to rat liver COMT (11), or as a control, in normal rabbit serum similarly diluted, a 1:10 dilution (in PBS) of goat anti-rabbit serum (Polyscience Inc., Warrington, Pa.) for 30 min at room temperature and then in a 1:10 (in PBS) dilution of peroxidase–antiperoxidase complex (PAP) (Polyscience Inc., Warrington, Pa.) for 45 min at room temperature. All slides were washed for 15 min each in three changes of PBS between each step. Peroxidase reaction product was developed by incubation of sections for 5 min in 3,3′-diaminobenzidine (0.05%) in 0.05 *M* Tris buffer, pH 7.6, containing 0.01% H_2O_2. After a final wash in distilled water the sections were dehydrated through graded alcohols, cleared with xylene, and mounted in Canada Balsam.

Atrophy of the parotid gland was produced by ligation of the excretory duct. The parotid glands were postganglionically sympathectomized by excision of the superior cervical ganglion. The operations were performed under Nembutal anesthesia. In all cases, the operation was unilateral, the intact side serving as a control.

Results. *Immunocytochemical reaction of COMT of the normal parotid gland (Figs. 1–4).* The sites of positive histological reaction product indicate the localization of interaction of the specific antiserum with tissue COMT and will be referred to as a COMT-positive reaction. COMT-positive product was observed in the cytoplasm of the myoepithelial cells of the acini and intercalated ducts, myoepithelial-like cells of the small excretory duct, and small basal cells of the large excretory duct. COMT was also present in the striated duct cells. Here, however, it appeared to be associated with basal striations. The differences in distribution were dramatically shown where transitions between striated and intercalated ducts were present in the sections (Fig. 1).

In the intralobular ducts the density of the COMT-positive reaction appeared much greater in the striated duct nearest the intercalated duct. The myoepithelial cells containing COMT-positive product were located at the bases of the acinar serous cells and intercalated duct (Fig. 1). Control sections of the parotid gland were negative (Fig. 2).

COMT-positive reaction was also present in myoepithelial-like cells located at the bases of small excretory ducts in the interlobular connective tissue (Fig. 3). Small basal cells containing COMT were found in the epithelium of the large excretory ducts (Fig. 4).

Immunocytochemical reaction of COMT following the surgical procedure (Figs. 5–9). 1. Superior cervical ganglionectomy (Figs. 5–7). Fourteen days after this procedure no histological changes were observed. COMT-positive product was present in the cytoplasm of the striated duct cells, myoepithelial cells (Figs. 5, 6), and small basal cells of the large excretory duct (Fig. 7). This distribution was similar to that of the normal parotid gland.

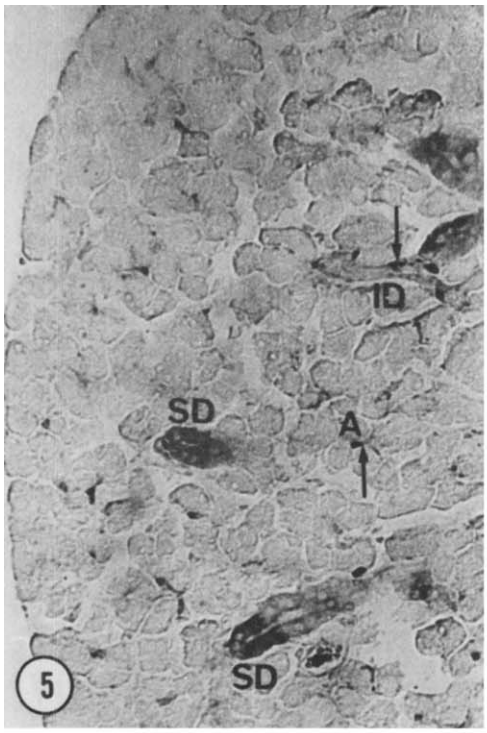
2. Ligation (Fig. 8). Ligation of the main excretory duct caused the expected histological changes in the parotid gland (see Refs. (10, 15)). The acinar cells not only

FIG. 1. Normal rat parotid gland. Strong specific staining for COMT is seen in the cytoplasm of the myoepithelial cells (arrows) and striated duct cells (SD) near the intercalated duct. A, acini; ID, intercalated duct. ×145.

FIG. 2. Normal rat parotid gland treated with normal rabbit serum as control. No staining for COMT is seen. ×145.

FIG. 3. Normal rat parotid gland. COMT is present in the myoepithelial-like cells of the small excretory duct (ED). ×410.

FIG. 4. Normal rat parotid gland. Small basal cells (arrows) of the large excretory duct (ED) contain COMT. ×655.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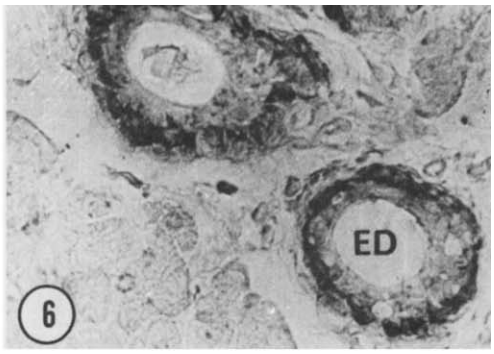
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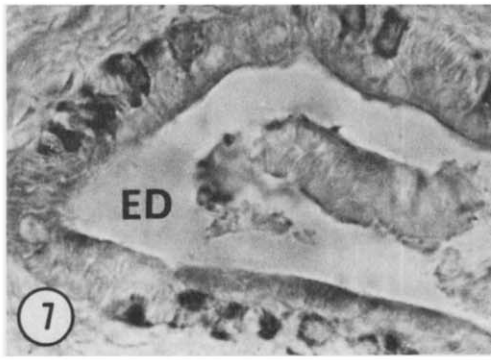
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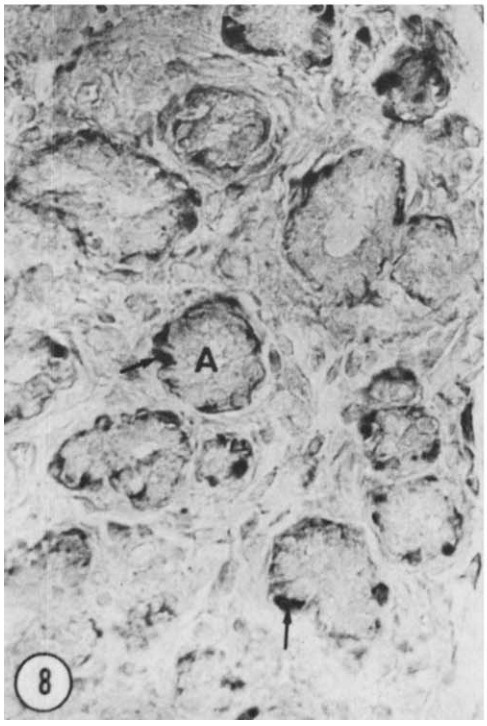
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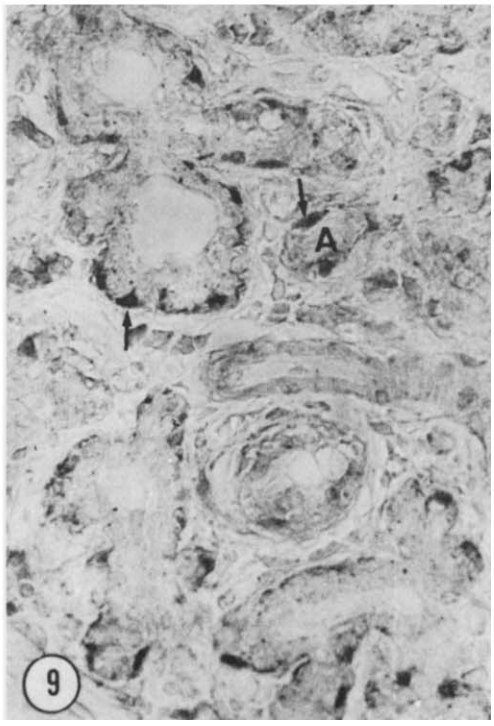
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atrophied, but also decreased in number. The intralobular and interlobular ducts had atrophied and their lumina were markedly dilated and the interstitial connective tissue had proliferated. The myoepithelial cells were intact and had COMT-positive reaction products (Fig. 8).

3. Ligation and postganglionic sympathectomy (Fig. 9). The glands that had been ligated and sympathectomized were similar to that seen after ligation alone. COMT was observed only in the myoepithelial cells (Fig. 9).

Discussion. In classical experimental approaches, salivary gland functions are differentiated by examination of the effects of ligation of the major excretory ducts or denervation of the gland. Following ligation the major secretory and ductal components of the gland, with the exception of the myoepithelial cells, undergo a striking atrophy (15, 16). After surgical, chemical, or immunological sympathectomy those functions of the gland dependent upon sympathetic innervation are lost (17, 18).

Previous studies have shown that rodent salivary glands contain both catecholamines and the enzymes, monoamine oxidase and COMT, responsible for their metabolic inactivation (17–20). Following sympathectomy, factors specifically related to intact sympathetic innervation such as the intraneuronal biosynthetic enzymes, tyrosine hydroxylase, and dopamine-β-hydroxylase, the tissue level of norepinephrine, and the high-affinity uptake system for norepinephrine all decline in parallel (18, 20). After atrophy due to duct ligation these factors remain essentially unchanged after appropriate adjustments are made for the reduction in glandular mass (10, 20).

Sympathectomy results in minimal reductions in monoamine oxidase levels while duct ligation leads to a profound decrease in enzyme levels. While it has been clearly established that monoamine oxidase is present in sympathetic neurons it is also widely distributed in other cell types in the salivary gland. Thus the minimal reduction in activity following degeneration of sympathetic neurons is consistent with the distribution of the enzyme. In the case of COMT, sympathectomy results in very minor changes in enzyme activity in the parotid gland (15). Similar studies in the submaxillary gland of rat indicate a sympathectomy-dependent decrease in COMT activity and suggest the presence of intraneuronal COMT (8).

However, duct ligation results in a partial and quite variable reduction of COMT activity ranging from 10 to 40% of the activity in unligated glands. A major fraction of COMT activity is unaffected in the presence of a major atrophy of the gland (8, 15, 20).

Our present results provide direct, visual confirmation of these conclusions. As in other tissues studied (11, 21, 22), we have found no evidence for a neuronal localization for COMT. In the salivary gland, COMT was found in the striated duct cells, the small basal cells of the excretory ducts, and in myoepithelial cells. In those locations the enzyme is not detectably affected by sympathectomy. Ductal ligation led to a variable loss in COMT located in striated duct cells and no detectable change in COMT in myoepithelial cells. The myoepithelial localization of COMT was unchanged following both duct ligation and sympathectomy. Thus the variable de-

FIG. 5. Rat parotid gland after removal of the superior cervical ganglion. COMT reaction products are seen in the cytoplasm of the myoepithelial cells (arrows) and striated duct cells (SD). A, acini; IR, intercalated duct. ×190.

FIG. 6. Rat parotid gland after removal of the superior cervical ganglion. Specific staining for COMT is observed in the myoepithelial-like cells of the small excretory duct (ED). ×425.

FIG. 7. Rat parotid gland after removal of the superior cervical ganglion. COMT reaction product are found in the small basal cells of the large excretory duct (ED). ×535.

FIG. 8. Rat parotid gland following ligation of the main excretory duct. The myoepithelial cells (arrows) contain COMT. A, acini. ×410.

FIG. 9. Rat parotid gland following ligation of the excretory duct and removal of the superior cervical ganglion. The myoepithelial cells (arrows) contain COMT. A, acini. ×400.

crease in COMT activity following duct ligations may reflect the loss of striated duct cells and possibly the small basal cells of the excretory duct. The major fraction of COMT activity which is unaffected by ligation-induced activity clearly appears to be associated with myoepithelial cells.

The precise function of COMT in these extraneuronal sites is problematic. There is little information about the functional role of basal cells in the salivary gland excretory ducts. A similar question can be raised concerning the role of COMT in the small, basal cells of the rat epididymis (11). With regard to myoepithelial cells on the other hand, conventional thinking suggests that the presence of COMT in contractile myoepithelial cells is related to the inactivation of norepinephrine released locally from noradrenergic varicosities or perhaps by direct sympathetic innervation of myoepithelial cells. Thus, by analogy with cholinergic nerve–muscle relationships, COMT has been presumed to be the noradrenergic analog of acetylcholinesterase.

Certain aspects of this analogy are of interest. The contractile function of myoepithelial cells, first suggested as early as 1661 (23), is well documented (24). These cells contain filaments similar to those of smooth muscle cells; they respond to sympathetic stimulation by contraction leading to increased ductal pressure. The response is mediated through an α-adrenergic receptor and has been observed directly in organ culture. Further, it is known that the acini of rat parotid glands are closely associated with catecholaminergic, fluorescent varicosities. It is of interest to note that a fraction of norepinephrine uptake by salivary gland is reserpine resistant, suggesting a significant participation of extraneuronal uptake sites for norepinephrine. Furthermore, reserpine treatment results in a partial loss of COMT activity within 6–18 hr (18). These findings suggest that both norepinephrine uptake and the reserpine-induced reduction of COMT activity may occur in myoepithelial cells. The possibility that the reserpine-sensitive fraction of COMT in salivary glands is present in myoepithelial cells is under examination by our immunocytochemical procedure to determine whether this effect occurs in myoepithelial cells or elsewhere.

Recent experimental results have greatly weakened any direct functional or morphological analogy between acetylcholinesterase and COMT. Localization of COMT in brain by fluorescent immunocytochemical techniques have demonstrated glial and ependymal cell localization rather than a neuronal localization. Other major sites of COMT are the choroid plexus (22) and the ciliary body of the eye (25). It has been suggested that in these sites COMT functions as a barrier or "enzymatic sink" to the passage of free catechols which restrict neuronally released catechols to a local area (25). Studies of the localization of COMT in the reproductive tract suggest a similar function of the enzyme as a protective barrier or "enzyme-sink" for catecholestrogens (26). Finally it should be noted that COMT has been demonstrated immunocytochemically in the ductal cells of the lactating rat mammary gland where it may play the same role as in the salivary gland (27, 28). Thus it is clear that further investigations will be required to define the nature of the role of COMT in salivary gland myoepithelial cells with certainty.

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