Fine Structural Localization of Peroxidase Activity in the Epithelium and the Gland of the Rat Larynx

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Summary. Endogenous peroxidase activity was demonstrated in ciliated cells and secretory cells of the laryngeal epithelium and gland of rats, using the diaminobenzidine method for cytochemical demonstration of peroxidase activity. The intensity of peroxidase activity was greatly varied from cell to cell, but the fine structural localization of the activity was similar in various cell types. The activity was localized in cisternae of rough-surfaced endoplasmic reticulum including nuclear envelope, some vesicles and saccules of the Golgi complex, large membrane-limited granules, multivesicular bodies and probable lysosomes. In secretory cells, the activity was also found in secretory granules.

The significance of peroxidase activity is not unclear, while the activity, at least a part of it, seems to be secreted into the cavity of the larynx. The possibility that peroxidase participates bactericidal mechanism, deserves further investigation.

Introduction

Endogenous peroxidase activity has been demonstrated in various cell types including leukocytes (Yamada, 1966; Ackerman, 1968; Cotran and Litt, 1969; Miller and Herzog, 1969; Bainton and Farquhar, 1970), epithelial cells of the uterus (Brökelmann, 1969), epithelial cells of the colon (Venkatachalam et al., 1970), follicular cells of the thyroid (Nakai and Fujita, 1970; Strum and Karnovsky, 1970), peritoneal macrophages (Fahimi et al., 1970) Kupffer cells in the liver (Fahimi, 1970) and acinar cells of the parotid gland (Herzog and Miller, 1970). In addition to these, the present author found endogenous peroxidase activity in the epithelium and glands of the rat larynx and upper part of the trachea. The activity was localized in cisternae of endoplasmic reticulum, perinuclear cisternae, some Golgi saccules and vesicles, secretory granules, and several large membrane-limited granules like other various cell types previously reported.

Materials and Methods

Small pieces of the larynx and the upper part of the trachea of normal adult rats were fixed in ice-cold 2.5% glutaraldehyde in 0.1 M phosphate buffer, following the perfusion of a same fixative from the left ventricle of the heart. After overnight-wash in the same buffer containing 7.5% sucrose, the specimens were incubated for 60 min at 20°C in a incubation medium, slightly modified after Graham and Karnovsky (1966) and Essner (1969) and composed of 20 mg of 3,3'-diaminobenzidine tetrahydrochloride (DAB) (Sigma Chemical Co.), 10 ml of 0.05 M Tris-HCl buffer (pH 8.0) and 0.1 ml of 2% H2O2. Then the specimens were washed in 7.5% sucrose solution and postfixed in buffered 1% O2O4 for 1 hour. They were dehydrated through graded concentrations of ethanol and embedded in Epon epoxy resin. Thin and thick sections were cut on a Porter-Blum ultramicrotome. Thin sections, either
Fig. 1. A light micrograph of a thick section of the larynx, reacted for peroxidase activity but not stained with any other dyes. Note the reaction product in some epithelial cells (arrows) and most glandular cells. Extremely dense staining of blood vessels (V) depends upon peroxidatic activity of hemoglobin. C cavity of larynx. ×100

unstained or stained with lead acetate, were examined in a Hitachi HU-11 D electron microscope. Thick sections were used for light microscopic examination. Controls:

The specimens, fixed in glutaraldehyde and washed in buffer, were incubated in several incubation media as described below.

a) The specimens were incubated in the absence of DAB or H2O2 from the regular incubation medium.

b) The specimens, boiled for 5 min in Tris-HCl buffer, were incubated in the regular incubation medium.

c) The specimens were treated for 10 min at room temperature in Tris-HCl buffer containing inhibitors (10⁻³—10⁻¹ M KCN, 10⁻³—10⁻¹ M NaN₃, 2% H2O2). Then they were incubated in the regular incubation media added the same concentration of each inhibitor.

After the incubation, the specimens were treated like regularly incubated ones.

Results

I. Light Microscopic Observations (Fig. 1)

Two types of epithelia, pseudostratified ciliated epithelium and stratified squamous epithelium, cover the surface of the larynx. The ciliated epithelial region and the laryngeal gland were examined in this study. By light microscopic examination of the larynx, dark brown reaction product was found in some epithelial cells and many glandular cells, but the intensity of staining was greatly varied from cell to cell. Red blood cells and some connective tissue cells exhibited reaction besides epithelial elements.

In the upper part of the trachea, similar results were obtained.