ENZYME HISTOCHEMISTRY OF HUMAN OLFACTORY MUCOSA

TETSUO ISHII

*Department of Otolaryngology, Teikyo University
School of Medicine, Tokyo*

In the human olfactory mucosa, the author demonstrated activities of oxidative enzymes succinic, lactic, malic dehydrogenases and NADH-diaphorase), lysosomal enzymes (acid phosphatase, β -glucuronidase, N-acetyl- β -glucosaminidase), alanyl- and leucyl-aminopeptidases, adenosine triphosphatase (ATPase) and cholinesterase (ChE). The olfactory and supporting cells and Bowman's gland cells showed intense activities of oxidative and lysosomal enzymes. The activity of aminopeptidases was found to be intense in Bowman's gland, while ATPase activity was present in the walls of arteries and veins. ChE activity was restrictedly observed in the nerve fibers around Bowman's gland and arteries. No ChE-active nerves were found in the olfactory epithelia and nerves.

Baradi & Bourne (1959) (4) reported cholinesterase (ChE) activity in the olfactory mucosa of rabbits, describing that ChE activity was present in the dendrites and axons of olfactory cells and in the olfactory nerves. In the olfactory mucosa of rabbits and pigs, Herberhold (1967) (9) demonstrated acid and alkaline phosphatases, adenosine triphosphatase (ATPase), esterase, lipase, cholinesterase (ChE), DOPA-oxidase and peroxidase.

The present study of human olfactory mucosa was designed to demonstrate activities of oxidative enzymes (succinic, lactic, malic dehydrogenases and NADH-diaphorase), lysosomal enzymes (acid phosphatase, β -glucuronidase and N-acetyl- β -glucosaminidase), ATPase, alanyl- and leucyl-aminopeptidases and ChE.

MATERIALS AND METHOD

The olfactory mucosa was removed from the ethmoid bones taken from 10 cadavers ranging 20–79 years of age.

(1) Fresh olfactory mucosa was instantly frozen-sectioned in a cryostat at 10 μ . The sections were thawed on the coverslips and dried at room temperature. The sections were incubated in the media for succinic, lactic, malic dehydrogenases, NADH-diaphorase (Balogh *et al.*, 1961) (3), alanyl- and leucyl-aminopeptidases (Nachlas *et al.*, 1957) (13). After incubation, the specimens were briefly rinsed with distilled water and mounted with glycerol jelly.

(2) After fixation with 4% ice-cold formol-calcium solution, pH 7.0–7.2 for 2 hours, the tissues were sectioned in a cryostat and incubated in the substrate solutions prepared for acid phosphatase (Barka & Anderson, 1962) (5), β -glucuronidase

(Hayashi *et al.*, 1964) (8), N-acetyl- β -glucosaminidase (Hayashi, 1965) (7), ATPase (Padykula & Herman, 1955) (14) and ChE (Karnovsky & Roots, 1964) (12).

RESULTS

As is shown in Fig. 1, the human olfactory epithelium consists of supporting, olfactory and basal cells. The olfactory cell is slender-shaped with an elongated nucleus, but in the histochemical specimen it is indistinct among supporting cells because of the thickness of frozen section. The histochemical findings are described below.

Oxidative enzymes succinic, lactic, malic dehydrogenases and NADH-diaphorase

The activities of these enzymes showed similar histochemical distribution. The most intense reaction was observed in the supranuclear portions of supporting cells (Fig. 2), while other areas of the epithelium showed moderate activities. Bowman's glands showed also intense activities.

Alanyl- and leucyl-aminopeptidases

Bowman's glands showed intense activities of aminopeptidases. Very weak activity was seen in the epithelium. The moderate activity was found in the connective tissue around Bowman's glands and beneath the epithelium.

Lysosomal enzymes (acid phosphatase, β -glucuronidase and N-acetyl- β -glucosaminidase)

The activities of these lysosomal enzymes showed essentially the same distributing patterns. Activities were intense in the supranuclear portions of olfactory and supporting cells (Fig. 3) and in Bowman's gland cells. The receptor dendrite of olfactory cell was indistinguishable among supporting cells.

Adenosine triphosphatase (ATPase)

The cell membrane of each cell showed ATPase activity. The intense reaction was found in the walls of arteries, arterioles, capillaries and veins. Bowman's gland and epithelium showed weak activity.

Cholinesterase (ChE)

ChE activity was restrictedly seen in the nerve fibers and erythrocytes in the blood vessels. No activity was found in the olfactory epithelium. ChE activity in the nerve fibers was specified to be acetylcholinesterase (AChE) by the inhibitory test (Ishii & Toriyama, 1972) (11). The olfactory nerve failed to show enzymatic activity, while anterior ethmoidal nerve was found to contain AChE-active fibers sparsely. AChE-positive nerve bundles were found to be distributed under the epithelium (Fig. 4). The arteries were innervated by dense AChE-positive nerve plexus. The dense AChE-active nerves were found on the anterior ethmoidal artery, before it passed the lamina cribrosa. Few AChE-active fibers were seen

FIG. 1. Human olfactory epithelium of 57 year-old woman, stained with hematoxylin-eosin. Paraffin section. Supporting, olfactory and basal cells are observed. $\times 410$

FIG. 2. Lactic dehydrogenase activity is the most intense at the supranuclear portion of supporting cells. Bowman's gland (B) shows also intense activity. $\times 330$

FIG. 3. The olfactory cells (arrows) show β -glucuronidase activity in the supranuclear areas. $\times 1120$

FIG. 4. ChE-active nerve fibers surround Bowman's gland (B). Arrow points to 2 ChE-active nerve bundles. Olfactory epithelium fails to show ChE activity. $\times 150$

around the duct of Bowman's gland and veins, though the gland was apparently surrounded by AChE-positive nerve fibers (Fig. 4).

DISCUSSION

Distribution of ChE activity in the present paper is different from that reported by Baradi & Bourne (1959) (4). The author could not find ChE-positive nerve observe in the olfactory nerves and epithelia. Though Herberhold (1967) (9) did not fibers acid phosphatase in the olfactory mucosa, the intense activity of this enzyme was found in this study.

Electronmicroscopically lysosome-like inclusions and extended Golgi-complex were observed in the supranuclear portions of supporting cells (Arstila & Wersäll, 1967) (2). This findings coincide well with the present observation that intense lysosomal enzyme activities were demonstrated in the same areas. These cells were considered to have secretory function (1, 6, 9, 15) to supply the secreted covering the surface of olfactory epithelia. Intense activity of oxidative enzymes was also confirmed in this portion.

The olfactory cells showed intense activities of lysosomal enzymes and moderate activities of oxidative enzymes in the supranuclear portions, though activities of other enzymes were not prominent. The olfactory dendrites were not discernible among supporting cells in the frozen-sectioned materials.

It is presumed that Bowman's glands are innervated by ChE-active nerve fibers. Since ChE-positive fibers were none in the olfactory nerve and few in the ethmoidal nerve, these ChE-positive nerves around the glands seemed to originate the sphenopalatine ganglion. It is reported that ChE-positive fibers were distributed around the nasal glands (Ishii, 1970) (10). It is also interesting to know that both Bowman's gland and nasal gland are innervated by the same origin.

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