Enzyme Histochemistry of the Olfactory and Vomeronasal Sensory Epithelia in the Golden Hamster

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ABSTRACT. Histochemical activities of several enzymes were investigated in the olfactory epithelium (OE) and vomeronasal organ (VNO) of the golden hamster. Activities of adenosine triphosphatase, lactate dehydrogenase and succinate dehydrogenase were intense in the OE, and the sensory (VSE) and respiratory epithelium (VRE) of the VNO. The activity of acid phosphatase was intense in both the OE and the VSE, while that of non-specific esterase was intense in the VSE alone. The activity of alkaline phosphatase was detectable only in the VRE. Activities of monoamine oxidase and acetylcholine esterase were negative in all of the OE, VSE and VRE. These similarities and differences in the histochemical distribution of enzymes between OE and VSE may reflect the common olfactory function and/or functional specialization in these epithelia. On the other hand, the VRE was considerably different from the OE and VSE in the enzymatic distribution. This may reflect the non-olfactory function of this epithelium.—**KEY WORDS:** enzyme histochemistry, golden hamster, olfaction, olfactory epithelium, vomeronasal organ.

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The olfactory (OE) and vomeronasal sensory epithelium (VSE) project centrally to the different sites and constitute 2 distinct olfactory systems, i.e., OE-main olfactory bulb and VSE-accessory olfactory bulb [4, 18, 22, 31, 38, 39, 42]. Although the significance of these 2 olfactory systems is not completely understood, morphological and experimental data suggest functional specializations in these systems [18, 20, 24, 42]. On the other hand, both the OE and the VSE are embryologically derived from the olfactory placode [10, 18, 46], and undergo some modifications during development [7, 11, 15, 25, 28, 33, 47]. These modifications may also be taken as an indication of functional specialization of these epithelia. Indeed, they are reported to show different sensitivities to odoriferous compounds [5, 13, 30]. There are, however, very few reports in the literature on the enzyme histochemistry of the 2 epithelia [9, 40, 41]. The present study was therefore designed to examine differences in the localization of several enzymes in these epithelia of the golden hamster and to discuss whether such differences reflect both metabolic and functional differences. The fine structure of these epithelia was also examined to consider the histochemical localization of enzymes in connection with cell organelles.

MATERIALS AND METHODS

A total of 10 adult golden hamsters of both sexes were used in the present study. Under ether anesthesia, animals were decapitated to remove the OE and vomeronasal organ (VNO) as materials. Histochemical studies were made on acid phosphatase (ACP), alkaline phosphatase (ALP), adenosine triphosphatase (ATPase), monoamine oxidase (MAO), non-specific esterase (NsE), acetylcholine esterase (AChE), lactate dehydrogenase (LDH) and succinate dehydrogenase (SDH). The histochemical procedures for the enzymes examined in the present study were detailed in Table 1. Control sections were incubated in substrate-free media for ATPase, MAO, NsE, LDH and SDH. Some inhibitors were used as shown in Table 1 for ACP, ALP and AChE.

For electron microscopy, a part of the materials was double-fixed in glutaraldehyde and osmium tetroxide and embedded in the epoxy resin by the routine procedures. Ultrathin sections were cut on a Porter-Blum MT-2 ultramicrotome, stained with uranyl acetate and lead citrate and examined with a Hitachi HS-7 electron microscope. Semithin sections were stained with Mallory's azur II-methylene blue for light microscopy.

Table 1. Histochemical methods used in the present study

Enzyme	Fixative	Fixation	Section	Substrate	Incubation	Inhibitor	References
ACP	2% glutar-aldehyde	4°C, 30 min	Frozen, 7 μ m	Sodium- β -glycero-phosphate	37°C, 1 hr	10 ⁻⁴ M sodium fluoride	17, 35.
ALP	80% ethanol	-20°C, overnight	Paraffin, 5 μ m	Sodium- β -glycero-phosphate	37°C, 1 hr	0.05M L-phenyl-alanine	17, 49.
ATPase	2% glutar-aldehyde	4°C, 30 min	Frozen, 7 μ m	Adenosine triphosphate	37°C, 30 min	×	34, 35.
MAO	×	×	Frozen, 7 μ m	Triptamine-HCl	37°C, 30 min	×	16.
NsE	80% ethanol	-20°C, overnight	Paraffin, 5 μ m	α -naphtyl acetate	20°C, 15 min	×	36.
AChE	2% glutar-aldehyde	4°C, 30 min	Frozen, 7 μ m	Acetyl-thiocholine	20°C, 30 min	10 ⁻⁴ M eserine	19, 23.
LDH	Acetone	-20°C, 1 hr	Frozen, 7 μ m	Sodium-DL-lactose	37°C, 30 min	×	21.
SDH	Acetone	-20°C, 1 hr	Frozen, 7 μ m	Sodium-succinate	37°C, 1 hr	×	32.

×: Not used (done).

RESULTS

Structure of the OE and VNO: The OE consisted of olfactory, supporting and basal cells, and was occasionally penetrated by ducts of the Bowman's glands underlying the epithelium. Olfactory and supporting cells were arrayed alternatively in the OE, while basal cells were situated sporadically on the basement membrane. The superficial layer of the OE was formed by the distal prolongations of olfactory and supporting cells. The upper third of the epithelium contained elliptical nuclei of elongated supporting cells. Middle and basal thirds were densely occupied by round nuclei of olfactory cells arranged in several layers.

The VNO was a tubular structure with a crescent lumen and enclosed by a bony capsule. The medial wall of the lumen was lined with the VSE, while the lateral wall was lined with the respiratory epithelium (VRE) typically ciliated pseudostratified. The VSE consisted of sensory, supporting and basal cells. Sensory and supporting cells were arranged as in the OE, though basal cells were restricted to the transitional region from VSE to VRE. On the other hand, the VRE consisted of ciliated epithelial cells and basal cells. Ciliated cells were elongated and possessed an elliptical nucleus in the middle portion of the cell. Basal cells were small and amorphous, and scattered on the basement membrane.

The fine structure of cells constituting the OE and

VSE was fundamentally similar with each other, and olfactory cells of the OE and sensory cells of the VSE were interpreted as olfactory receptor neurons. The fine structure of the OE and VSE was schematically illustrated in Fig. 1. On the other hand, ciliated cells of the VRE were covered with numerous cilia and microvilli and contained a large number of mitochondria and free ribosomes and poorly developed rER and Golgi apparatus. Mitochondria were inclined to cluster in the superficial layer of the cytoplasm near the free surface. Basal cells of the VRE possessed scanty cytoplasm and poorly developed organelles such as mitochondria, free ribosomes and rER.

In the present study, the OE and VSE were arbitrarily divided into several portions for the convenience of explanations as follows. The apical part corresponded to the uppermost cytoplasm including olfactory vesicles of olfactory cells and microvilli of supporting cells in the OE, and cytoplasmic protrusion and microvilli of sensory cells and microvilli of supporting cells in the VSE, respectively. The superficial layer corresponded to a portion over the level of elliptical nuclei of supporting cells and included aggregations of mitochondria in the receptor neurons. The upper third corresponded to a portion over the level of the supranuclear cytoplasm of receptor neurons and consisted of the dendrites of receptor neurons rich in microtubules and the nuclei and supranuclear cytoplasm of

Fig. 1. Schematic drawings of the fine structure of the olfactory epithelium and vomeronasal sensory epithelium.

Fig. 2. Intense activity of acid phosphatase in the middle to basal region of the olfactory epithelium (OE). $\times 200$.

Fig. 3. Intense activity of acid phosphatase in the middle to basal region of the vomeronasal sensory epithelium (VSE). The activity is negative in the vomeronasal respiratory epithelium (VRE). $\times 200$.

supporting cells. The middle to basal region consisted of cell bodies of receptor neurons containing rER, lysosomes and Golgi apparatus and infranuclear cytoplasm of supporting cells. In addition, the VRE was divided into the brush border consisting of cilia and microvilli of epithelial cells, the superficial layer containing a large number of mitochondria of

epithelial cells, the middle layer including nuclei of epithelial cells and the basal layer including scattered basal cells.

Acid phosphatase (ACP) (Figs. 2, 3): In the OE, the intense activity of ACP was observed in its middle to basal region, especially in the supranu-

Fig. 4. Intense activity of alkaline phosphatase on the brush border and basal region of the vomeronasal respiratory epithelium (VRE). The activity is negative in the vomeronasal sensory epithelium (VSE). $\times 200$.

Fig. 6. Intense activity of adenosine triphosphatase in the apical part of the vomeronasal sensory epithelium (VSE) and on the brush border and in the superficial layer of the vomeronasal respiratory epithelium (VRE). $\times 200$.

Fig. 5. Moderate to intense activity of adenosine triphosphatase in the apical part of the olfactory epithelium (OE). The activity is moderate in the other parts of the epithelium. $\times 200$.

Fig. 7. Negative activity of non-specific esterase in the olfactory epithelium (OE). $\times 200$.

clear cytoplasm of the olfactory cells. The staining reaction was distinctly granular in these cells. No activity was observed in the supporting and basal cells.

In the VSE, the intense activity was observed in

the supranuclear cytoplasm of the sensory cells as in the olfactory cells of the OE. No activity was observed in the VRE.

Alkaline phosphatase (ALP) (Fig. 4): In the OE, the activity of ALP was almost negative throughout

Fig. 8. Moderate to intense activity of non-specific esterase in the apical part and superficial layer of the vomeronasal sensory epithelium (VSE). The activity is weak on the brush border of the vomeronasal respiratory epithelium (VRE). $\times 200$.

Fig. 10. Intense activity of lactate dehydrogenase in the apical part and superficial layer of the vomeronasal sensory epithelium (VSE). The activity is also intense on the brush border of the vomeronasal respiratory epithelium (VRE). $\times 200$.

Fig. 9. Extremely intense activity of lactate dehydrogenase above the upper third of the olfactory epithelium (OE). $\times 200$.

Fig. 11. Intense activity of succinate dehydrogenase in the superficial layer of the olfactory epithelium (OE). $\times 200$.

the epithelium.

The activity of ALP was almost negative in the VSE as in the OE, while it was intense in the VRE on its brush border and in the basal layer, especially in basal cells.

Adenosine triphosphatase (ATPase) (Figs. 5, 6): In the OE, the activity of ATPase was moderate to intense in its apical part, seemingly localized in that of the supporting cells, and moderate in the other parts of the epithelium.

Fig. 12. Intense activity of succinate dehydrogenase in the apical part and superficial layer of the vomeronasal sensory epithelium (VSE). The activity is also intense in the superficial layer of the vomeronasal respiratory epithelium (VRE). $\times 200$.

In the VSE, the activity was intense in its apical part, seemingly localized in that of the supporting cells as in the OE, but weak through the other parts of the VSE. In the VRE, the intense activity was also observed on its brush border and in its superficial layer, but almost negative in the other parts of the epithelium.

Monoamine oxidase (MAO): The activity of MAO was negative in all of the OE, VSE and VRE.

Non-specific esterase (NsE) (Figs. 7, 8): Although the activity of NsE was intense in the Bowman's glands in the submucosa, it was negative throughout the OE.

In the VSE, the distribution of the enzyme activity was entirely different from the OE. The moderate to intense activity was present in the apical part and superficial layer of the VSE and appeared to be localized in the dendrites of the sensory cells. The activity was weak in the other parts of the epithelium. In the VRE, the activity was weak on its brush border, but negative in the other parts of the epithelium.

Acetylcholine esterase (AChE): The activity of AChE was negative in all of the OE, VSE and VRE.

Lactate dehydrogenase (LDH) (Figs. 9, 10): The activity of LDH was extremely intense above the upper third of the OE, and moderate to weak in the middle to basal region.

In the VSE, the activity was intense in the apical part and superficial layer, but completely negative in the other parts of the epithelium. In the VRE, the activity was intense in the superficial layer, negative on the brush border and moderate in the other parts of the epithelium.

Succinate dehydrogenase (SDH) (Figs. 11, 12): The intense activity of SDH was densely accumulated in the superficial layer of the OE. The activity was moderate in the apical part and upper third, and weak in the middle to basal region.

In the VSE, the intense activity was accumulated in the apical part and superficial layer, but almost negative in the other parts of the epithelium. In the

Table 2. Distribution and intensity of enzymatic activities in the olfactory epithelium (OE), vomeronasal sensory epithelium (VSE) and vomeronasal respiratory epithelium (VRE)

	ACP	ALP	ATPase	NsE	LDH	SDH
OE	Apical part	—	—	++~+++	—	++++
	Superficial layer	—	—	++	—	++++
	Upper third	—	—	++	—	++++
	Middle to basal region	+++	—	++	—	++
VSE	Apical part	—	—	+++	++~+++	+++
	Superficial layer	—	—	+	++~+++	+++
	Upper third	—	—	+	—	—
	Middle to basal region	+++	—	+	—	—
VRE	Brush border	—	+++	+++	+	—
	Superficial layer	—	—	+++	—	+++
	Middle layer	—	—	—	++	+
	Basal layer	—	+++	—	++	+

++++: Extremely intense, +++: Intense, ++: Moderate, +: Weak, —: Negative.

VRE, the activity was intense and accumulated in the superficial layer, but weak or negative in the other parts of the epithelium.

Histochemical findings on the distribution and intensity of enzymatic activities in the OE, VSE and VRE were summarized as shown in Table 2. No difference was observed in enzymatic activities between the male and the female. Since MAO and AChE were negative in all of the OE, VSE and VRE, they were excluded from Table 2.

DISCUSSION

The present findings on the histological and fine structure of the OE and VSE were similar with each other and agreed well with those of previous authors [1–3, 6, 8, 14, 26, 27, 29, 37, 43–45, 48, 50]. As for the histochemical distribution of enzymes, however, the OE and VSE showed some similarities and differences in the present study. Although it is rather difficult to interpret the functional significance of histochemically demonstrated enzymes, these similarities and differences in the OE and VSE may reflect the common olfactory function and/or functional specialization in these epithelia. On the other hand, the VRE, simultaneously examined as a representative of non-olfactory epithelium, was considerably different from the OE and VSE in the histological and fine structure as well as the enzymatic distribution. This may support the probability that the histochemical distribution of enzymes reflect at least a part of the function of an epithelium.

Acid phosphatase (ACP): Among enzymes examined in the present study, ACP showed the same distribution pattern and granular reaction in the OE and VSE, and was localized in the supranuclear cytoplasm of olfactory and vomeronasal sensory cells. Because ACP is a well-known marker enzyme of lysosomes, and because lysosomes are abundant in the supranuclear cytoplasm of these olfactory receptor cells [6, 7, 26, 27, 43–45], the histochemical reaction of ACP can be attributable to lysosomes. The present results on the histochemical distribution of ACP agree well with the previous reports [9, 40, 41]. Functionally, ACP is supposed to participate in the lysosomal destruction of aged or damaged organelles and subsequent cell repair in the OE and VSE [9]. The absence of ACP reaction in the VRE may suggest the slow turnover of epithelial cells in the VRE, since the VRE, different from the other

nasal respiratory epithelium, is derived from the olfactory placode [10, 18, 46] and less exposed to the airflow than the usual respiratory epithelium due to the functional mechanism of the VNO [13, 45].

Alkaline phosphatase (ALP): Although the physiological significance of ALP is still unknown, ALP is often observed on the plasma membrane in various tissues [17, 49]. In the present study, however, the distribution of ALP was completely different between the olfactory (OE and VSE) and non-olfactory (VRE) epithelia, especially on their free surface. Although the OE was covered with olfactory cilia and microvilli, the VSE with microvilli, and the VRE with cilia and microvilli, the histochemical reaction of ALP was observed only on the cilia and microvilli of the VRE. Since the cilia of the OE and microvilli of the VSE are supposed to be the first site of olfactory perception [7, 8, 18, 24, 26, 27], these results may highlight the functional differences between cilia of the OE and the VRE, and the functional similarity of the former with microvilli of the VSE.

The absence of ALP on the free surface of the OE in the present study agrees well with the previous reports [9, 40, 41]. In the VNO, however, results vary among investigators. Cushieri [9] reported the ALP activity in the uppermost layer of the VSE, and not in the VRE of the mouse. Shapiro [41], on the other hand, observed its basal localization in the VSE of fetal rats and mice. Although such differences appear to be species related, further investigations are warranted. The significance of ALP activity in the basal cells of the VRE in the present study is also further to be investigated.

Adenosine triphosphatase (ATPase): This enzyme is generally responsible for the breakdown of ATP (adenosine triphosphate) to ADP (adenosine diphosphate) with the release of free energy [35]. There are several kinds of ATPase with different responses to inhibitors and activators [34, 35]. In the present study, however, the histochemical examination was restricted to calcium-activated ATPase, which is generally localized in myosin, plasma membrane, or mitochondria [34, 35], because there are some problems on the reliability or significance of histochemical demonstration of the other kinds of ATPase [35]. Previous reports on the activity of the calcium-activated ATPase are not consistent. In the OE of the rhesus monkey [40], the activity of calcium-activated ATPase was slight in the olfactory vesicles, absent in the rest of the receptor or

supporting cells, and intense in the basal cells. In mice [9], however, the ATPase activity was intense in the superficial layer of the OE where mitochondria were aggregated, moderate in the basal cells, and intense in the supporting cell bodies and distal parts of sensory cells in the VSE, while it was absent from the basal layer. In the present study, ATPase showed the similar distribution pattern in all of the OE, VSE and VRE, and most intensely observed in the apical part of these epithelia. Because ATPase is often associated with the plasma membrane and mitochondria [34, 35], and because mitochondria are not restricted to the apical part of the epithelia to be distributed to their still lower parts [6, 7, 26, 27, 43–45], the activity of ATPase seems to be localized in the apical cell membrane and vesicles of supporting cells of the OE and VSE and epithelial cells of the VRE. The presence of the ATPase activity in both olfactory (OE and VSE) and non-olfactory (VRE) epithelia suggests that ATPase is not related to the olfactory function but to more fundamental function of these epithelia. The inconsistency of the present results with the previous reports seems to be species related, but is further to be investigated.

Non-specific esterase (NsE): While NsE is localized in various organelles such as endoplasmic reticulum, Golgi apparatus, mitochondria and lysosomes [12], its physiological significance is still unclear. The activity of NsE was reported to be intense in the superficial layer of the VSE in the mouse [9]. In the OE, however, the intense activity was found in this layer in the mouse [9], while it was only slight or absent in all layers in the rhesus monkey [40]. In the present study, the activity of NsE was intensely observed in the apical part and superficial layer of the VSE as reported in the mouse, but negative throughout the OE as in the rhesus monkey. These inconsistencies may reflect differences in species, fixatives, histochemical procedures and perhaps the nature of substrates.

The presence of the NsE activity in the apical part and superficial layer of the VSE in the present study may suggest that NsE is localized in mitochondria of the dendrite of sensory cells. This may also suggest the high metabolic activity in this part of the cells [9]. Since the NsE activity is observed only in the sensory cells of the VSE, it is possible that NsE plays a role in the specialized olfactory function unique to the VSE.

Lactate dehydrogenase (LDH): LDH takes part in

the final step of glycolysis, catalyzes the reaction between pyruvic acid and lactic acid, and is localized in the cytoplasm [21, 35]. The activity of this enzyme was reported in the rhesus monkey to be intense in the upper third of the OE and diffusely positive in the rest of the OE [40]. In the present study, it was also intense in the OE, VSE and VRE and showed a similar distribution pattern as in the rhesus monkey. As the olfactory perception occurs at the free surface of the epithelium and is transmitted downwards in the epithelium [7, 8, 18, 24, 26, 27], the distribution of LDH in the upper third of the epithelium may suggest the high metabolic rate in this part of the epithelium. The conspicuously intense activity of LDH in the OE may suggest more active metabolism or cellular turnover in this epithelium than that of the VSE and VRE.

Succinate dehydrogenase (SDH): SDH is a well-known marker enzyme of mitochondria [32, 35] and catalyzes the reaction between succinic acid and fumaric acid in the citric acid cycle (Krebs' cycle). As mitochondria are abundant in the upper part of the OE and VSE [6, 7, 26, 27, 43–45], the intense activity of SDH in the superficial layer of the OE and VSE of the mouse and rhesus monkey [9, 40] are consistent with ultrastructural findings. The present results are also consistent with these findings, and the intense activity of SDH is observed in the superficial layer of the OE and the VSE. Since the activity of SDH is also observed in the VRE as in the OE and VSE, SDH seems to be engaged in a fundamental function other than olfaction in these epithelia.

Monoamine oxidase (MAO) and acetylcholine esterase (AChE): MAO is engaged in the oxidative degradation of several monoamines [16, 35], while AChE hydrolyzes acetylcholine ester [19, 23, 35]. Since monoamines and acetylcholine are reported to play a role as neurotransmitters [18, 24, 38], the histochemical activities of MAO and AChE were examined in the OE and VSE in the present study to search for the possibility that monoamines and acetylcholine are engaged in the olfactory neurotransmission. As the results, the activities of MAO and AChE were negative in both the OE and the VSE. These findings seem to minimize the possible participation of monoamines and acetylcholine in the olfactory transmission and agree well with the absence of previous reports on the positive activity of these enzymes in the OE and VSE.

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