Comparative Aspect on the Innervation of Submandibular Glands in Cat and Rabbit; an Electron Microscopic Study

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KAGAYAMA, M. and NISHIYAMA, A. Comparative Aspect on the Innervation of Submandibular Glands in Cat and Rabbit; an Electron Microscopic Study. Tohoku J. exp. Med., 1972, 108 (2), 179-193 — The innervation of the submandibular glands of cat and rabbit was studied by electron microscopy. The acini of the submandibular glands of both species were composed of myoepithelial cells and two types of secretory cell; namely, acinar and demilunar cells in the cat, and neck and acinar cells in the rabbit. In the peri-acinar connective tissue there were both adrenergic and cholinergic nerve endings which were differentiated by their contents of synaptic vesicles, especially of small granular vesicles. These nerve endings were surrounded partly or completely by Schwann cell cytoplasm. On the other hand, only one type of nerve ending (cholinergic) was observed within the acini. These intra-acinar nerve endings were devoid of Schwann cell sheath, contacted directly with the plasma membranes of the myoepithelial cells and of the one type of secretory cell that was close to the intercalated duct; namely, the acinar cells in the cat and the neck cells in the rabbit. The occurrence of the nerve endings within the acini was seen more frequently in young cats but somewhat less in adult cats. The functional significance of the intra-acinar nerve endings is discussed. —— nerve-glandular relationship; submandibular gland

There are some conflicting views about the existence of the intra-acinar nerve endings in the submandibular glands. Intra-acinar nerve endings, which show direct contact with a parenchymal element, have been illustrated in human (Tandler 1965), cat (Creed and Wilson 1969, Shackleford and Wilborn 1970) and monkey submandibular glands (Cowley and Shackleford 1970, Kagayama 1971). On the other hand, only extra-acinar nerve endings have been described in the submandibular glands of rat (Scott and Pease 1959), dog (Fujita et al. 1964), cat (Garrett 1966b, d), and man (Ferner and Gansler 1961, Garrett 1967, Norberg et al. 1969).

Kagayama (1971) previously found the intra-acinar nerve endings in the monkey submandibular glands, which showed a restricted localization within the acini; namely they were observed only between myoepithelial and mucous cells, but not between serous cells. Generally the acini of the submandibular glands

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are composed of two types of secretory cell and myoepithelial cells. Histochemical nature and population of both types of secretory cell vary among animal species. In a histochemical study of Shackleford and Klapper (1962), both types of secretory cell were reported as mucous in cat and both as serous in rabbit submandibular glands. It would, therefore, be worthy to elucidate the distribution of intra-acinar nerve endings in the submandibular glands of both species.

MATERIALS AND METHODS

The submandibular glands of adult males and females were examined in rabbit and cat. Also were studied the submandibular glands of young animals, weighing 750 to 2,500 g in cat and 1,500 to 2,500 g in rabbit. The tissues were fixed in 5% glutaraldehyde in Millonig's (1962) phosphate buffer for 1 hour and post-fixed in 1% osmium tetroxide in the same buffer for 1 hour. Following fixation, the tissues were dehydrated in graded ethanols, passed through propylene oxide and embedded in Epon 812 (Luft 1961). Specimens were cut on a Porter-Blum MT-I microtome with glass knives. Thin sections were doubly stained with uranyl acetate and lead solution of Reynolds (1963). A Hitachi HS-8 electron microscope was used for observation. For light microscopy, thick sections were made from the epon blocks and stained with toluidine blue.

RESULTS

The secretory and myoepithelial cells

The acini of the submandibular glands of both cat and rabbit were composed of myoepithelial cells and two types of secretory cell. In the adult cat, almost all of the acini showed demilune formations and most portion of each acinus was occupied by acinar cells (Fig. 3). However, the acinar cells were not fully developed in the young cat. They took only confined portion near the intercalated duct (Figs. 1 and 2). Ultrastructural characteristics of both types of secretory cell were similar to those described by Shackleford and Wilborn (1970). The secretory granules of the demilunar cells mostly showed electron lucent matrix and some of the granules contained one or two smaller electron dense subunits (Fig. 5). The secretory granules of the acinar cells were more dense than those of the demilunar cells and had not intra-granular subunits (Fig. 5).

Contrary to the acini of the cat submandibular gland, the demilune formation was not obvious in the rabbit gland. Most portions of the acini were composed of acinar cells and only small segments of the acini just distal to the intercalated ducts were occupied by special granulated cells (Fig. 4). These granulated cells are termed "neck cells" in this paper. The population of neck cells in young rabbit was similar to that of adult one.

The secretory granules of the acinar cells had electron lucent matrix and contained fine filaments. Sometimes a relatively dense accumulation of fine filaments was observed in it (Fig. 6). The granular endoplasmic reticulum was well developed throughout the cytoplasm and the cisternae took lamellar arrangement. A few cytoplasmic folds were formed at the lateral and basal portion of the acinar cells. Intercellular canaliculi, lined with short microvilli and
limited by a junctional apparatus were found between adjacent acinar cells.

The secretory granules of the neck cells differed in structure from those of the acinar cells. They were round and contained relatively electron dense material (Fig. 6). None of the intra-granular structure was observed in them. The cisternae of granular endoplasmic reticulum were characteristically dilated. Cytoplasmic folds were scarce and intercellular canaliculi were not observed between adjacent neck cells.

In both species, myoepithelial cells lay between the basal surfaces of secretory cells and the basal lamina. They were characterized by the presence of fine filaments in the cytoplasm except near the nucleated portion (Figs. 9, 10 and 12). The cytoplasmic processes of the myoepithelial cells were frequently found near the acinar cells in the cat, especially in the young animal, and near the neck cells in the rabbit.

**The nerve endings**

Two different locations of nerve endings were distinguished, namely intra-acinar and extra-acinar positions which were divided by the basal lamina surrounding the acini. Extra-acinar nerves consisted of many small axons embedded in typical Schwann cell envelopment. The axons formed terminal swellings near the acini. These nerve endings contained numerous synaptic vesicles and were covered partly by Schwann cells. By their contents of synaptic vesicles, two types of nerve ending were differentiated. One contained numerous small agranular vesicles together with a few large granular vesicles. The other type contained many small granular vesicles mixed with a few small agranular vesicles and large granular vesicles (Figs. 7 and 8). Both types of nerve ending were observed in the peri-acinar connective tissue of both cat and rabbit submandibular glands.

Also the intra-acinar nerve endings were found in the submandibular glands of both species. These intra-acinar nerve endings had no Schwann cell sheath around them. They lost their Schwann cell sheath where they penetrated the basal lamina (Fig. 9). The nerve endings showed restricted localization within the acini. In the rabbit submandibular gland, they contacted directly with the plasma membranes of myoepithelial cell and neck cell (Figs. 12 and 13). None of them has been observed among acinar cells in the rabbit submandibular gland.

In the adult cat, intra-acinar nerves were scarce and only a few axons or axonal swellings were found in the course of this study (Figs. 9 and 10). However, many nerve endings were observed in the acini of the young cat (Fig. 11). The plasma membranes which were in direct contact with nerve endings were of the myoepithelial cells and of the acinar cells in both young and adult cats. Thus, acinar and neck cells in the rabbit, and demilunar and acinar cells in the cat apparently differ in their topographical relationship to intra-acinar nerve endings.

Nerve endings within the acini were of only one type which contained many small agranular vesicles together with a few large granular vesicles (Figs. 10, 11, 12).
Apposed plasma membranes of the nerve endings and the terminal cells were separated by a narrow distance of about 200 Å. No specialization of the membrane or of the subjacent cytoplasm has been observed on either side of the contact area between nerve endings and secretory or myoepithelial cells, in both species.

**Discussion**

There are some conflicting views about the existence of intra-acinar nerve endings in the exocrine glands especially in the submandibular glands. Nerve terminals inside of the acinar basal lamina were first observed in rat salivary glands by Scott and Pease (1959). They reported that while intra-acinar nerves had not been observed in the submandibular gland they were fairly common in the parotid gland. Garrett (1966 a, b, c, d, 1967) studied the innervation of the parotid and submandibular glands in the cat and man by means of various methods. He stated that nerve endings were not present in the acini, but only the extra-acinar nerve endings were found. Similar results have been repeatedly reported in the submandibular glands in the dog (Fujita et al. 1964), rat (Tamarin 1966), and man (Ferner and Gansler 1961, Norberg et al. 1969).

Contrary to these observations, the intra-acinar nerve endings were illustrated in the submandibular glands of the cat (Creed and Wilson 1969, Shackleford and Wilborn 1970), monkey (Cowley and Shackleford 1970, Kagayama 1971), and man (Tandler 1965). Kagayama (1971) previously revealed the existence of intra-acinar nerve endings, which were in direct contact not only with the myoepithelial cells but also with the mucous cells in the monkey submandibular gland. Nerve endings were not found among the serous cells. Similar nerve-glandular relationship was also described in the submandibular glands of the squirrel monkey (Cowley and Shackleford 1970). The present study verified the existence of intra-acinar nerve endings in both cat and rabbit submandibular glands. These intra-acinar nerve endings did not occur next to the demilunar cells in the cat and acinar cells in the rabbit submandibular glands. Since both types of the secretory cell had been reported as mucous in the cat and both as serous in the rabbit submandibular glands (Shackleford and Klapper 1962), it may not always be true that intra-acinar nerves tend to innervate the mucous cells as postulated by Cowley and Schakleford (1970). It would be suggested that the nerves within the acini tend to contact with the one type of secretory cell that is located next to the intercalated duct, and also with the myoepithelial cells, but not with the other type of secretory cell.

Yohro (1971) stated, in his postnatal developmental study of the mouse submandibular glands, that nerve endings had been already established in the terminal tubules of the full-term embryos and that the adult intercalated ducts were densely innervated. The developmental changes in distribution of the intra-acinar nerves were also found in the cat submandibular glands; namely the frequent occurrence of them in the young animals and less frequent in the adult. Creed
and Wilson (1969), who found intra-acinar nerves in the cat submandibular glands, used adult cats and kittens ranging in body weight from 1 to 3 kg., but they did not refer to the developmental changes of innervation. Conflicting views about the existence of the nerve endings within the acini may be partly due to these developmental changes in the innervation of this gland as suggested by Yohro (1971).

Abundant evidence for the association of small granular vesicles with the presence of catecholamines has been presented (De Robertis and De Iraldi 1961, Wolfe et al. 1962, Richardson 1964), and autonomic nerve endings can be distinguished into two types by their different populations of synaptic vesicles, at least when potassium permanganate (Richardson 1966, Hökfelt 1967) or glutaraldehyde and osmium tetroxide (Bennett and Rogers 1967, Tranzer and Thonen 1967) are used as fixatives. According to this classification, the intra-acinar nerve endings have always been reported as cholinergic in the submandibular glands of cat (Creed and Wilson 1969) and monkey (Cowley and Shackleford 1970, Kagayama 1971), and also in other exocrine glands such as the lacrimal gland of monkey (Russell 1968) and exocrine pancreas of bat and dove (Watari 1968). The present study also confirmed this. On the other hand, nerve endings in the peri-acinar connective tissue were both adrenergic and cholinergic in both species. Similar results were reported in the submandibular glands of man (Norberg et al. 1969) and monkey (Kagayama 1971). Furthermore, studies using histochemical methods of Falck (1965) for the localization of catecholamines and of Koelle (1951) for the cholinesterase activity demonstrated the presence of both divisions of autonomic nerve around the acini of the submandibular glands of rabbit (Freitag and Engel 1970) and man (Garrett 1967, Norberg et al. 1969).

In his electrophysiological studies on the cat submandibular gland, Lundberg (1955, 1958) showed that stimulation of either the sympathetic or parasympathetic nerve supply to the gland produced a secretory potential across the outer membrane of individual secretory cells. These responses occurred only after a long latency (0.2 to 0.4 for parasympathetic and 0.6 to 1.0 sec for sympathetic stimulations). Creed and Wilson (1969) re-examined the innervation of the same gland and suggested that the parasympathetic innervation of the secretory cell was closer than the sympathetic innervation. Our electrophysiological studies on cat and rabbit submandibular glands (Nishiyama et al. 1971, Kagayama and Nishiyama 1971) were in accord with Creed and Wilson's result (1969). In both cat and rabbit submandibular glands, there was a response to a single parasympathetic stimulation, which has been regarded as an effective in close innervation. On the other hand, the potential was set up only by repetitive stimulation for sympathetic nerve and the mean latency of the secretory potential to sympathetic stimulation was longer than that to parasympathetic stimulation in cat submandibular gland. This suggests that sympathetic nerve does not make direct contact with the secretory cells. Thus, these electrophysiological results are consistent with the present morphological observation that only cholinergic nerve penetrates into the acini.
We could not exclude a possibility of the electrotonic spread of the secretory potentials through the tight junction from the adjacent cells to the cell in which the potential was recorded, since the secretory potentials were easily recorded in a number of cells, whereas the intra-acinar nerve endings were found in the restricted site within the acini. This will be the subject for future studies.

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References

17) Koelle, G.B. (1951) The elimination of enzymatic diffusion artifacts in the histo-


Figs. 1–4. Light micrographs of epoxy resin sections stained with toluidine blue. × 350.

Fig. 1. A section from a young cat (750 g body weight) submandibular gland. Darker stained acinar cells, surrounded by lighter stained demilunar cells, occupy very small portion near the intercalated duct.

Fig. 2. A section from a young cat (1,300 g) submandibular gland. Acinar cells are more densely stained with toluidine blue and occupy larger portion than Fig. 1. Note typical demilune formations.

Fig. 3. A section from an adult cat (3,200 g) submandibular gland. Note the marked predominance of the acinar cells than the demilunar cells.

Fig. 4. A section from an adult rabbit submandibular gland. Granular segments contain several neck cells which are packed with densely stained secretory granules. They locate between acinar cells and intercalated duct.
Fig. 5. An electron micrograph of a young cat (2,500 g) submandibular gland. Demilunar cells (right) are characterized by cytoplasmic folds, and typical secretory granules which contain densely stained subunits. The cytoplasm of acinar cell (left) is filled with homogeneously stained secretory granules. A cytoplasmic process of myoepithelial cell is seen at basal portion of the acinar cell. × 12,000.

Fig. 6. Apical portions of neck cells (left) and acinar cells (right); an adult rabbit submandibular gland. Granules of neck cell are homogeneously dense. On the other hand, granules of acinar cells contain fine filaments and some of them show a few accumulations of filaments. The glandular lumen surrounded by two neck cells and three acinar cells is provided with a few microvilli. × 21,000.
Fig. 7. A small nerve bundle in the peri-acinar connective tissue of an adult rabbit submandibular gland. Among these axons, two are crowded with small agranular vesicles. \(\times 14,500\).

Fig. 8. Cross section of a small nerve in the peri-acinar connective tissue of an adult rabbit submandibular gland. Three axons contain many small granular vesicles mixed with small agranular vesicles and large granular vesicles. One axon is partly bared of Schwann cell sheath. \(\times 34,000\).

Fig. 9. An axon penetrating through the basal lamina to invaginate into the cytoplasm of myoepithelial cell an adult cat submandibular gland. The axon can be differentiated by a few neurotubules and its Schwann cell sheath. \(\times 30,000\).
Fig. 10. An intra-acinar nerve ending between myoepithelial cell and acinar cell; an adult cat. It contains a large number of small agranular vesicles and a few large granular vesicles. Secretory granules of the acinar cell are stained dense and cisternae of granular endoplasmic reticulum are characteristically dilated. $\times 22,000$.

Fig. 11. Two axons within an acinus of a young cat submandibular gland (1,300 g). Their structures are similar to Fig. 10. $\times 20,000$.

Fig. 12. An intra-acinar nerve ending interlocked within the cytoplasm of myoepithelial cell; an adult rabbit. Many small agranular vesicles and a few large granular vesicles are contained in it. $\times 31,000$.

Fig. 13. Two nerve endings between neck cells; an adult rabbit. Note the absence of small granular vesicles in them. Neck cells are characterized by their dilated granular endoplasmic reticulum and densely stained secretory granules. $\times 18,500$. 

