Sensory Innervation of Gingival and Alveolar Mucosa of the House Musk Shrew (*Suncus murinus*)

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YAMAMOTO, T. and SAKADA, S. Sensory Innervation of Gingival and Alveolar Mucosa of the House Musk Shrew (*Suncus murinus*). Tohoku J. exp. Med., 1986, 150 (3), 327-336 —— The lingual gingival and the alveolar mucosa of mandible of the house musk shrew (*Suncus murinus*) were stained by methylene blue vital staining or osmic acid staining, and mounted as whole thickness preparations. The sensory innervation and the distribution of sensory receptors were investigated with a light microscope. The nerve fibers supplying these regions derive from the sublingual nerve, which ascend in the mucosa as they branch out. Sensory receptors found in the present study are of four kinds; free nerve endings, bush-like nerve endings, Merkel cell-neurite complexes and encapsulated corpuscles. The Merkel cell-neurite complexes were scarce and localized in the upper margin of gingival mucosa. The bush-like nerve endings were distributed preferentially in the alveolar mucosa, in which their maximum density was 9-23 per mm². Among the organized receptors, the encapsulated corpuscles appeared most frequently throughout the mucosal area investigated, and their maximum density amounted to 27-56 per mm² in the gingival mucosa. These corpuscles were relatively small and poorly differentiated. Although the bush-like nerve endings and the encapsulated corpuscles were fewer in the third molar region, there was no obvious regional difference in their distribution densities from the premolar region to the second molar region. —— sensory innervation; sensory receptors; distribution density; oral mucosa; house musk shrew

In comparison with the primates, carnivora and rodents, a little information is available on the insectivores concerning the morphology of sensory receptors in the oral mucosa. Early light microscopic studies have shown the characteristics of sensory receptors in the hard palate (Botezat 1907) and the gingiva (Lewinsky and Stewart 1939; Stewart and Lewinsky 1939) of the mole, and the hard palate (Ogasawara et al. 1954) and the soft palate (Ohtomo 1955) of the hedgehog. These studies have also mentioned the location of receptors in relation to the epithelial layer. Osanai (1975) has demonstrated the various kinds of sensory receptors in the several parts of oral cavity of the Japanese shrew-mole, and given some brief comments on their distribution patterns. As far as can be ascertained, no quantitative investigation ever has been made of the distribution density of
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sensory receptors in the oral mucosa of the insectivores. The purpose of the present investigation was to demonstrate the sensory innervation and the distribution of sensory receptors in the gingival and the alveolar mucosa of the house musk shrew.

MATERIALS AND METHODS

Twenty adult male house musk shrews (Suncus murinus), weighing 58–70 g, were obtained from a colony maintained in the Central Institute for Experimental Animals (Kawasaki). After each animal was anesthetized with sodium pentobarbital, the lingual gingival and the alveolar mucosa of the mandible were dissected out as completely as possible under a stereoscopic dissecting microscope and stuck on a soft resin plate with the lamina propria outward using fine steel pins. The fresh tissues were treated immediately, in the manner described previously (Yamamoto and Sakada 1983), by the vital methylene blue staining method (Meyling 1953) or the osmic acid staining method. In the former method, the specimens were stained with a freshly prepared 0.01% solution (pH 6.0) of methylene blue for 10–20 min at 37°C. Meanwhile, the extent of the staining was examined several times with a microscope. The tissue were then immersed in a chilled 8% ammonium molybdate solution for 18–24 hr and washed in running tap water for 30 min. In the latter method, the specimens were stained with a 1% solution of osmium tetroxide in 0.1 M phosphate buffer (pH 7.4) for 30–60 min at 4°C, rinsed in buffer, and immersed in a 5% solution of pepsin for 3–5 days at 37°C. After staining, all specimens were placed on glass slides and mounted in a 50% glycerin for investigation with a light microscope. The preparations stained by methylene blue were used for the examination of the shape of unmyelinated axon terminals and the distribution of sensory receptors, and those stained by osmic acid were used for the examination of the innervation, the shape of the capsule surrounding axon terminals and the size of encapsulated receptors. Both the charts showing the mucosal innervation and the distribution of sensory receptors were made by tracing an enlarged micrograph of each preparation as confirming the details through a microscope.

RESULTS

The side view of the mandible of the house musk shrew is shown by the micrograph in Fig. 1. The lingual gingival mucosa adjoins the alveolar mucosa with an indistinct sulcus and the crest of the free gingiva is underdeveloped. Both the gingival and the alveolar mucosa were observed to be densely innervated and to contain numerous sensory receptors. The diagram in Fig. 2 indicates the appearance of the innervation of these regions, which was made by tracing the micrograph of the whole-mounted preparation stained by osmic acid. The area enclosed by a dotted line is shown by the original micrograph in Fig. 3. As shown in Fig. 2, the small fasciculi which derive from the sublingual nerve ascend in the alveolar mucosa as they branch out repeatedly, then penetrate into the gingival mucosa. Within the alveolar mucosa, nerve fibers usually wind their ways remarkably. Although some of the gingival nerve fibers were observed to enter the interdental papillae, it could not be confirmed whether they extend to the periodontal membrane. Moreover, no periodontal nerve fibers supplying the gingival mucosa were detected in this study.

In these regions, the myelinated nerve fibers were observed to terminate as
free nerve endings, bush-like nerve endings, Merkel cell-neurite complexes or encapsulated corpuscles. Among them, the Merkel cell-neurite complexes were located only in the upper margin of gingival mucosa, while other sensory receptors were ubiquitous throughout the mucosal area investigated. The free nerve endings can be identified as fine non-myelinated axon terminals ending blindly within the connective tissue of lamina propria (Fig. 4). Whole course of their axon terminals could rarely be followed. Careful focusing with a microscope revealed that a part of free nerve endings penetrate into the epithelium to form intra-epithelial fibers (Fig. 5), which course their ways toward the surface of mucosa and often end in a small bulb-like swelling.

The bush-like nerve endings were well demonstrated by means of the methylene blue vital staining as a complex arborization of fine axon terminals
Their terminal branches have varicose swellings and small expanded tips. No capsules were discerned around the axon terminals in spite of the application of the osmic acid staining. The methylene blue technique showed also the existence of Merkel cell-neurite complexes in the gingival mucosa (Fig. 7). They consisted of a single Merkel cell and neurite or a grouping of 2–7 Merkel cells and neurites. The expanded disc-like nerve endings which end in close association with the Merkel cells were well recognized.

The encapsulated corpuscles were densely distributed in these regions. Fig. 8 shows the arrangement of these corpuscles in relation to their parent fibers at a lower magnification. The encapsulated corpuscles consist of a non-myelinated axon terminal surrounded by an inner core and a thin capsule. The stem axon and its terminal appearance were seen clearly in the preparation stained by methylene blue (Fig. 9), while the non-neural materials surrounding the axon terminal could be well demonstrated by osmic acid staining (Fig. 10). Several different types of encapsulated corpuscles could be defined by light microscopy. The simple corpuscles with a non-branched axon terminal, the typical form of which is shown in Fig. 10, were dominant accounting for 78.2% of the whole. The branched-axon type whose axon terminal ramifies into several branches, such as shown in Fig. 9, were only 17.6%. The remaining 4.2% were of other types and of those indistinct. The encapsulated corpuscles found in this investigation

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were relatively small. Their diameter and length measured on the specimens stained by osmic acid were 14–35 μm (22.1 ± 4.8 μm, n = 82) and 17–55 μm (34.5 ± 7.4 μm), respectively.

Three kinds of organized receptors were observed to be quite different in their distribution pattern. A quantitative investigation was made using ten whole thickness preparations stained by methylene blue. After an enlarged micrograph of each preparation was prepared, the positions of sensory receptors were mapped in it as confirming their kinds under a microscope. Consequently, the distribution charts were made by tracing those micrographs. Fig. 11 shows the typical chart of the location of each receptors examined in the oral mucosa of right mandible. As shown in the chart, the Merkel cell-neurite complexes were scarce, and located only in the upper margin of gingival mucosa excepting the interdental papillae. The bush-like nerve endings were distributed preferentially in the alveolar mucosa, in which their maximum number per mm² was 9–23. A small number of these nerve endings were scattered also in the gingival mucosa. Among the organized receptors, the encapsulated corpuscles appeared most frequently in both the gingival and the alveolar mucosa of house musk shrew. The
maximum number of these corpuscles per mm² amounted to 27-56 in the gingival mucosa. Although the bush-like nerve endings and the encapsulated corpuscles were relatively fewer in the third molar region, there was no obvious regional difference in their distribution densities over the mucosal area neighboring the premolar, the first molar and the second molar.
DISCUSSION

Earlier investigators (Lewinsky and Stewart 1939; Stewart and Lewinsky 1939) have reported that the gingival mucosa of the mole has a very rich nerve supply. The results of the present study have also shown that both the gingival and the alveolar mucosa of the house musk shrew are richly innervated by the trigeminal sensory nerve fibers. The gingival innervation of the cat (Lewinsky and Stewart 1937) and rat (Bernick 1956) has been reported to be derived from two sources, nerve fibers arising from the periodontal membrane and those coming from the alveolar or palatal mucosa. It was observed in the house musk shrew that the lingual gingival mucosa including the interdental papillae was innervated by the alveolar nerve fibers originating from the sublingual nerve. However, another source of the gingival innervation, the nerve fibers originating from the periodontal nerve bundles, was not detected in the present materials.

Only a few investigations are available on the morphology of sensory receptors in the oral mucosa of the insectivores (Botezat 1907; Lewinsky and Stewart 1939; Stewart and Lewinsky 1939; Ogasawara et al. 1954; Ohtomo 1955; Osanai 1975). For the most peculiar one among those sensory receptors, the Eimer's organs are observable in the hard palate of the mole (Botezat 1907), and the gingiva, hard palate and labial mucosa of the Japanese shrew-mole (Osanai 1975). The Eimer's organs are known to be abundant in the mole's snout, which consist of an epithelial peg containing a cluster of the intra-epithelial fibers and several Merkel cell-neurite complexes in the stratum basale (Armstrong and Quilliam 1961; Halata 1972a). Although the Merkel cell-neurite complexes as well as the intra-epithelial fibers were found in the gingival mucosa of the house musk shrew, it could not be recognized that they composed the Eimer's organs.

The bush-like nerve endings are well distinguishable from the free nerve endings or the intra-epithelial fibers by means of the whole thickness preparations stained by methylene blue (Yamamoto and Sakada 1983; Yamamoto et al. 1986a, b). Botezat (1907) and Ohtomo (1955) have mentioned the appearance of the branched terminations in the oral mucosa of insectivores, which possibly correspond to the bush-like nerve endings. We have just demonstrated that the bush-like nerve endings are distributed abundantly in the oral mucosa of the house musk shrew, the density of which is higher than that in the alveolar mucosa of the mouse (Yamamoto et al. 1986b).

The most conspicuous feature of the gingival and alveolar mucosa of the house musk shrew is the dense distribution of encapsulated corpuscles. The axon terminals and the capsules of these corpuscles are rather poorly differentiated. Among the several types of the encapsulated corpuscles, the simple type amounted to 78.2% as described above, while no clew-like corpuscles were detected in these regions. This findings in the present study agree with those reported on the oral mucosa of the Japanese shrew-mole (Osanai 1975) and the snout of the mole.
By contrast, the clew-like corpuscles have been shown to appear frequently in the oral mucosa of primates (Stewart and Lewinsky 1939; Dixon 1962; Winkelmann 1962; Taylor et al. 1964; Tolman et al. 1965; Desjardins et al. 1971) and lower mammals (Stewart and Lewinsky 1939; Dixon 1962; Sakada 1980; Yamamoto and Sakada 1983; Byers and Yeh 1984; Chan and Byers 1985).

The distribution density of sensory receptors in the oral mucosa is revealed to be higher in proportion to the increase in number and size of the papillae (Gairns 1955; Dixon 1962; Winkelmann 1962; Seto 1972; Byers and Yeh 1984; Chan and Byers 1985). Accordingly, the sensory receptors are generally more numerous in the anterior part of the oral mucosa (Dixon 1962, 1963; Taylor et al. 1964; Byers and Yeh 1984; Chan and Byers 1985). The organized sensory receptors in the human gingiva have been reported to increase in their distribution densities from the molar region to the incisor region (Tolman et al. 1965; Seto 1972). The encapsulated corpuscles in the alveolar mucosa of the mouse are concentrated in the mucosal rugae where the papillae are well developed (Yamamoto et al. 1986b). On the contrary, the density of nerve distribution shows no particular pattern of regional change in the gingival mucosa of the cat (Winkelmann 1962) and in the molar junctional epithelium of the rat (Byers and Holland 1977). Moreover, Desjardins et al. (1971) have reported that the number of organized receptors in the human mandibular gingiva is greater in the molar region than in the incisor region. According to their opinion, the density of sensory receptors is in proportion to the richness of gross anatomical innervation.

Turner (1983) described from his findings in the gingival mucosa of the monkey that the Merkel cell-neurite complexes are located mainly in the masticatory mucosa in intimate contact with the tongue. In the present study, the organized receptors were detected abundantly throughout the mucosal area investigated, and their distribution densities are high even in the molar region. These mucosal area of the house musk shrew is considered to receive the external stimuli frequently during the mastication. It is, therefore, suggested that the sensory receptors in the oral mucosa are located preferentially where the external stimulation is frequent, and concerned not only in the oral sensation but also in the proprioceptive mechanisms which control the movement of the tongue and the jaw.

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References


