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Nerve cell bodies and small ganglia in the connective tissue stroma of human submandibular glands

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ABSTRACT

The objective of the study was to investigate the presence and distribution of nerve cell bodies and small ganglia in the stroma of human submandibular gland. A retrospective immunohistochemical study in 13 human submandibular glands, fixed in neutral buffered formalin and embedded in paraffin wax, was undertaken. Six glands were excised in the course of radical neck dissection for oral squamous cell carcinoma and were disease-free, six showed sialadenitis, and one was involved by tuberculosis. Primary antibodies applied were neuron specific enolase, synaptophysin, and glial fibrilliary acidic protein. Neuron specific enolase and synaptophysin positive nerve cell bodies and small ganglia were found in 8/13 and 13/13 glands, respectively. They were found in the interlobular connective tissue stroma of human SMG, in close association to salivary parenchymal cells and blood vessels, and some of them were incorporated in GFAP positive peripheral nerves. To our knowledge, nerve cell bodies and small ganglia have been described only in the connective tissue stroma of autotransplanted human SMG and their functional importance is not clear.

In rats and mice ganglion cell or ganglioneurons, i.e. nerve cells located outside the central nervous system, are commonly found in the submandibular gland (SMG), where they constitute the submandibular ganglion (SG), also called Langley's ganglion, sublingual ganglion, and lingual ganglion [2]. SG is a discrete cholinergic, parasympathetic extracranial ganglion, that along with the ciliary, pterygopalatine, and otic ganglia comprises the cranial section of the autonomic nervous system [2,12,10]. It has been extensively studied and utilized in neurophysiology studies, as it is one of the most easily accessible ganglia of the peripheral nervous system [4,13,5]. Besides the SG, ganglion cells isolated or in small groups called ganglia, are found along the ducts of submandibular and sublingual glands (Stensen's duct), intra- and extra-glandularly [7], in particular at the branching sites of the ducts [13]. Those ganglia are so numerous in rats that contribute approximately 1/2–1/3 of the total ganglion cell population of the SG [8].

Descriptions of the human SG are found in classical anatomy textbooks [12], and detailed descriptions if its anatomy and histology is found in the papers of Siessere et al. [10] and Moriyama et al. [6], respectively. SG is located above the deep part of the human SMG, laterally and superiorly to the hyoglossus muscle and under the lingual nerve with which it may be fused [12]. It carries secremotor preganglionic fibers to the SMG, sublingual gland, and probably the minor salivary glands of the lips, cheeks, floor of mouth, and tongue [12,6], through its motor or parasympathetic root originating in the superior salivary nucleus; and sympathetic fibers through the sympathetic root from the sympathetic plexus that are vasomotor and also terminate in the SSG [12]. It also receives sensory fibers from the trigeminal nerve. Microscopically, it is a small ganglion composed of rather circular ganglion cell with eccentrically located nucleus and medium sized, evenly distributed Nissl bodies, surrounded by small satellite cells with dark nuclei [6]. Isolated ganglion cells or small ganglions have been described in the area of the hilum of the SMG [12], but there is only one report on their presence in the stroma of SMG [6,3].

As we have occasionally encountered ganglion cells and small ganglia in surgically excised human SMG, we report their presence and distribution in the connective tissue stroma of human SMGs.

Thirteen totally excised human submandibular glands were retrospectively collected and immunohistochemically studied. Six glands were excised in the course of radical neck dissection for oral squamous cell carcinomas and were disease-free, six showed sialadenitis, and one was involved by tuberculosis. Tissues had been fixed in neutral buffered formalin and embedded in paraffin wax (FFPE). Two paraffin blocks with adequate material were randomly

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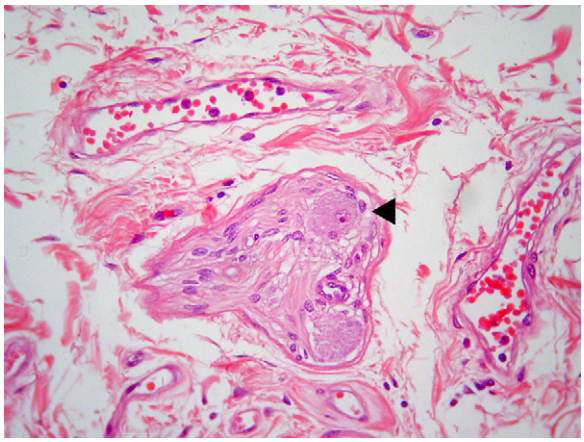

Fig. 1. Nerve cell body embedded in a peripheral nerve (arrowhead) (hematoxylin and eosin stain, original magnification 400×).

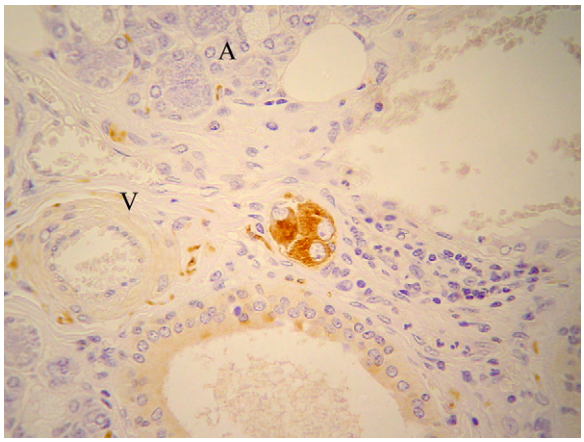

Fig. 3. Three synaptophysin positive nerve cell bodies arranged in a small ganglion in the vicinity of a striated duct (SD), a medium-sized vessel (V) and serous acini (A) (synaptophysin immunohistochemistry, original magnification 400×).

collected from each gland. The specific anatomical site of the tissue in each block within the gland could not be defined. Three FFPE tissue sections from each block were immunohistochemically stained with a standard streptavidin–biotin–peroxidase method, after antigen retrieval in Citra® solution (HK086-9K, Biogenex, San Ramon, CA, USA) in a microwave oven (2 high pressure cycles of 15 min each). Primary antibodies applied were neuron specific enolase (NSE, MIG-N3, Biogenex, 1:100), synaptophysin (SNP88, Biogenex, 1:50), and glial fibrilliary acidic protein (GFAP, GA5, Novocastra, 1:100). The sections were treated with 3% hydrogen peroxide for endogenous peroxidase blocking, and universal blocking reagent (HK085-5K, Biogenex) for the abolishment of non-specific binding of the primary antibodies. Staining reaction was visualized with 3′,3 diaminobenzidine (Sigma DAB, D-5637, St. Louis, MO, USA). Counterstain was developed with Mayer's hematoxylin. For negative controls, substitution of primary antibodies by non-immune human serum was utilized. The presence of ganglion cells and their distribution in relation to the parenchymal and stromal elements of the SMG were recorded.

Nerve cell bodies were recognized in conventional hematoxylin and eosin stained sections as large cells with lightly-stained, finely granular cytoplasm and a large, round, euchromatic nucleus with a dense, prominent nucleolus (Fig. 1). They appeared circular in shape and the nucleus was eccentrically placed. They reacted intensely for NSE (Fig. 2) and synaptophysin (Fig. 3), but NSE showed intense background staining. NSE positive ganglions cells were found in 8/13 glands, and synaptophysin positive cells in all SMGs studied, regardless of the underlying pathology. They were occasionally arranged in small ganglia of 2–4 nerve cell bodies. Nerve cell bodies were situated in the interlobular connective tissue stroma, in the vicinity of interlobular ducts, and close to acini and blood vessels. Most of them were embedded in GFAP positive peripheral nerves (Figs. 2 and 4).

We have confirmed the presence of NSE and/or synaptophysin positive ganglion cells in the interlobular connective tissue stroma of human SMG. A similar observation has been made by Geerling et al. [3] in human SMG autotransplanted in the temporal fossa for the alleviation of severe xeropthalmia, before and after transplantation.

The morphology of the ganglion cells reported herein is comparable to that described by Moriyama et al. [6] for ganglion cells of the human SG. Satellite cells [6] were not obvious, possibly due to the fact that most of the ganglion cells and ganglia were embedded in peripheral nerves, surrounded by the perineurium. Further morphometric analysis was not undertaken, as our material did not fulfill the defined criteria [6].

The functional role of the intrasalivary neuron cells and ganglia is not clear [4,11]. In general, parasympathetic innervation of SMG is responsible for fluid and electrolytes secretion, regulation of salivary secretion, as well as regeneration of the SG [11,9]. Ganglion cells are considered responsible for the innervation of the SMG [4,11], and in rats and ferrets, ganglia in the interstitial

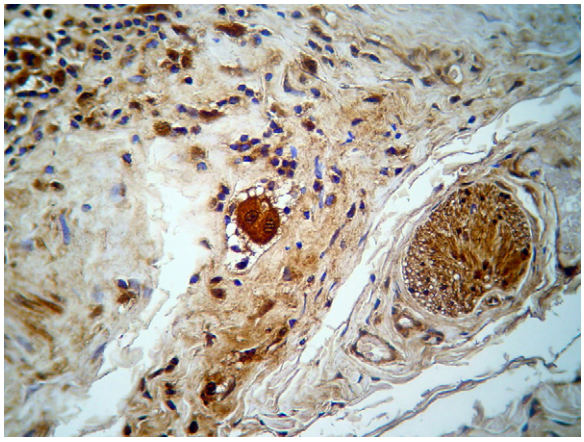

Fig. 2. Two NSE positive nerve cell bodies close to a peripheral nerve (NSE immunohistochemistry, original magnification 400×).

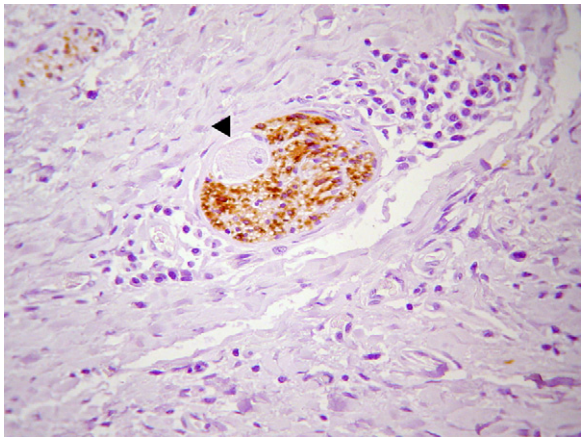

Fig. 4. Nerve cell body (arrowhead) embedded in a GFAP positive peripheral nerve (GFAP immunohistochemistry, original magnification 400×).

tissue of the hilar regions of SMG and sublingual gland express nitric oxide synthase immunoreactivity and NADPH-diaphorase enzyme staining, supporting a role in parasympathetic secretion and vasodilatation of the glands [1]. In the present study, some nerve cell bodies were seen within GFAP positive peripheral nerves, but most were seen in the connective tissue stroma, close to striated ducts, acini, and medium-sized vessels. However, the results of a morphological study, like the one presented herein, are not sufficient to elucidate the possible function of interlobular ganglion cells in SG.

Geerling et al. [3] noticed that parasympathetic ganglion cells and their nerves survive after autologous free transplantation, despite the severance of normal parasympathetic and sympathetic innervation by the surgical procedure. Furthermore, nerves sprouting from adjacent sites developed functional connections with them. Those findings were considered to implicate that they may facilitate external reinnervation of the gland and functioning of the autografts long after transplantation.

As nerve cell bodies and small ganglia in the interlobular connective tissue stroma of human SMG are common and may be actively involved in salivary function, their alterations in pathologic conditions associated with decreased salivary secretion, i.e. xerostomia would be of interest to be studied.

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References

[1] P. Alm, J. Ekstrom, B. Larsson, G. Tobin, K.E. Andersson, Nitric oxide synthase immunoreactive nerves in rat and ferret salivary glands, and effects of denervation, Histochem. J. 29 (9) (1997) 669–676.
[2] G.S. Crouse, A.J. Cucinotta, Progressive neuronal differentiation in the submandibular ganglia of a series of human fetuses, J. Comp. Neurol. 125 (2) (1965) 259–271.
[3] G. Geerling, J.R. Garrett, K.L. Paterson, P. Sieg, J.R. Collin, G.H. Carpenter, S.G. Hakim, I. Lauer, G.B. Proctor, Innervation and secretory function of transplanted human submandibular salivary glands, Transplantation 85 (1) (2008) 135–140.
[4] K. Kawa, S. Roper, On the two subdivisions and intrinsic synaptic connexions in the submandibular ganglion of the rat, J. Physiol. 346 (1984) 301–320.
[5] J.W. Lichtman, The reorganization of synaptic connexions in the rat submandibular ganglion during post-natal development, J. Physiol. 273 (1) (1977) 155–177.
[6] H. Moriyama, K. Shimada, N. Goto, Morphometric analysis of neurons in ganglia: geniculate, submandibular, cervical spinal and superior cervical, Okajimas Folia Anat. Jpn. 72 (4) (1995) 185–190.
[7] Y.K. Ng, W.C. Wong, E.A. Ling, The intraglandular submandibular ganglion of postnatal and adult rats. I. A light and electron microscope study, J. Anat. 180 (Pt. 2) (1992) 305–314.
[8] Y.K. Ng, W.C. Wong, E.A. Ling, The intraglandular submandibular ganglion of postnatal and adult rats. II. A morphometric and quantitative study, J. Anat. 181 (Pt. 2) (1992) 249–258.
[9] G.B. Proctor, G.H. Carpenter, Regulation of salivary gland function by autonomic nerves, Auton. Neurosci. 133 (1) (2007) 3–18.
[10] S. Siessere, M. Vitti, L.G. Sousa, M. Semprini, M.M. Iyomasa, S.C. Regalo, Anatomic variation of cranial parasympathetic ganglia, Braz. Oral. Res. 22 (2) (2008) 101–105.
[11] T. Suzuki, S. Sakada, Synaptic transmission in the submandibular ganglion of the rat, Bull. Tokyo Dent. Coll. 13 (3) (1972) 145–164.
[12] P.L. Williams, R. Warwick, Gray's Anatomy, 36 edition, Churchil Livingstone, 1980.
[13] M. Yamakado, T. Yohro, Population and structure of nerve cells in mouse submandibular ganglion, Anat. Embryol. (Berl.) 150 (3) (1977) 301–312.