A STUDY OF MORPHOLOGICAL AND PHYSIOLOGICAL CHANGES IN THE MANDIBULAR GLAND OF THE SHEEP ASSOCIATED WITH EATING AND DIRECT STIMULATION

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ABSTRACT

A STUDY OF MORPHOLOGICAL AND PHYSIOLOGICAL CHANGES IN THE MANDIBULAR GLAND OF THE SHEEP ASSOCIATED WITH EATING AND DIRECT STIMULATION

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This study was undertaken to investigate relationships between the structure of the mandibular gland of the sheep and its secretory activity in response to feeding, direct stimulation of autonomic nerves, or pharmacological agents that mimic the action of autonomic transmitters. Forty-five crossbred Romney ewes and wethers were used in acute experiments and twenty-two in chronic experiments.

Histochemical and electron microscopical examinations of the structure of mandibular glands confirmed that their secretory endpieces are composed of mucous tubulo-acinar cells and seromucous demilunes. The mucous acini contained a single type of electron lucent granules, whereas the granules of demilunes typically exhibited a tripartite structure. The intercalated ducts were relatively short and lined by non-secretory, simple cuboidal cells and occasional basal cells. Striated ducts were numerous and lined by four cell types, the most common of which (type-I) were tall, columnar, electron lucent cells with well developed membrane infoldings basally with associated mitochondria and small, dense, apical bodies. Myoepithelial cells were distributed densely around the secretory endpieces and within the basement membranes. Myoepithelial cells were also found embracing the intercalated duct cells.

Both AChE-positive and biogenic-amine fluorescent nerve fibres were present around the secretory endpieces and the walls of blood vessels. Fewer biogenic-amine fluorescent fibres were seen in relation to duct cells. Electron microscopy showed unmyelinated fibres in both epilemmal and

hypolemmal sites. The epilemmal axons were frequently found close to a variety of effector cells, while hypolemmal axons were observed occasionally in the intercellular space between adjacent striated duct cells and between intercalated duct and mucous cells. Axons containing large granular vesicles were also found within interstitial nerve bundles.

Mandibular secretion was studied after cannulation of the mandibular duct in both acute and chronic experiments. In anaesthetized animals, stimulation of either the chorda lingual nerve (3-8V, 5-10Hz, 0.2 msec) or injection of carbachol (40 $\mu g\ kg^{-1}$ body weight, iv) within 10-25 sec caused a copious secretion (0.33-0.74 $g\ min^{-1}$) of low protein content (0.44-1.56 $mg\ ml^{-1}$). This response was completely blocked by atropine (0.1 $mg\ kg^{-1}$ body weight). In contrast, stimulation of cervical sympathetic trunk (3-8V, 5-10 Hz, 0.2 msec) after a latency of 35-102 sec caused a meagre secretion (0.01-0.06 $g\ min^{-1}$) of high protein concentration (4.02-25.68 $mg\ ml^{-1}$). Isoprenaline had similar effects. Secretory responses to sympathetic stimulation were blocked by propranolol (1.0 $mg\ kg^{-1}$ body weight). Studies involving gel electrophoresis demonstrated major protein bands exclusively in the sympathetic nerve or isoprenaline stimulated saliva. These major protein components (both soluble and insoluble) were found by immunocytochemical studies to be localized in the demilunes and some striated duct cells of the resting gland.

It was found that in sheep fed lucerne chaff (ca. 1,000 g daily) a rapid and sustained mandibular flow only occurred during eating, although, short term increases were seen, for example, during drinking. Flow was absent during rumination and slight ($0.95 \pm 0.09\ g\ h^{-1}$) or absent at rest. Saliva produced during eating had its highest protein concentration almost immediately as eating commenced ($1.65 \pm 0.06\ mg\ ml^{-1}$) and remained at a high level during the first hour of eating ($1.55 \pm 0.06\ mg\ ml^{-1}$). Propranolol (1.0 $mg\ kg^{-1}$ body weight, iv) caused significant reductions

in protein secretion during eating ($p<.001$) without associated changes in flow. Gel electrophoretic studies confirmed the presence of a major protein band similar to soluble protein band X found in sympathetically evoked saliva. The intensity of this major protein band in saliva collected during eating was also reduced after propranolol treatment. Saliva collected during teasing had a high protein concentration (2.73 ± 0.20 mg ml^{-1}). It is concluded that sympathetic activation was involved mainly early in the eating period and that parasympathetic nerves were active throughout. The latter was confirmed by a great reduction in flow after injection of atropine (0.1 mg kg^{-1}, iv).

Morphological studies of the glands of sheep whose food had been withheld for 20 hours revealed that both the mucous acini and seromucous demilunes were filled with secretory granules. Stimulation of the chorda lingual nerve for 2-4 hours caused acini to discharge their contents of secretory granules, but no appreciable changes in the demilunes. On the other hand, stimulation of the cervical sympathetic trunk produced varying degrees of degranulation in the demilunes, with, in some cells, vacuolation. Infusion of isoprenaline (2h; 0.3 µg kg^{-1} min^{-1}) produced similar changes in demilunes. Striated duct cells showed reduced PAS-staining, and disruption of their basal regions, particularly after stimulation of sympathetic nerves. Concurrent stimulation of both sympathetic and parasympathetic nerves resulted in a combination of the above separate effects.

Eating led to extensive degranulation and greater evidence of synthesis in the mucous acini than parasympathetic nerve stimulation, the changes increasing with the duration of eating, and a depletion of secretory granules in demilunes that could be prevented by propranolol. (1.2 mg kg^{-1} body weight, iv). The morphological changes in demilunes were not proportional to the duration of eating but were greatest in its early phases. Evidence of small dense bodies which were apparently discharged *via* the apical membrane of striated duct cells and a loss of PAS-staining in these cells suggest that they secrete during eating. However, neither damage

to striated duct cells nor secretory endpieces was evident.

The results suggest that the sheep mandibular gland is naturally stimulated by both divisions of the autonomic nervous system, with acinar cells predominantly under the parasympathetic and demilunes under the sympathetic control. The sympathetic stimulation of salivary protein secretion appears to be mainly mediated *via* a β-adrenergic mechanism whereas the secretion of fluid and probably also mucus glycoproteins is an atropine-sensitive parasympathetic effect.

On both morphological and physiological grounds it is suggested that in sheep mandibular glands, myoepithelial cell contraction is important in assisting the secretion of viscous saliva.

Further studies on the following areas would seem appropriate: (i) systematic morphological studies using stereological analysis of changes in the acinar cells, demilunes, striated ducts and their cytoplasmic components; (ii) ultrastructural examinations of the innervation pattern in this gland under normal conditions, after specific denervation and reinnervation; (iii) studies of the nature and origin of the salivary proteins secreted during eating and nerve stimulation and (iv) the use of chronically cannulated animals for studies of the influence of different conditions of feeding.

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