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Acetylcholine synthesis, muscarinic receptor subtypes, neuropeptides and secretion of ferret salivary glands with special reference to the zygomatic gland

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ABSTRACT

Studies on salivary secretion are usually focused on parotid and submandibular glands. However, the film of mucin, that protects the oral structures and is responsible for the feeling of oral comfort, is produced by the submucosal glands. The submucosal zygomatic and molar glands are particularly large in carnivores such as the ferret. Comparisons between the mucous sublingual, zygomatic and molar glands, serous parotid and seromucous submandibular glands showed the acetylcholine synthesis, in terms of concentration, to be three to four times higher in the mucous glands than in the parotid and submandibular glands. Bromoacetylcholine inhibited 95–99% of the synthesis of acetylcholine in the incubates of the five types of glands, showing the acetylcholine synthesis to depend on the activity of choline acetyltransferase. The high acetylcholine synthesis in the zygomatic gland was of nervous origin, since cutting the buccal nerve, aiming at parasympathetic denervation, and allowing time for nerve degeneration, reduced the acetylcholine synthesising capacity of the gland by 95%. A similar reduction (96%) in the parotid gland followed upon the avulsion of the parasympathetic auriculo-temporal nerve. Zygomatic saliva was very viscous. The salivary flow rate in response to electrical stimulation (20 Hz) of the buccal nerve (zygomatic gland), expressed per gland weight, was one-third of that to stimulation of the auriculo-temporal nerve (parotid gland) or the chorda-lingual nerve (submandibular gland). As previously shown for the parotid and submandibular gland, a certain fraction (25%) of the parasympathetic secretory response of the zygomatic gland depended on non-adrenergic, non-cholinergic transmission mechanisms, probably involving substance P and vasoactive intestinal peptide and possibly calcitonin gene-related peptide. Particularly, high concentrations of vasoactive intestinal peptide were found in the sublingual and molar glands, and of substance P in the submandibular, zygomatic and molar glands; notably, the concentration of calcitonin gene-related peptide of the sublingual gland was not detectable. All five muscarinic receptor subtypes were detected in the five glands. The receptor protein profile, as judged by immunoblotting and semi-quantitative estimations, was about the same in the glands: high level of M3, low level of M2 and levels roughly in the same range of M1, M4 and M5. Compared to the parotid and submandibular glands, the M5 receptor level was particularly low in the mucin-secreting glands. The present study

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points out both similarities and dissimilarities between the five types of glands investigated. The zygomatic gland, in particular, appears to be a suitable model for future studies aiming at causing relief of dry mouth by local pharmacological treatment.

1. Introduction

Studies on salivary secretion are usually focused on the large parotid and submandibular glands.[1–3] And yet, the feeling of mouth moistness is associated with the continuous secretion of mucin from minor glands, located just beneath the mucosa and with their secretory ducts opening up at the mucosal surface, rather than with deficits in the watery secretion from the parotid and submandibular glands during meals.[4–7] A novel approach to the treatment of dry mouth by local pharmacological stimulation of the submucosal minor glands, minimizing systemic effects, was suggested from observations in the ferret and its zygomatic gland (also called the orbital gland), using the cholinesterase inhibitor physostigmine as test drug.[8,9] Applied on the oral mucosa, the inhibitor transverses the mucosal layer and prevents the breakdown of acetylcholine released from the intraglandular cholinergic nerve endings, thereby enhancing the cholinergic drive on the secretory cells. In the ferret, as in other carnivores,[10,11] the zygomatic gland, located in a space in the antero-lateral part of the soft palate in the infratemporal fossa, and the molar gland, located opposite to the lower molar teeth, less than a mm beneath the mucosa, are large well-defined mucin-secreting glands suitable for experimental studies.[9,12]

In the present study on the ferret, the cholinergic innervation of the zygomatic and molar glands was investigated and comparisons were made with the more well-studied serous parotid and sero-mucous submandibular glands as well as with the mucous sublingual gland[9,13,14] with focus on: secretory capacity to nerve stimulation, acetylcholine synthesis, muscarinic receptor subtypes and neuropeptides (known to evoke secretion of saliva and/or to potentiate the secretory action of acetylcholine[13]).

2. Materials and methods

2.1. Animals

A total of 32 adult female ferrets, weighing between 0.7 and 1.5 kg, were used. Animals were anaesthetised with pentobarbitone (50 mg/kg intraperitoneal). In case of secretory studies supplementary doses of pentobarbitone were given intravenously as required. The animals were divided into the following study groups: (1) seven animals subjected to unilateral avulsion of the auriculo-temporal nerve and assessment of the acetylcholine synthesis in both parotid glands and in the submandibular, sublingual, zygomatic and molar glands on the non-operated side; (2) four animals subjected to unilateral division of the buccal branch of the mandibular nerve and assessment of the acetylcholine synthesis in the zygomatic and molar glands of both sides (as to molar glands only two animals were examined); (3) five unoperated animals and the assessment of muscarinic subreceptor types in all five types of glands; (4) seven unoperated animals and the assessment of neuropeptide contents in all five types of glands; (5) nine animals and secretory studies on the zygomatic gland and, in addition, in some of these animals on the parotid and submandibular glands as well. The animals, under deep anaesthesia, were killed by exsanguination. Glands were removed, weighed and stored at −70 °C until analysed. The protocols were carried out according to local ethical committee guidelines.

2.2. Preliminary surgery

The animals were anaesthetised with sodium pentobarbitone (25 mg/kg intraperitoneal) and ketamine (50 mg/kg intramuscular). The operations were performed unilaterally. In one group of four animals, the buccal branch of the mandibular nerve was approached from the mouth and cut as it appears between the pterygoid muscles.[15] In another group of seven animals, the auriculo-temporal nerve was avulsed, where it emerges from the base of the skull. The wound was sutured and the animals were allowed to recover. They were examined 7 days postoperatively.

2.3. Secretory studies and nerve stimulation

Under pentobarbitone anaesthesia, the femoral vein was cannulated. A tracheal cannula was fitted. The body-temperature was maintained by an electric blanket at approximately 38 °C, thermostatically controlled by a rectal probe. The lateral duct of the zygomatic gland (draining 80% of the gland[9]), opening on a mucosal ridge postero-medial to the parotid duct, was cannulated from the mouth. The parotid duct was exposed externally on the chin, close to the mouth. The submandibular duct was exposed in the neck, and cannulated anterior to the chorda-lingual nerve. A fine polyethylene tube (outer diameter of 0.61 mm) was inserted into each duct. Saliva appearing was collected in ice-chilled pre-weighed vials usually over 5 min periods; the vials were then re-weighed. The buccal nerve was dissected as it appeared between the pterygoid muscles. The auriculo-temporal nerve was dissected medial of the mandible. The chorda-lingual nerve was dissected as far as possible from the submandibular duct. Each nerve was cut and the peripheral nerve end was stimulated with a ring electrode at 20 Hz (6–8 V) either intermittently in periods usually of 5 min or continuously up to 80 min. The response of the initial 5 min period of stimulation at 20 Hz was used for comparisons between the glands. The stimulations were performed in the presence of the α-adrenoceptor blocker phentolamine and the β-adrenoceptor blocker propranolol (1 mg/kg intravenous of each). When appropriate, atropine (1 mg/kg intravenous) was given to block the muscarinic receptors; the glands involved in this