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Organic secretory products, adaptive responses and innervation in the parotid gland of ferret: A histochemical study

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Summary To qualify cellular events of possible pathophysiological significance in the parotid of ferret, tissue obtained post-mortem from mature animals of either sex was examined by light microscopical histochemistry for calcium, protein, amino acids, mucosubstances and hydrolases, and by neurohistology. Calcium was localised in acinar cells replete with granules containing protein, disulphides and usually carboxylated mucosubstances. Acid phosphatase activity was basally concentrated in the acinar cells. The granular luminal region of striated ductal cells showed protein, tryptophan, disulphides, neutral mucosubstances, and E600-sensitive esterase and Naphthol AS-D chloroacetate esterase activities, whereas their basal region showed acid phosphatase activity. Strong periluminal activity of acid phosphatase and E600-resistant esterase characterised the collecting ducts. Cholinesterase activity was associated with an extensive network of nerve fibres embracing parenchyma. Catecholamine fluorescence was not seen. β-glucuronidase reactive macrophages abounded in the interstices. The results suggest that while the acini in the parotid of ferret secrete polyionic glycoproteins, shielded by calcium, the striated ducts secrete tryptophan-rich products comprising neutral glycoproteins and showing proteolytic activity. Innervation is of the cholinergic type and parenchymal lysosomal activity, possibly related to autophagy of stored secretory products and heterophagy of luminal material, is brisk. Macrophages contribute to maintaining the glandular microenvironment, wherein secretory activity appears to be lethargic.

Introduction

Work in our laboratory with the use of histochemistry and neurohistology has led to interesting observations on secretory events in the submandibular and lingual glands of ferret (*Mustela putorius furo*).[1–4] We have now extended our studies to

Abbreviations: AB, Alcian Blue; DMAB, *p*-dimethylbenzaldehyde; GBHA, glyoxal-bis-(2-hydroxyanil); HID, high-iron diamine; PAS, periodic acid-Schiff

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the parotid gland of that species. An early observation has been that the parotid of ferret often contains microscopic concretions, usually calcified. Similar concretions, known as microliths,[5] occur naturally in human salivary glands and are of significance in the genesis of chronic sialadenitis.[6–8] Natural occurrence of salivary microliths has also been recognised in cat, wherein experimental manipulation of secretion has yielded a wealth of information on their natural history.[9–12] Whilst microliths of man and cat are most commonly found in submandibular and sublingual glands, respectively, they are almost absent from parotid.[6,7,11] The increased occurrence of microliths in the parotid of ferret is therefore exceptional and deserves investigation. This requires a broad approach starting with a careful assessment of the gland using both conventional histology and special histochemical methods. Since microliths contain calcium, relate to autophagy of secretory glycoprotein and are influenced by nerve-mediated secretory activity,[5,7,11,12] we applied light microscopical histochemical techniques for calcium, protein, mucosubstances, lysosomal hydrolases and nerve fibres, in an attempt to qualify the parotid parenchyma. Such qualification should shed some light on the cellular processes influencing the occurrence of microliths in the ferret parotid. The results are reported herein.

Materials and methods

Seven mature ferrets of either sex, that had been fasted overnight, were killed with an overdose of pentobarbitone sodium given intraperitoneally. Pieces of tissue were removed from the apex of 11 parotid glands, which is located between the submandibular salivary gland and submandibular lymph node.[13] They were either immersion fixed in a formaldehyde solution and paraffin-embedded or were quenched in isopentane cooled by solid carbon dioxide.

Sections of paraffin-embedded tissue were stained with haematoxylin and eosin to confirm the identity of parenchymal constituents of the glands.

Sections of quenched tissue were cut at a thickness of 20 μm on a cryostat and were stained with GBHA for ionised and ionisable calcium.[14,15]

The following protein and amino acid histochemical techniques were applied to sections of paraffin-embedded tissue: ninhydrin-pararosanilin (SO_2)-Schiff for protein,[16] the Millon reaction for tyrosin,[16] the DMAB nitrite reaction for tryptophan, and the coupled maleimide reaction to demonstrate thiol groups staining blue and disulphide groups staining red.

The following mucosubstance-histochemical techniques were applied to sections of paraffin-embedded tissue: AB at pH 2.5 followed by PAS to demonstrate neutral mucosubstances staining red (periodate-reactive) and acid mucosubstances staining in varying shades of purple and royal blue (periodate-reactive and variably Alcianophilic), and the HID technique followed by AB at pH 2.5 to demonstrate sulphated mucosubstances staining brown or black (HID-reactive) and nonsulphated carboxylated mucosubstances staining sky blue (Alcianophilic).

Techniques for the following hydrolytic enzymes were applied to sections of quenched tissue: acid phosphatase, β-glucuronidase, and non-specific esterase with and without E600 as inhibitor. In addition, esteratic activity was sought in 5 μm thick sections of paraffin-embedded tissue incubated with the substrate Naphthol AS-D chloroacetate.[17]

The following neurohistological techniques were applied to sections of quenched tissue: cholinesterase with and without eserine as inhibitor to survey cholinergic-type innervation, and the glyoxylic acid method to demonstrate catecholamine fluorescence.

Details of the preservation and histochemical methods, unless otherwise specified, are reported elsewhere.[1–3]

Results

Histology

The glandular parenchyma was arranged in lobules that were widely separated by fibrofatty stroma with neurovascular bundles. Acini of uniform appearance, intercalary ducts and striated ducts comprised the lobules. The acini featured a tiny central lumen surrounded by basophilic pyramidal cells with variably distinct borders, a prominent luminal region replete with granules and a basally aligned rounded or ovoid and variably dense nucleus with a fine nucleolus (Fig. 1a). The intercalary ducts were inconspicuous (Fig. 1a). The cells lining the obvious lumens of striated ducts were columnar with distinct borders, and showed a prominent luminal region containing palely stained or lightly eosinophilic granules and a narrow basal eosinophilic region containing the densely stained nucleus (Fig. 1b). At the periphery of lobules, the striated ducts extended for a varying distance into the interstices and there was a progressive change to double layered collecting ducts (Fig. 1c). The inner layer of the collecting ducts consisted of cuboidal cells resembling those of striated ducts but shorter;