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Comparative histochemistry of posterior lingual salivary glands of mouse

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ABSTRACT

Normal posterior deep and superficial salivary glands of tongue were examined in male mice by means of light microscopical histochemistry and neurohistology. Both glands showed acini and simple ducts. Demilunes were present in the superficial gland. Disulphides and neutral mucosubstances occurred in acini and demilunes. Tryptophan staining was seen in acini of the deep gland and demilunes, whereas acid mucosubstances were exclusively localised in the superficial gland. Dehydrogenase activities were widespread. Strong esterase activity occurred throughout the parenchyma of the deep gland and in demilunes; it was variably inhibited by E600, apart from acinar apical regions in the deep gland. Lipase was confined to acini of the deep gland and demilunes. Acid phosphatase staining was similarly localised; it was also seen in periluminal ductal rims of the deep gland, in which ouabain-sensitive Na,K-ATPase was localised basolaterally. Staining for alkaline phosphatase decorated occasional myoepithelial-like arrangements and interstitial capillaries. Acetylcholinesterase was associated with nerve fibres embracing glandular parenchyma. Adrenergic fibres were not seen. The results suggest that the acini of the posterior deep lingual gland secrete neutral glycoproteins, whereas the ducts transport ions and absorb luminal material. The posterior superficial lingual gland mainly secretes acid glycoproteins. Both glands produce lingual lipase, receive cholinergic-type innervation and have inconspicuous myoepithelium.

1. Introduction

The deep and superficial, minor salivary glands in the posterior part of the tongue, which were identified by von Ebner and Weber respectively, are of significance in digestion, taste and protection of taste buds (Nagato et al., 1997). They have been variously investigated in small carnivores and bats (Poddar and Jacob, 1980; Tandler et al., 1997; Triantafyllou et al., 1999); and intensely in man and rat (see: Hand et al., 1999; Redman, 2012). Less attention has been paid to the posterior lingual glands of mouse (*Mus musculus*), which are useful for studying glandular sexual dimorphism (Hanker et al., 1980) and harbouring amastigotes in experimental American trypanosomiasis (Lopes et al., 1991a, 1991b). Burstone (1953) applied early histochemical techniques to localise mucosubstances and nucleic acids in those glands; and the use of autoradiography, various sequences of basic dyes and *vic*-glycol methods in conjunction with pre-digestion and blocking of reactive moieties, and lectins enabled a more refined characterisation of carbohydrate residues therein (Spicer and Duvenci, 1964; Stoward et al., 1980; Schulte and Spicer, 1983). A more detailed histochemical profiling is, however, desirable. This prompted the present investigation wherein posterior lingual glands of male mouse were examined with the use of histological, protein, mucosubstance and enzyme histochemical, and neurohistological techniques, which have been found valuable while exploring minor salivary glands in other species (Harrison, 1974; Triantafyllou et al., 2001).

2. Materials and methods

2.1. Animals, glands and preservation

Five mature male CD1 mice that had been fasted overnight and killed by neck dislocation (schedule 1) as part of an *in vitro* investigation (Smith et al., 2000), became available. Surplus tissues were donated for additional investigations to ensure best use of the material. Ethical committee approval was not required.

The tongues of the mice were rapidly removed and cut into transverse, frontal slices. Posteriorly situated slices including the vallate papilla were further bisected. Halves were immediately quenched in isopentane cooled by solid carbon dioxide and then

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stored at −70 °C. Other halves were immersion fixed in a formaldehyde solution and then processed for paraffin blocks.

2.2. Histology

Sections of fixed tissue were cut at a thickness of 5 mm and were stained with haematoxylin and eosin.

2.3. Protein and amino acid histochemistry

Sections of fixed tissue were cut at a thickness of 5 mm and were stained with the *p*-dimethylbenzaldehyde nitrite reaction for tryptophan (Adams, 1957) and the coupled maleimide reaction for thiol groups (staining blue) and disulphide groups (staining red) (Sippel, 1978).

2.4. Mucosubstance histochemistry

Sections of fixed tissue were cut at a thickness of 5 mm and were stained with alcian blue at pH 2.5 followed by periodic acid-Schiff 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) (Mowry, 1956; Spicer et al., 1967). The high-iron diamine technique followed by alcian blue at pH 2.5 was used to demonstrate sulphated mucosubstances staining brown/black (high-iron diamine-reactive) and non-sulphated carboxylated mucosubstances staining sky blue (alcianophilic) (Spicer, 1965).

2.5. Enzyme histochemistry

Sections of quenched pieces were cut at a thickness of about 10–12 mm on a cryostat and were incubated with substrates for the following oxidative enzymes: peroxidase with and without KCN to inhibit peroxidatic responses of respiratory enzymes (Graham and Karnovsky, 1966; Garret and Kidd, 1976), cytochrome oxidase (Kugler et al., 1988), succinate dehydrogenase (Nachlas et al., 1957), lactate dehydrogenase (Bancoft and Hand, 1987), and NADH and NAD(P)H dehydrogenases (Scarpelli et al., 1958).

Cryostat sections were also incubated with substrates for the following hydrolytic enzymes: thiamine pyrophosphatase (Novikoff and Goldfischer, 1961), alkaline phosphatase (Stutte, 1967), Na,K-ATPase with and without ouabain to inhibit activity (Mayahara and Ogawa, 1988), acid phosphatase (Barka and Anderson, 1962), β-glucoronidase (Hayashi et al., 1964), non-specific esterase with and without E600 inhibitor (Davis, 1959; Bancoft and Hand, 1987), and lipase with and without sodium taurocholate activator (Triantafyllou et al., 2002).

2.6. Neurohistology

Sections of quenched pieces were cut at a thickness of about 10–12 mm on a cryostat and were incubated for cholinesterases with and without eserine to inhibit activity (Karnovsky and Roots, 1964).

In addition, cryostat sections of 30 mm thickness were treated by the glyoxylic acid method for catecholamines and were examined by fluorescence microscopy (de la Torre and Surgeon, 1976).

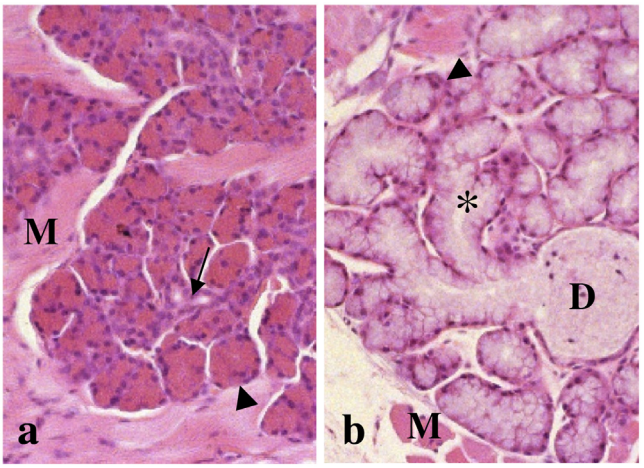

Fig. 1. Paraffin sections stained with haematoxylin and eosin. (a) Deep (von Ebner) gland; collections of acini (arrowhead) surround ducts (arrow) and are between skeletal muscle fibres (M). (b) Superficial (Weber) gland; tubulo-acini (asterisk), demilunes (arrowhead) and dilated duct (D) draining from two tubules. Objective magnification ×20.

3. Results

3.1. Histology

The parenchyma of both glands was arranged in non-encapsulated lobular clusters interspersed between bundles of skeletal muscle (Fig. 1).

The deep (von Ebner) gland showed acini and few intralobular ducts. The acini showed inconspicuous lumina and were composed of usually pyramidal cells with variably distinct borders, a prominent apical region replete with strongly eosinophilic granules and a basally aligned rounded vesicular nucleus with dispersed chromatin and inconspicuous nucleoli. The ducts were lined by simple small cuboidal cells with scanty, agranular, eosinophilic cytoplasm and central nuclei with dispersed chromatin (Fig. 1a).

The superficial (Weber) gland showed acini and tubulo-acini opening into occasionally dilated, intralobular ducts lined by flattened epithelium. The acini and tubulo-acini were of a mixed appearance showing palely stained central cells with basal flattened nuclei, which were often capped by small, more densely stained demilunes (Fig. 1b).

3.2. Protein and amino acid histochemistry

Variably intense, disulphide staining was localised in the apical region of acini of the deep gland, and in central cells and demilunes of the superficial gland; the remainder of the parenchyma showed moderate diffuse thiol staining (Fig. 2a and b).

Moderate tryptophan staining was confined to acini and periluminal ductal rims of the deep gland, and demilunes of the superficial gland (Fig. 3a and b).

3.3. Mucosubstance histochemistry

Neutral mucosubstances were present in both glands. Acid mucosubstances were localised in the superficial gland only (Figs. 4 and 5).

The demilunar cells showed considerable variation in the staining with the high-iron diamine–alcian blue technique (Fig. 5). A few cells had a strong affinity for alcian blue alone, whereas others had a varying affinity for high-iron diamine. In some cells the affinities coexisted. The central cells were constantly stained by periodic acid–Schiff, and showed variable alcianophilia and moderate affin-

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