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Histochemical study on the intestine goblet cells in cichlid and poecilid species (Teleostei)

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ABSTRACT

Histochemical properties of intestine goblet cells in firemouth cichlid, zebra mbuna, freshwater angelfish and platyfish are described. Goblet cells occurred regularly in the epithelial cell layer throughout the entire intestine, they were strongly coloured by alcian blue at pH 2.5. This colour got gradually weaker when the pH was reduced, but still after alcian blue at pH 0.2 these cells displayed a distinct blue colour. When the goblet cells were treated with periodic acid-Schiff (PAS), they displayed a strong purple-magenta colour. The findings that a number of goblet cells displayed various colours between blue and purple-magenta when acidic alcian blue was followed by PAS, and between blue and red-brown when acidic alcian blue was followed by neutral red, may reflect different ages or stages of development and differentiation for these cells. However, such results may also suggest a true cellular heterogeneity in the present population of goblet cells, reflecting that the intestine mucus layer has a number of roles in teleosts like lubrication, protection, immunological defence, digestion and absorption.

In the ferritin injected specimens of firemouth cichlid and platyfish, a number of macrophage-like cells in intestine wall displayed Prussian blue precipitations in tissue treated with acid ferrocyanide, suggesting that these cells play a cleansing role in the intestinal wall. No ferritin uptake was seen in the intestine goblet cells and eosinophilic granule cells.

1. Introduction

Recently, the amount and composition of the mucin throughout the digestive tract in teleosts have been investigated by means of various histochemical techniques in species of evolutionary old as well as in modern teleosts (cf. Sarasquete et al., 2001; Diaz et al., 2003, 2006).

Firemouth cichlid, freshwater angelfish and zebra mbuna, family Cichlidae, are freshwater teleosts belonging to the order Perciformes, which originated about 15 million years ago (Colbert and Morales, 1991; Helfman et al., 1997; Nelson, 2006). Firemouth cichlid and freshwater angelfish are native to Central America, whereas zebra mbuna has originated in Lake Malawi, Africa (Lee et al., 1980; Konings, 1990; Page and Burr, 1991). Thus zebra mbuna is endemic to this lake, closely adapted to areas with rocky bottom, feeding on various particles from the biocover on the ground or on plankton particles in the open water (Konings, 1990).

Freshwater angelfish inhabits mainly swamps or flooded grounds with dense aquatic and riverine vegetation, feeding mainly on small fish and invertebrates, whereas firemouth cichlid prefers slow-moving waters in canals and ponds, feeding mainly on algae (Lee et al., 1980). Platyfish, family Poeciliidae, belongs to a much older teleost order, Cyprinodontiformes, which originated about 45 million years ago (Colbert and Morales, 1991; Helfman et al., 1997; Nelson, 2006). It is a freshwater species native to Central and North America, where it occurs in canals and ditches with slow-moving water, feeding mainly on small animals and plant matter (Allen et al., 2002).

The aim of the present study was to reveal and compare types of goblet cells and mucus in the epithelial cell layer throughout the intestine in species from both an evolutionary young and an older teleost order. We also intended to relate the present findings to the diet and feeding habits of each species, and to compare the results with those previously published about intestinal goblet cells and mucus in teleosts belonging to very old orders like Siluriformers, originating about 150 million years ago (Carroll, 1988; Colbert and Morales, 1991; Nelson, 2006).

It is widely held that the intestine from small teleosts is often damaged when using standard fixatives. We therefore also aimed to find a fixative with high ability to preserve the various details in this type of delicate tissue.

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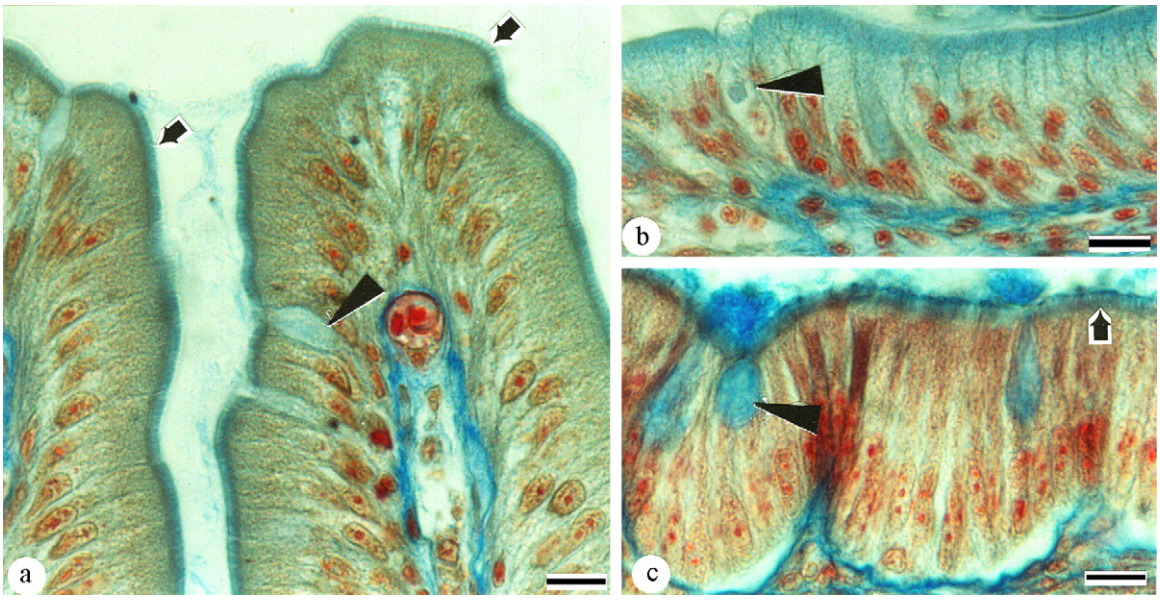

Fig. 1. Platyfish intestine fixed in ethanol–formaldehyde and stained with Heidenhain's Azan. (a) Epithelial cell layer in anterior intestine. The brush border (arrows) is thick and well preserved. A goblet cell (arrowhead) occurs between ordinary epithelial cells. Scale bar: 20 μm. (b) Epithelial cell layer in posterior intestine, filled by numerous supranuclear vacuoles. The brush border is poorly developed. Goblet cell (arrowhead). Scale bar: 20 μm. (c) Epithelial cell layer in anterior intestine of 6 mm larvae. Brush border (arrow) and goblet cells (arrowhead) are well developed. Scale bar: 20 μm.

2. Materials and methods

Fifteen specimens of firemouth cichlid (*Thorichthys meeki*) and platyfish (*Xiphophorus maculatus*) and five specimens of zebra mbuna (*Maylandia zebra*) and freshwater angelfish (*Pterophyllium scalare*), 1–3 years old, mass about 2–5 g, kept in well aerated aquaria at 21–25 °C were used in this study. In addition 10 platyfish prenatal larvae, total length about 6 mm, were used.

The fishes were killed with an overdose of chlorobutanol, the intestine was sectioned into three parts with equal lengths: the anterior, middle and posterior intestine, and fixed at 4 °C for a week in 4% formaldehyde, made up from paraformaldehyde 24 h before use, in phosphate buffer (pH 7.4); alternatively in a fixative composed of 100% ethanol, 37% formaldehyde and 100% acetic acid in a volume ratio 85:10:5 (Leknes, 1980; Reite, 1996). The platyfish larvae were treated with the same fixative as the adults.

After cleansing in buffer or ethanol depending on the fixative, the tissues were dehydrated in ethanol, treated with xylene, embedded in paraffin wax and sectioned.

Dewaxed sections (4 μm) were treated with Mayer's haemalum and eosin, Heidenhain's azan, alcian blue (pH 2.5, 1, 0.5 or 0.2) and/or periodic acid-Schiff (PAS) solution followed by Mayer's haemalum or neutral red followed by eosin (Grimstone and Skaer, 1972; Pearse, 1980).

Four specimens of firemouth cichlid and platyfish were injected intraperitoneally with 0.03–0.05 ml 10% solutions of horse-spleen ferritin (Sigma) by means of 0.5 ml tuberculin syringes (Becton Dickingson). After 8 h, the fishes were killed as described above, and the intestine was incubated in buffered fixative, dehydrated, embedded and cut. Dewaxed sections (4 μm) were treated with a ferrocyanide solution, made by dissolving 2 g potassium ferrocyanide in 100 ml 0.75 M hydrochloric acid solution, in order to visualize ferric ions, and then stained in 1% aquatic solution of neutral red followed by 1% aquatic solution of eosin (Pearse, 1980). Control sections of intestine from uninjected firemouth cichlid and platyfish were also treaded in this way.

The number of injected specimens was low, the injection needles were thin and the amount of injected ferritin was small in accordance with the established national ethical guidelines, rules and legislations for this type of experiments (Leknes, 2007).

3. Results

The epithelial cell layer in intestine from firemouth cichlid (*Thorichthys meeki*), zebra mbuna (*Maylandia zebra*), freshwater angelfish (*Pterophyllium scalare*) and adult and larval platyfish (*Xiphophorus maculatus*) was much better preserved in ethanol–formaldehyde than in the traditional buffered formaldehyde solutions (Figs. 1a and 2a). In particular, details in the epithelial brush border, nucleus and supranuclear vacuoles are distinctly seen in tissue fixed in this way (Fig. 1a–c).

The present cichlids contained a short, intestine-shaped stomach whereas platyfish was stomach-less. The anterior part of the intestine in the present cichlids and poecilid was much wider, had a thicker brush border and contained a more folded epithelial cell layer than in middle and posterior intestine (Fig. 1a and b). The epithelial cell layer in posterior intestine nearly lacked brush border, but contained numerous supranuclear vacuoles, except in the platyfish larvae (Fig. 1b). Goblet cells occurred frequently in the epithelial cell layers throughout the entire intestine, included in the platyfish larvae (Figs. 1a–c and 2a). Normally, there occurred some capillaries and lymphocytes in the subepithelial connective tissue, i.e. in lamina propria. Eosinophilic granule cells were seen regularly in this layer throughout the entire intestine, particularly in firemouth cichlid and adult platyfish, and usually in close proximity to capillaries (Fig. 2g). Generally, the total thickness of the lamina propria and inner circular and outer longitudinal intestine muscle layers was about the half the thickness of the intestine epithelial cell layer in the present species.

The colouring-reactions described below were most prominent and distinct in tissue fixed in buffered formaldehyde (Table 1). The goblet cells were strongly coloured by periodic acid-Schiff (PAS) solutions (Fig. 2b). These cells were also strongly coloured by alcian blue at pH 2.5; this colour was somewhat fainter when pH was reduced, but still at pH 0.5 or 0.2 these cells were distinctly coloured by this dye (Fig. 2f). When the alcian blue pH