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On endocytosis of foreign ferritin and occurrence of phagolysosomes in fish heart endothelial cells

Ingvar Leiv Leknes

Faculty of Teacher Education and Sport, Sogn og Fjordane University College, Box 133, N-6851 Sogndal, Norway

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ABSTRACT

In the present study the ultrastructure and function of the endothelial cells enveloping the muscle trabeculae in heart in two teleosts, platyfish and firemouth cichlid, are described and discussed. These cells displayed a structure making them able to take up large amounts of foreign ferritin particles from the blood stream. The ferritin particles were assembled into huge phagolysosomes. Large amounts of Prussian blue were precipitated throughout these lysosomes when treated with acid ferrohexacyanide solution. The occurrence of Prussian blue precipitations in the control heart endothelial cells after Schmorl's solution, suggests that these cells normally contain undigestible material, a finding which strengthens the view that this tissue is involved in blood clearance in the present species. In conclusion, these heart endothelial cells seem able to perform a very efficient blood clearance of scavenger and foreign macromolecules and particles in the present species.

1. Introduction

The endothelial cell layers enveloping the muscle trabeculae in the spongy heart wall of gadoids and other modern teleosts are suggested to play important roles in the clearance of scavenger and foreign macromolecules and particles from the blood stream (Sætersdal et al., 1974; Ferguson, 1975; Lemanski et al., 1975; Leknes, 1980, 1983, 1987, 2011; Nakamura and Shimozawa, 1994). Modern teleosts like wild and farmed gadoid and perciform species play a very important role as food supply for a rapidly growing human population, and detailed knowledge about the blood clearing mechanisms in such fish is therefore of great value and interest. The order Perciformes alone consists of about 12 000 species, i.e. about 40% of all known teleostean species today (Nelson, 2006).

The main aim of the present work was therefore to reveal more exactly the capability and capacity of the endothelial cell layers in the spongy heart wall of platyfish (*Xiphophorus maculatus*) and firemouth cichlid (*Thorichthys meeki*), i.e. species from the teleost orders Cyprinodontiformes and Perciformes, respectively, to take up foreign ferritin particles from the blood stream. Perciformes evolved about 15 million years ago, whereas Cyprinodontiformes emerged about 30 million years earlier (Nelson, 2006). Thus, a goal for this study was to compare the blood clearance in heart from two teleosts which are regarded to have developed a number of modern characters, but appear unrelated in evolutionary terms.

This study also intended to find out how foreign ferritin particles are taken up, transported, treated and stored by this type of cells. Finally, it was also an aim to try out methods which make it practical possible to study the endocytic capability and the number and size of phagolysosomes in these cell layers throughout a large volume of heart tissue in an optical microscope.

2. Materials and methods

Fifteen specimens of platyfish (*X. maculatus*, Günther) and fifteen specimens of firemouth cichlid (*T. meeki* Brind), 1–3 years old, 1.5–2.0 g in weight and 4.0–5.0 cm in length, supplied by a local aquarium store, were used in this study. They were kept in two well-aerated glass aquaria ($30 \times 35 \times 60\,cm^3$) at 26 °C, and regularly fed with TetraMin (Tetra Werke, Melle, Germany). Eight specimens from each species were injected intraperitoneally with 0.02–0.03 ml of a 10% solution of horse-spleen ferritin (Sigma, St. Louis, MO, USA) by means of 0.5 ml tuberculin syringes (Becton Dickinson and Company, Franklin Lakes, New Jersey, USA). After ¼, 6, 8, or 24 h fish were euthanized with an overdose of 0.5% chlorobutanol.

Hearts from four injected specimens from each species and from four uninjected control specimens from each species were fixed at 0 °C for 20 h in 2 parts 0.2 mol/l cacodylate buffer (pH 7.2), 6 parts modified Ringer's solution (Leknes, 1980), 1 part 2.50 mol/l

E-mail address: ingvar.leknes@hisf.no

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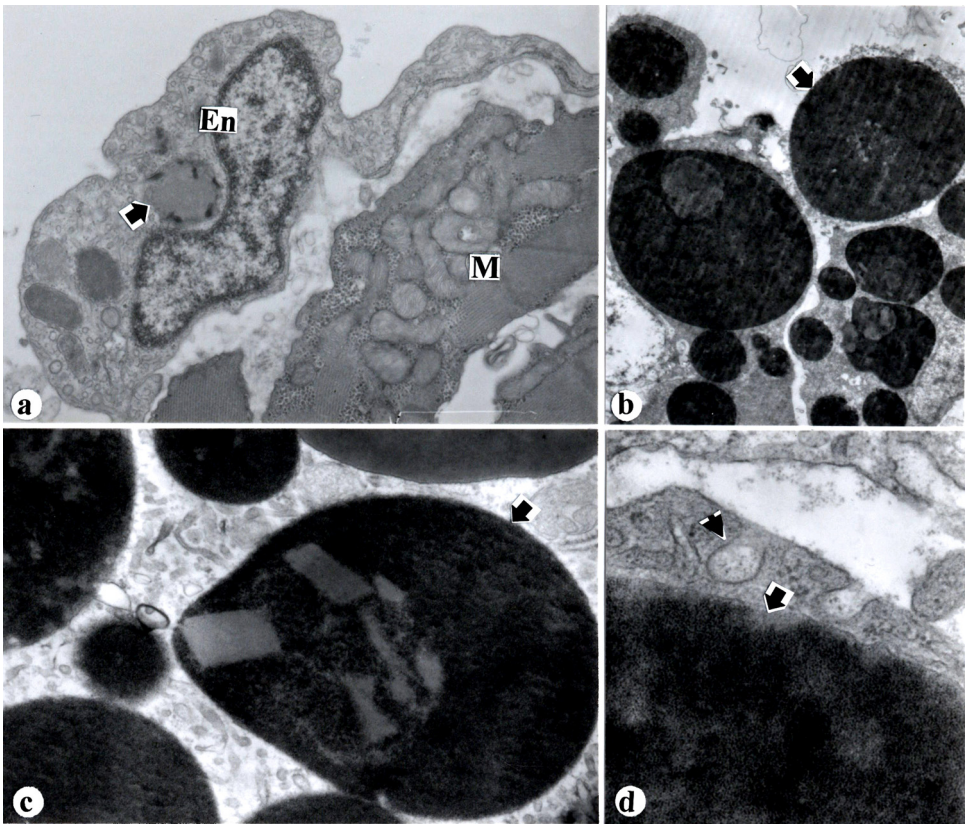

Fig. 1. (a) Part of endothelial cell layers enveloping a muscle trabecula in heart ventricle from firemouth cichlid (*Thorichthys meeki*). The endothelial cell (En) contains several moderate dense bodies (arrow) and clathrin-coated vesicles (left, lower corner of picture), but is elsewhere strongly attenuated. The muscle cell (M) contains a core of mitochondria. ×5200. (b–d) Part of endothelial cell in heart atrium from platyfish (*Xiphophorus maculatus*) euthanized 24 h after intraperitoneal injection of horse-ferritin. The originally moderate dense bodies have increased much in both size and electron density (arrows), as they are packed by huge numbers of ferritin particles, each with an electron dense core of iron ions. The individual ferritin particles are seen in (d) in the greatly enlarged phagolysosome (arrow). In addition, several particles with size and electron density similar to ferritin cores, occur in the two clathrin-coated pits (arrowhead) depicted in (d). ×16,600, ×32,000 and ×66,000, respectively.

glutaraldehyde and 1 part 3.33 mol/l formaldehyde (made up from paraformaldehyde 24 h before use). The present Ringer's solution was composed of: 152 mM Na^+, 1.9 mM K^+, 1.1 mM Ca^{2+}, 154 mM Cl^-, 2.4 mM HCO_3^-, 14.1 mM glucose, 14,6 mM sucrose. After washing in a mixture of buffer and Ringer's solution, the tissue was post-fixed at 0 °C for 3–4 h in 0.0526 mol/l OsO_4 in 0.2 mol/l cacodylate buffer, pH 7.2 (Grimstone and Skaer, 1972; Culling, 1974; Bancroft, 2008). It was then cleansed in buffer, followed by distilled water, dehydrated at 0 °C through a graded acetone series, embedded in Epon plastic and sectioned (Hayat, 1972). Ultrathin sections were layed on formvar films on 3 mm wide copper grids, stained with 2% uranyl acetate for 1 h, and with lead citrate for 15 min (Reynolds, 1963; Hayat, 1972), were viewed with a Jeol (JEM-100CX) transmission electron microscope operated at 80 kV.

Hearts from four injected specimens from each species and from three uninjected control specimens from each species were fixed at 4 °C in 1.333 mol/l formaldehyde, made from paraformaldehyde within 24 h before use, in phosphate buffer, pH 7.4 (Leknes, 1980). After cleansing in buffer, the tissues were dehydrated through a graded ethanol series, treated with xylene, embedded in paraffin wax and sectioned (thickness 4 μm). Dewaxed sections were incubated with a solution made by dissolving 2 g potassium ferrohexacyanide in 100 ml 0.75 mol/l hydrochloric acid solution, in order to visualize ferritin iron ions (Pearse, 1985). Then, the sections were treated with 1% aqueous solution of neutral red followed by 1% aqueous solutions of eosin, dedydrated through a graded ethanol series and mounted under cover slips in Sigma Mountex medium or Merck Neo-Mount medium (Merck, Darmstadt, Germany) (Grimstone and Skaer, 1972; Culling, 1974; Bancroft, 2008).

The injection needles were thin and the amount of injected ferritin was small in accordance with established national ethical guidelines, rules, legislations and approvals for this type of experiments (Leknes, 2015).

Some sections of the control hearts were dewaxed by xylene and incubated in Schmorl's solution, composed of 75 ml 1% ferric chloride, 10 ml 1% potassium ferrichexacyanide and 15 ml distilled water, treated with 1% aqueous solution of neutral red followed by 1% aqueous solution of eosin, dehydrated in graded ethanol series and mounted under cover slips in Sigma Mountex medium or Merck Neo-Mount medium (Grimstone and Skaer, 1972; Culling, 1974; Pearse, 1985; Bancroft, 2008).

3. Results

3.1. Electron microscopic level

The entire heart wall in platyfish (*X. maculatus*) and firemouth cichlid (*T. meeki*) was spongious, composed of 10–15 μm thick muscle trabeculae enveloped by an endothelial cell layer, which was up to 4–6 μm thick in atrium and flat in ventricle (Fig. 1a). This endothelium contained numerous lysosome-like moderately dense bodies (MDB, diameter up to 2 μm), clathrin-coated pits and vesicles and tubules of agranular endoplasmic reticulum in both species (Fig. 1a). In heart from ferritin-injected specimens, euthanized 6, 8 or 24 h after the injection, the endothelial cells enveloping the muscle trabeculae, were filled by granules, width 3 μm and more,