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ORIGINAL ARTICLE

Morphology and changes of chloride cell of *Rutilus rutilus Caspicus* (Cyprinidea, teleost) in Caspian sea

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Abstract An ultrastructural study was performed on chloride cells of euryhaline R.r. Caspicus of south of Caspian Sea. The chloride cells are distributed in the interlamellar region of filaments. They are oval to elongated form with an apical positioned nucleus, expanded tubular system and heteromorphic mitochondria. These cells are surrounded by pavement cell and accessory cell. A small and depressed surface formed by pavement cells is in contact with the aquatic milieu. There is also channel system in accessory cells. One of the typical features was the important changes in microtubules and mitochondria of chloride cells in some fishes. Swelling and rupture of cristae and degeneration of microtubules were from these changes.

Keywords Gill · Fish · Chloride cells · Rutilus · Rutilus · Caspicus

Introduction

The Rutilus rutilus Caspicus (Talaji in Mazandarani), belongs to cyprinidea family is one of the most important endemic species of fishes in south of Caspian Sea.

This species is eurytopic, living in rivers, streams, lakes, reservoirs and fresher parts of seas. Because of the variability in structure of tissues among different fish species (Wilson and Laurent 2002), clarification of the normal histology of a particular species is important to interpret the morphological and functional modifications occurring under pathological conditions. The gills are exposed to direct contact with pathogenic agents that can enter the aquatic milieu as a result of natural occurrence or anthropic action (Austin 1999) and caused by ventilatory movements, a particularly high water volume bypasses the large gill

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surface. Therefore, the gills are one of the most frequently affected organs under these circumstances (Lindesjoo and Jhulin 1994; Vigliano et al. 2006). Since relevant physiological processes take place in the gills, such as gas exchange, osmoregulation, excretion of nitrogenous waste products and acid-base balance, disturbance of the structure of this organ compromises the survival of fish. In addition, the gills represent an appropriate model for the study of the potention environment effects on the fish organism and, indirectly an indicator of degree of environmental contamination.

The aim of the present study was to study using transmission microscopy, the structure of chloride cells of gill epithelia in R.r.Caspicus under seawater conditions in south of of Caspian Sea to a salinity of 12/48–12/64 gr/lit.

Materials and methods

Six speciemens of rutilus rutilus caspicus ranging in body mass from 800–1500 gr were caught from south of Caspian sea. The gill arches were dissected out and fixed by immersion in 2% phosphate-buffered glutaraldehyde for 24 h. After rinsing with 0/1 M PB, the speciemens were postfixed with 1% osmium tetroxide in 0/2 M PB for 1 h. After rinsing with PB and dehydration with ethanol and acetone, the speciemens were infiltrated and embeded in resin. Ultrathin sections were cut with glass or diamond knives in an ultramicrotome (Leica ultracut R).

Sections were mounted on grids, double stained in Uranyl acetate and lead citrate and examined with a transmission electron microscope (TEM, LEO).

Results

The gills of R.r.Caspicus consist of two sets of four holobranchs, each of them situated on either side of the pharynx in the right and left opercular cavities. Each holobranch is composed of an arch showing caudal convexity. From which two rows of branchial filaments project caudolaterally. The lamellae radiate out from the dorsal and ventral surfaces of each filament..

The chloride cells were most frequently observed in interlamellar regions of the filament and at the base of the lamellae (Fig. 1).

These cells are oval to elongated in shape and shown an elliptical euchromatic nucleus, apical positioned and an abundant cytoplasm. They are surrounded by the pavement cells and accessory cells (Fig. 2). The apical plasma membrane of Cc is covered by lateral cytoplasmic projections of adjacent pavement cell. So a small area of the cell is exposed to the aquatic milieu (Fig. 3). The most important feature of chloride cells is the great abundance of mitochondria and intracellular tubular system. The profile of mitochondria varied from round or ovoid to elongated with electro-dense matrix and abundant tubular crystae (Fig. 4). The extensive tubular system consisting of a network of tubules which connected with basolateral plasma membrane and mitochondria (Figs. 3, 4). An accessory cell is also next to chloride cell, and the two cells share a shallow junction. The most distinctive feature of AC is the presence of some widened channels. These channeles which are originated from the plasma membrane appeared to be in continuity with intercellular area (Fig. 5).

One favorite point in this study was the changes in chloride cells of three specimens. So some cells exhibite signs of sever damage in microtubules and mitochondria (Fig. 6).

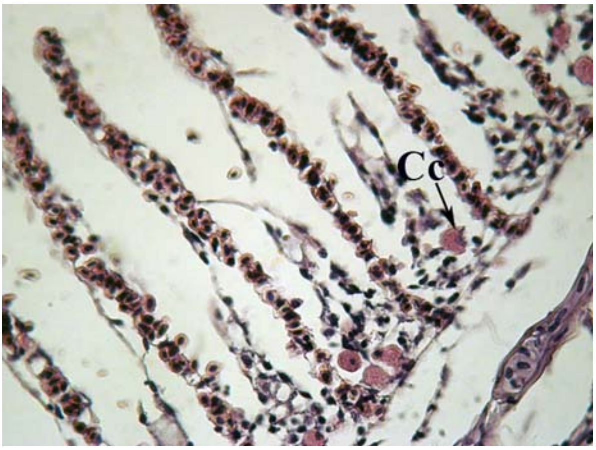

Fig. 1 Gill filament showing lamellae and chloride cells are at the base of lamellae as eosinophilic cells

Discussion

The gill in fishes, are the main osmoregulation organ that is highly sensitive to many factors, including changes in salinity, pollution and stress (Kuitz et al. 1995; Uchida et al. 2000). Importance of the role of gills on gas exchange (Hughes 1984), ion and acid-base equilibrium (Evans 1980), and nitrogen excretion (Haswell et al. 1980) is proportional to their susceptibility to changes in the environment.

Gill chloride cells, appear mostly in the epithelia of filamens, and when necessary may appear on lamellae (Sakamoto et al. 2001). In R.r.Caspicus, also this cell appears mostly on the interlamellar region and near the base of the secondary lamellae. This has been observed in many other teleosts (Wilson and Laurent 2002) of both seawater (Sw) and freshwater (Fw) adapted fishes. However, in many species, Brachdanio rerio (Isisag and Karakisi 1998), Oreochromis mossambicus (Kuitz et al. 1995), Solea solea (Dunel and Laurent

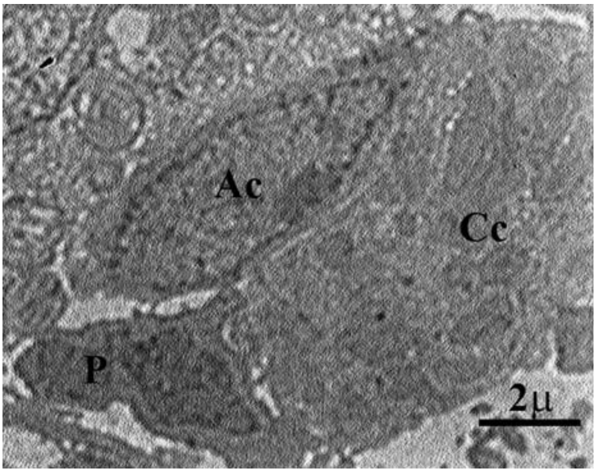

Fig. 2 General appearance of chloride cell (Cc) covered by pavement cell (P) Accessory cell are in close contact with chloride cell

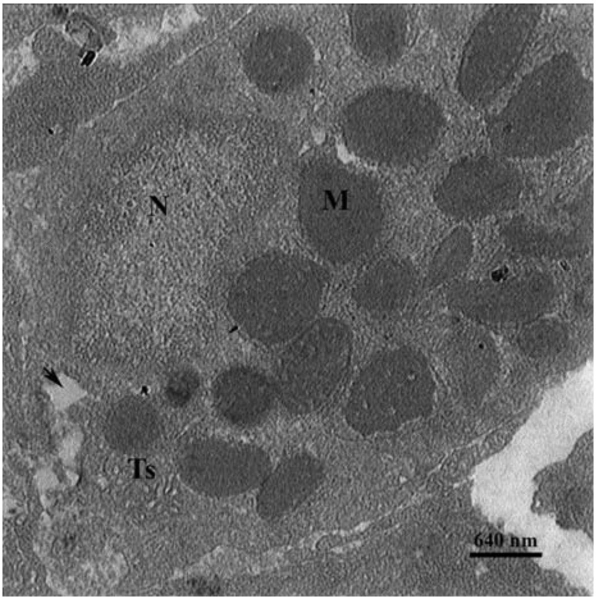

Fig. 3 An electron micrograph of a chloride cell. An euchromatic nucleus positioned apical cell. Note the tubular network (Ts) and apical crypt in this cell (arrowhead)

1980), Onchorhynchus mykiss (Bindon et al. 1994) and euryhaline fish (Sakamoto et al. 2001; Varsamos et al. 2002) it is on both in the filament and lamellar epithelia. The occurrence of lamellar chloride cell is thought to satisfy the physiological demand of ion uptake in some euryhaline teleosts (Sasai et al. 1998; Hirai et al. 1999) but not in others (Lin and Sung 2003).

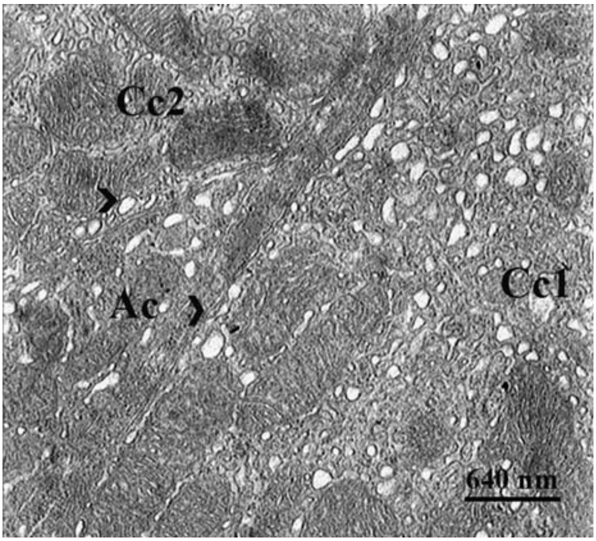

Fig. 4 this micrograph show two chloride cell and the relationship between these cell and accessory cell (Ac) Tubulo-vesicular network comes into close contact with mitochondria (arrowhead) and accessory ce

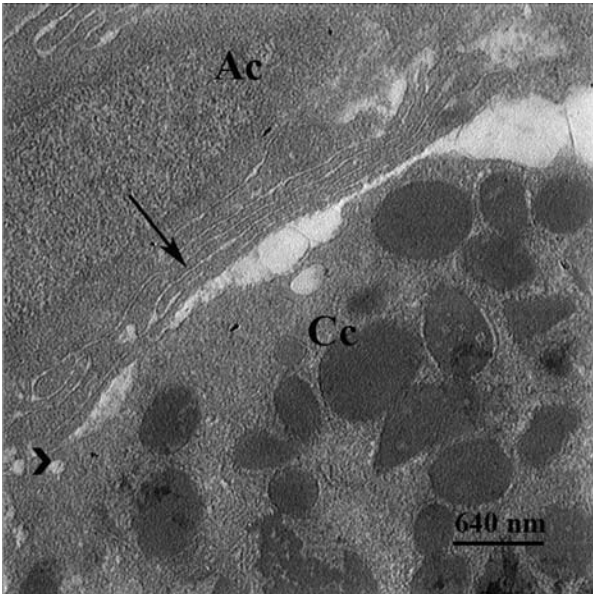

Fig. 5 The channels of accessory cells forming intercellular channel system (arrow). Note the relation between vesicles of chloride cell and intercellular area

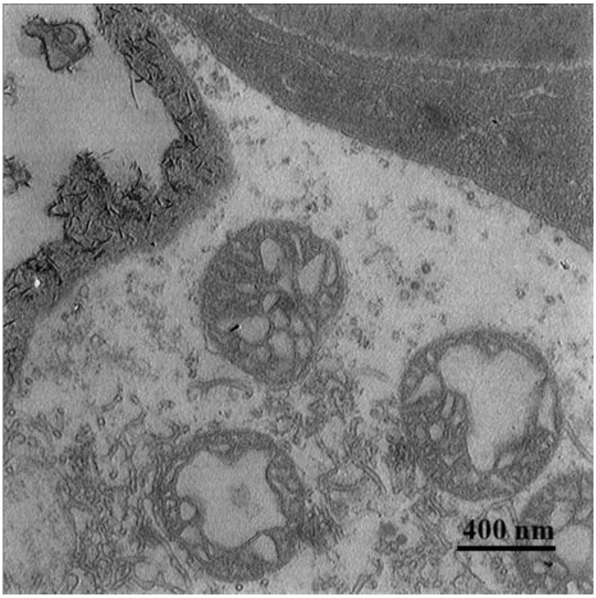

Fig. 6 Ultrastructural changes in chloride cells displayed a necrosis. Mitochondria displayed swelling, matrix vacuolization and lysis of cristae. Microtubules are also distrupted

Chloride cells, are mitochondrial rich cells exhibited the ultrastructural features typical of this cell population which is mainly involved in the active transport of ions (Laurent and Perry 1991). For this function, they are endowed with a large amount of mitochondria, an extensive network of tubules and vesicles and invaginations of basolateral membrane for widening ionic exchange area. The apical surface of this cell has different features in species. In this study chloride cells have crypts and invaginations in contact with the free surface. Although this characteris is typical for marine teleost (Laurent and Dunel 1980; Olson 1996, 2000), this can occurs when a euryhaline species passes from fresh to saltwater (Fishelson 1980; Wendelaar Bonga and Van der Meiji 1989).

Another typical feature of these cells indicates that there is one type of chloride cell. Ultrastructural studies of other fishes, have found more than one type of chloride cell, distinguished by different electrodensity of their cytoplasm (Wendelaar Bonga et al. 1990) or by their different in apical membrane characteristics (Pisam et al. 1995). The larger with lower mitochondria seemed this cell in R.r.Caspicus to B-cell and this can be consistent with this finding that B-cell is specifically important in freshwater invironment (Chris et al. 1999). Cytoplasmic tubules associated with numerous mitochondria, have been considered important for the osmoregulatory function, because they have represented sodium pump proteins involved in active ion excretion. These reticulated cytoplasmic tubules which anastomosed with each other to form a network, often continous with the basolateral plasma membrane and their lumenopen to the intercellular space. This has been frequently observed in many ion-transporting cells and is probably involved in active ion excretion for the regulation of body fluid osmolarity. The membranous systems in R.r.Caspicus seem to tubular system in freshwater species and formed a broad and loose network (Pisam 1981). Although accessory cells are usually found in marine species, they also were found in the gill epithelium of euryhaline Salmonids in freshwater (Pisma et al. 1989), as well as two species of tilapia (Cioni et al. 1991). It has been proposed that accessory cells are merely young stages in chloride cell development (Wendelaar Bonga and Van der Meij 1989), but their function is still unknown. Importantly accessory cell and chloride cell share a single-strand shallow junction, suggesting that a leaky paracellular patway is present betweenthe cells. This is thought to be the morphological basis for the relatively high ionic permeability of the gill (Karnaky 1992).

The most important ultrastructural changes in some of the gill chloride cells of R.r. Caspicus were in mitochondria and microtubule system. The mitochondria showed swelling and rupture of mitochondrial cristae and Microtubules displayed distruption. According to previou studies, so changes can be related to toxic action of compounds deriving from nitrite. Caspian sea is a closed water body which connected to open sea through the Volga river. This makes it very vulnerable to the effects of industrial pollution. Oil exploration activities, by the sea littoral countries, have increased in the past decade. There are also international plans to transfer oil and gas through underwater pipelines in the Caspian Sea. These activities will certainly have adverse effects on marine ecosystems of Iran. The rivers also contribute the greatest proportion of all types of pollution to the sea, carring pollutants that have originated from domestic and industrial outfalls and also direct run off of agriculturalpollutants (Ayati 2003).

It must be considered that the environmental Cl^{-} concentration is a determining factor influencing NO_2^{-} toxicity(Eddy et al. 1983). Because Cl^{-} is a competitive inhibitor of No_2^{-} uptake (Bath and Eddy 1980). R.r.Caspicus speciemens, were obtained from south of Caspian sea, a freshwater system with Salinity 12/64–12/68 gr/lit, and this can provide a suitable condition for toxic effects of nitrite. Although, this changes were observed in the gill chloride cell of three speciemens from six total that we have studied. The critical questins must be answered: Do the the chloride cells of other species displayed the ultrastructural changes? The period of No_2^-exposure for exhibiting signs of degeneration? And other questions that must be answered with more experiments.

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