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The Relationship Between Chloride Cells and Salinity Adaptation in the Euryhaline Teleost, *Lebistes reticulatus*

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Abstract: The present study elucidates, the relationship between chloride cells and salinity adaptation in the guppy, *Lebistes reticulatus*. Guppies were exposed to different salinities (2, 5, 8 and 11%) for 2 weeks to adapt them to salt water with the density of chloride cells was examined by light microscopy. The chloride cells of the gills were selectively stained by the fixator of Champy's modified by Maillet. The chloride cell density increased significantly in fish adapted to 8 and 11% salt concentration, which suggests that the densities of branchial chloride cells increased with environmental salinity.

Key words: Chloride cell, salinity, *Lebistes reticulatus*, gill, euryhalinity, teleost

INTRODUCTION

Euryhaline teleosts possess the capacity to osmoregulate under various environmental conditions (freshwater to hypersaline water). The ability of aquatic organisms to tolerate wide variations of salinity without compromising life processes is called euryhalinity (Boutet *et al*., 2006).

It is well known that the most important osmoregulatory organ in these animals is the gill, which is characterized by a relatively slow absorption of Na and Cl in low salinity environments and a relatively rapid secretion of Na and Cl in high salinity environments (Evans, 1993; Karnaky *et al*., 1976a). The branchial epithelium is important to euryhaline fish for both salt uptake in freshwater (Evans, 1984) and salt secretion in seawater (Maetz, 1974). The cellular basis for Na and Cl transport across the teleost gill epithelium is believed to be the chloride cell, so named because of its large, secretory-like appearance and by the demonstration of active chloride excretion by this cell in the teleost gill (Keys and Wilmer, 1932; Karnaky *et al*., 1976b).

Chloride cells have been implicated in many ultrastructural and biochemical studies as the cell type responsible for the salt secretion (Karnaky *et al*., 1976a; Foskett *et al*., 1981; Lin *et al*., 2003; Saquragui *et al*., 2007; Laiz-Carrion *et al*., 2005). Variations in the morphology and number of chloride cells thus, reflect adaptive responses to particular environments (Lee *et al*., 2003).

Teleost models of metabolic and cellular adaptation to varying environmental salinity have long been based on studies conducted on a restricted number of euryhaline species (Lin *et al*., 2003). The species studied are the pupfish *Cyprinodon variegatus* (Karnaky *et al*., 1976a), the killifish *Fundulus heteroclitus* (Karnaky *et al*., 1976b), the gobby *Gillichthys mirabilis*, the cichlid *Sarotherodon mossambicus* (Foskett *et al*., 1981), the guppy *Poecilia reticulatus* (Pisam *et al*., 1995) and the milkfish *Chanos chanos* (Lin *et al*., 2003). Most studies on these model species, show a similar positive correlation between environmental salinity and chloride cell density.

To study the relationship between the chloride cells and the salinity adaptation, we chose the euryhaline teleost *Lebistes reticulatus* for our experiments. This guppy is a euryhaline form, suitable for rearing in the laboratory in freshwater and capable of adaptation to high salinity environments. In this study, we wanted to evaluate the response of *Lebistes reticulatus* branchial tissue to salinity changes. To focus exclusively on the chloride cell proliferation of the gills of the guppies, the chloride cells of the gills are selectively stained by the fixator of Champy's modified by Maillet, which has made it possible to identify 'chloride cells' in gill tissue that are unidentifiable using other histological techniques.

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MATERIALS AND METHODS

Fish and experimental environments: Guppies (*Lebistes reticulatus*) from our own stock with a mean weight of 0.94 g (0.71-1.16) and a mean length of 4.4 cm (3.8-5.0) were used for this study. Fish were held in a 50×15×30 cm glass aquarium supplied with dechlorinated tap water in the laboratory. Experimental environments of different salinities were made with local tap water using proper amounts of salt rock (NaCl) and gypsum ($CaSO_4$). The fish used were adapted to different salinities 0, 2, 5, 8 and 11% with a daily photoperiod of 12 h for at least 2 weeks (Pisam, 1981). Controls were treated in the same way without the addition of NaCl and $CaSO_4$ to the test water. Aquaria were controlled continuously for the first 12 h to monitor possible unexpected conditions and fish deaths.

Water Temperature (T), specific Electrical Conductivity (EC), Salinity (S), Dissolved Oxygen (DO) and pH were measured in the laboratory. Water samples from different salinities were analyzed for major ions and nutrients. All analyses were made according to APHA-AVVA-WPCF standards (1989).

Histological investigations: Live fish were killed by taking from the aquarium to an aerobe condition at the end of the experiment. For microscopic examination, gill tissue samples in sterile medium were fixed in Champy's-Maillet solution for 18 h (Garcia-Romeu and Masoni, 1970). After the fixation, tissue samples were dehydrated, cleared with xylene and embedded in paraffin. A rotary microtome was used to cut sections (5 μ), which were examined under a light microscope.

RESULTS AND DISCUSSION

Chemical characteristics of the different salinities made in the laboratory are summarized in Table 1. The differences in EC, Cl and SO_4 were monitored since they are indicators of salinity (chloride from salt rock and sulfate from gypsum).

In the present study, the gill tissues of the guppies adapted to different salinities (2, 5, 8 and 11%) were investigated. Salinity, effects on the branchial epithelia were assessed by observing the densities of chloride cells fixed by Champy-Maillet fixator which, selectively stains chloride cells black or brown color. The use of this technique has made it possible to distinguish chloride cells from other cells in the branchial epithelium.

The histological structure of control fish gill epithelium is shown in Fig. 1. The gill epithelium of teleost fish is made of a mosaic of respiratory cells interrupted by chloride cells generally situated at the base of the secondary lamellae with a few mucousal cells present. Histological examination of gill tissues of guppies revealed that the filament epithelium of *Lebistes reticulatus* adapted to various salinities is different with fewer chloride cells observed in gill epithelium of fish maintained in 2 and 5% salinities when compared to fish adapted to 8 and 11% salinities. Abundance of branchial chloride cells increased with environmental salinity, with the densities of the chloride cells of fish adapted to 8 and 11% salinities were significantly higher than the other groups (Fig. 2).

Although, ion regulation in fish is mediated by a group of structures including the gastrointestinal epithelium and the kidney, the gill is the major site involved in balancing ion movement between diffusional gains or losses (Evans, 1993). In the branchial epithelium, chloride cells play a prominent role in teleost fish osmoregulation. Indeed, the permeability of this cell type is modified according to the salinity of the external environment (Pisam, 1981). Evans (1984) has estimated that 5% of teleost species are euryhaline, having the capacity to withstand large changes in environmental salinity. This capacity to evolve euryhalinity may be one reason that teleosts can be found in almost all aquatic environments (Lin *et al*., 2003). Among them, the guppy *Lebistes reticulatus* is a euryhaline form, suitable for rearing in the laboratory in freshwater and capable of adapting to high salinity environments. The present study confirms, the capacity of guppies to adapt to abrupt changes in environmental salinity.

The morphological adaptation of the gill structure to variable salinities has been the focus of intense research for many years, especially with respect to the role of chloride cells. For most euryhaline teleosts examined to date, branchial mitochondria-rich cells or chloride cells

Table 1: Physical and chemical properties of water used in rearing guppies

Salinity concentrations (%)	Water Temp (°C)	Dissolved oxygen (mg L^{-1})	pH (25°C)	EC (25°C)	Ca (mg L^{-1})	Mg (mg L^{-1})	Cl (mg L^{-1})	SO_4 (mg L^{-1})
Control	25.0	7.8	8.4	366	26.1	24.3	8.2	19.7
2	25.0	7.2	8.4	2800	92.2	18.2	2810.0	182.5
5	25.0	7.5	8.4	7000	140.3	23.1	3700.0	302.5
8	25.0	7.0	8.4	14164	150.3	31.6	4027.1	330.0
11	25.0	6.9	8.3	15000	172.3	30.4	5267.9	375.0

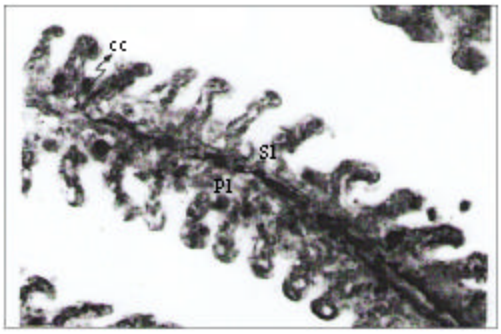

Fig. 1: Histological structure of control fish gill epithelium with fewer chloride cells cc: chloride cell, S1: Secondary lamellae, P1: Primary lamellae (X450)

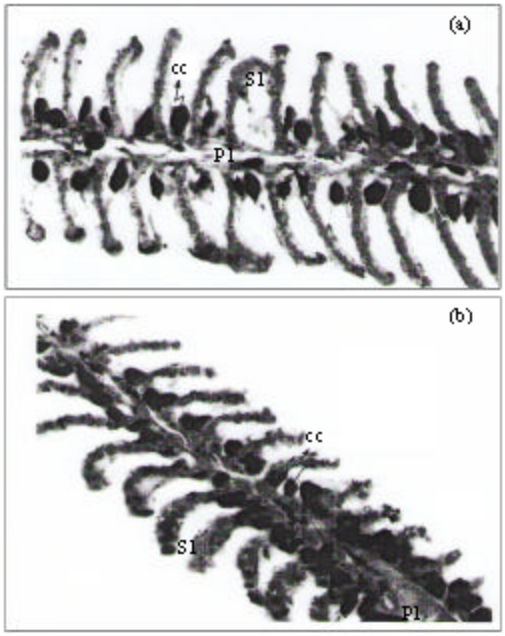

Fig. 2: Increased density of chloride cells selectively stained by Champy-Maillet fixator in longitudinal sections of gill tissue from *Lebistes reticulatus* adapted to 8% (a) and 11% (b) salinities cc: chloride cell, P1: Primary lamellae, S1: Secondary lamellae (X450 a; X720 b)

change in density, size and morphology (ultrastructure) in response to alterations in environmental salinities (Shirai and Utida, 1970; Foskett *et al*., 1970, 1983; Pisam, 1981; Chretien and Pisam, 1986; Hwang, 1987; Pisam *et al*., 1995; Lee *et al*., 2003). Since, chloride was localized to the chloride cells, this cell type has been considered the site of excretion in salt water (Sardet *et al*., 1979). They may also, perform salt absorption in freshwater. Similar results were found in the present study; raising environmental salinities resulted in the increased density of chloride cells at the base of the secondary lamellae in euryhaline guppy, *Lebistes reticulatus*. According to Eckert and Randall (1983), the number of chloride cells can change with varying external salinity such that exposure to high salinities results in an increase in the number of chloride cells. Moreover, our previous study in Kizilirmak river, Turkey, demonstrated that in *Capoeta tinca*, the number of chloride cells increased to meet physiological demands and adapt to salinity changes in the environment (Erkmen and Kolankaya, 2000).

The ability of fish to inhabit diverse and oscillating environments arises from a variety of adaptive physiological mechanisms. Salts dissolved in the water easily permeate into the body of fish, mainly through their gills and salt water adaptation is characterized by the insertion of new cells into the surface epithelium. There are indications that these cells, adjacent to typical chloride cells, are young chloride cells. The young cells interdigitate with typical chloride cells, creating a composite apex (Sardet *et al*., 1979). In the present study, the increase of chloride cells in the base of the secondary lamellae of guppies adapted to high salinity indicates that it may induce these cells in the gill filament epithelium, perhaps as an adaptation for osmoregulation. The osmotic gradient across the integument of these fish is very high and the key to their survival appears to be the gill's enhanced ability to excrete excess NaCl.

CONCLUSION

Density of chloride cells in euryhaline guppy, *Lebistes reticulatus* was modulated by environmental salinity. It is known that the external salinity induces osmoregulatory changes through endocrine mechanisms that influence epithelial differentiation and metabolism (Eckert and Randall, 1983; Fosket *et al*., 1983; Dean *et al*., 2003; Lee *et al*., 2006). These changes that chloride cells undergo during adaptation to high salinities indicates that they are the major site of active exchange in the gills of euryhaline teleost fishes.

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