Effects of Prolactin on α and β Chloride Cells in the Gill Epithelium of the Saltwater Adapted Tilapia "*Oreochromis niloticus*"

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ABSTRACT Tilapia (*Oreochromis niloticus*), 21 g average body weight, were divided into two groups. A group was maintained in fresh water, whereas another group was adapted for 2 weeks to 20‰ salt water. Among the latter, fishes were injected every 2 days for a week with tilapia prolactin (ti-PRL I). Gills were prepared for electron microscopy in order to determine the types and surface areas of chloride cells in each experimental condition. Two types of chloride cells, the α and β cells were easily distinguished on the basis of their location and ultrastructural features in the gills of freshwater fishes, while only one type of cell, the saltwater α cells presumably derived from the transformation of the freshwater α cells, were encountered in saltwater adapted animals. After PRL injection of saltwater adapted fishes, small chloride cells, which displayed ultrastructural features similar to those of β cells in freshwater tilapia, reappeared in interlamellar regions of the gills. In the same experimental conditions, the voluminous saltwater α cells showed a tendency to resume ultrastructural features more characteristic of the freshwater α cells from which they were derived. These observations tend to indicate that prolactin behaves as a "freshwater adapting hormone" and that β cells are specifically involved in fish adaptation to freshwater living conditions. © 1993 Wiley-Liss, Inc.

Key words: Prolactin, Gill, Chloride cells

The "chloride cells" of the gill epithelium are thought to play a major role in the hydromineral regulation of teleostean fishes (Keys and Willmer, 1932; Philpott and Copeland, 1963; Maetz, 1971). They contain numerous mitochondria and an extensive intracytoplasmic membranous tubular system which is an expansion of their basolateral plasma membrane (Doyle and Epstein, 1972; Karnaky et al., 1976; Philpott, 1980; Pisam, 1981). In the gill epithelium of some stenohaline and euryhaline freshwater adapted fishes, two types of chloride cells, the α and β cells, may be distinguished on the basis of their respective location and ultrastructural features (Pisam et al., 1987, 1990). Thus, while the α cells are located at the base of the gill secondary lamellae in close contact with the basement membrane of the pillar capillary, the β cells are usually observed in the interlamellar region of the primary epithelium. The β cells display in their apical cytoplasm numerous membrane-bound bodies of various sizes and shapes which are absent in the α cells. The β cells are found exclusively in freshwater adapted fishes and disappeared during seawater adaptation. They are therefore thought to be implicated mainly in freshwater adaptation (Pisam and Rambourg, 1991).

Morphological changes in chloride cells during fish adaptation to seawater or to freshwater are under con-

trol of both external and hormonal factors (Lahlou, 1980; Foskett et al., 1983; Mayer-Gostan et al., 1987). Following the original finding by Pickford and Phillips (1959) stressing the necessary presence of prolactin (PRL) for survival of hypophysectomized *Fundulus* in fresh water, the role of this hormone in freshwater osmoregulation has been established in several euryhaline species including the tilapia *Oreochromis mossambicus*. A major modification following transfer of teleosts into seawater is a drastic reduction in prolactin secretion (Dharmamba and Nishioka, 1968; Nicoll et al., 1981; Prunet et al., 1985; Auverin et al., 1992). Conversely, several prolactin injections into seawater-adapted fishes induce both an inhibition of gill Na^+ net excretion fluxes (Dharmamba and Maetz, 1976) and a significant inhibition of the current generated by an isolated opercular membrane (Foskett et al., 1982). It was therefore of interest to study the morphological effects of this so-called "freshwater adapting hormone"

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Figs. 1-3.

(Bern, 1975) on gill chloride cells of seawater adapted fishes. Recently, recombinant tilapia PRL I was produced with full biological potency (Swennen et al., 1991). It is the purpose of the present study to examine the effect of this hormone on the chloride cells of the saltwater-adapted *Oreochromis niloticus* and, more specifically, to investigate whether or not such treatment will induce the reappearance of β chloride cells.

MATERIAL AND METHODS

Tilapia (*Oreochromis niloticus*) of 21 g average weight were divided into two groups: the first group was maintained in fresh water (26°C; 12L/12D photoperiod), whereas the second group was adapted to 20‰ salt water (26°C; 12L/12D photoperiod) during 2 weeks. Among this last group, fishes were injected intraperitoneally every 2 days over an 8 days period either with a single injection of saline (NaCl 9‰) or a single injection of tilapia PRL I (188 amino acids variant). One day after the last injection, blood was collected with a heparinized syringe and the fishes were killed. Gills were quickly dissected out and fixed for 1 hr at room temperature in 2% glutaraldehyde buffered with 0.08M sodium cacodylate pH 7.5. In order to increase the contrast of apical bodies in the cytoplasm of chloride cells, gill fragments were immersed for 2 hrs in 0.1 M tris-maleate buffer containing 0.006 M $MnCl_2$ and 0.14% lead citrate. This technique will be referred to as the Mn-Pb technique (Pisam et al., 1987). The fragments were then postfixed for 1 hr at room temperature in a 1:1 mixture of 2% aqueous osmium tetroxide and 3% aqueous potassium ferrocyanide according to the method of Karnovsky (1971). Dehydration was in alcohol and embedding in Epon 812. Thin sections running parallel to the long axis of the primary lamellae and perpendicular to the secondary lamellae were stained for 2 min with lead citrate and examined at 60 kV with a Philips CM 12 electron microscope.

The surface areas of chloride cells were measured in thin sections running through the nucleus. They were calculated using a Kontron M 15 image analyser on micrographs at a final magnification of 3,000. The modifications of surface areas according to physiological conditions were assessed using the Student's t test.

Blood Cl^- and Na^+ levels were measured using respectively a chlorimeter (Radiometer) and an atomic absorption spectrophotometer (Perkin Elmer).

RESULTS

The gill of *Oreochromis niloticus* consists of several arches bearing primary lamellae (or filaments) from

TABLE 1. Mean surface areas (in $\mu m^2 \pm SD$) of chloride cells in sections of the primary gill epithelium from freshwater or saltwater (with or without PRL) adapted *Oreochromis niloticus*¹

	α Chloride cells		β Chloride cells
Freshwater	71 $\pm$ 16 n = 16	NS	59 $\pm$ 12 n = 11
Saltwater	147 $\pm$ 53 n = 22	$P < 0.001^*$	
Saltwater + PRL	122 $\pm$ 32 n = 11	NS	47 $\pm$ 10 n = 11
		$P < 0.005^*$	

¹n, number of cells; *, Student's t test; NS, not significant.

which radiate the secondary (or respiratory) lamellae. The regions of the primary lamellae separating the bases of adjacent secondary lamellae are referred to as "interlamellar regions." Each secondary lamella is vascularized by an arterial pillar capillary interconnecting an afferent and an efferent arteriole.

Two Types of Chloride Cells in Freshwater Adapted Fishes

Two types of chloride cells the α and β cells were easily distinguished according to their location and ultrastructural features.

As usual, the α cells were located at the base of the secondary lamellae in close contact with the basement membrane of the pillar capillary (Fig. 1). They were pale, elongated or ovoid cells with a mean surface area of 71 $\pm$ 16 μm^2 (Table 1). Their indented apical surface was slightly depressed to form a shallow apical cavity containing an extensive filamentous coat (Fig. 4). At their apical pole, numerous electron dense bodies were heterogeneous in shape. They comprised dark tubules 200–300 nm in length and 70–100 nm in diameter as well as more lightly stained cupule-like bodies 140–200 nm in diameter. The underlying cytoplasm contained numerous mitochondria and exhibited a tubular system chiefly made up of loosely anastomosed membranous tubules. As already shown by Wendelaar-Bonga and Van der Meij (1989), small accessory cells (40 $\pm$ 9 μm^2) were occasionally appended to the apical portion of the α cells (Fig. 4). In such cases, there were no apical interdigitations between the two cell types and apical junctions, when present, were shallow (40–50 nm in length). At the apex of the accessory cells a vesiculo-tubular system consisting of vesicles (50–80 nm in diameter) and short tubules (120–160 nm in length) was observed just beneath their flattened apical surface. Intermingled with elements of the endoplasmic reticulum, a well-developed tubular system was evenly distributed throughout the cytoplasm, which, in this cell type, was filled with glycogen particles.

The β cells were usually located in the interlamellar region. Cuboidal in shape, they were darker and slightly smaller (Table 1) than the α cells. Their wavy apical surface was endowed with a fuzzy coat (Fig. 5). Loosely anastomosed membranous tubules pertaining to the tubular system penetrated their apical cyto-

Figs. 1–3. Low-magnification photographs of the gill epithelium of *Oreochromis niloticus* in the three experimental conditions. $\times 3,000$.

Fig. 1. In the primary epithelium of a freshwater living fish (FW), two α cells (α) are observed at the base of two secondary lamellae (SL), in close contact with the pillar capillaries (PC). A smaller β cell (β) is present in the interlamellar region.

Fig. 2. In 20‰ saltwater adapted fish (SW), only voluminous saltwater α cells are present. They are located at the base of the secondary lamellae and exhibit an apical cavity (ac). PC, pillar capillary.

Fig. 3. In a 20‰ saltwater adapted fish injected with PRL (SW + PRL), the gill epithelium still contains voluminous saltwater α cells (α) but displays in addition smaller β cells (β). PC, pillar capillary.

Figs. 4 and 5.

TABLE 2. Effects of prolactin treatment on blood plasma Na^+ , Cl^- concentration¹

	Controls	ti-PRL I
n	9	9
Na^+	154.3 ± 3.5	$199.0 \pm 6.4^*$
Cl^-	148.2 ± 3.2	$205.1 \pm 8.1^*$

¹Values are means $\pm$ SD; n, number of animals. Plasma mineral content is expressed in mEq/l.

* $P < 0.01$.

plasm and intermingled with numerous electron-dense bodies mainly consisting of straight or curved tubular structures 350–400 nm in length and 70–90 nm in diameter (Fig. 5). In contrast to the α cells, the β cells were never associated with accessory cells.

One Type of Chloride Cell in Salt Water Adapted Fishes

After 2 weeks in 20‰ salt water, fishes were fully adapted to their hyperosmotic environment as indicated by plasma Cl^- and Na^+ levels (Table 2). In such fishes, only one type of chloride cell was present in the gill epithelium. They were ovoid, voluminous (Table 1) typical saltwater α cells (Philpott and Copeland, 1963) exclusively located at the base of the secondary lamellae (Fig. 2). Their apical surface was deeply invaginated to form an apical cavity filled with an extensive filamentous coat. The apical cytoplasm was deprived of cell organelles except for many electron-dense bodies consisting exclusively of more or less incurvated plate-like membranous structures 200–300 nm in diameter and 20 nm in thickness (Fig. 6). The extensively developed tubular system formed a tight network with numerous small and regular polygonal meshes (Fig. 7). The saltwater cells were always associated with accessory cells (Figs. 6,7) resembling those associated with the α cells in freshwater adapted fishes. Yet, in 20‰ salt water, the accessory cells sent into the apex of the adjacent chloride cells numerous interdigitations that reached the apical cavity. The contorted and shallow (30–40 nm in depth) apical junction between accessory and chloride cells was in contrast with the deep (280–

300 nm) narrow junctions binding chloride (or accessory cells) to adjacent pavement cells.

Reappearance of β Cells in Salt Water Adapted Fishes Injected With PRL

After PRL injection of 20‰ salt water adapted fishes, there was a significant increase of plasma Cl^- and Na^+ levels (Table 2) and the striking fact was the reappearance of small chloride cells (Table 1), mainly located in the gill interlamellar regions (Fig. 3), which displayed ultrastructural features similar to those of β cells in gills of freshwater adapted fishes (Fig. 1). Thus, as in β cells, loosely anastomosed elements of the tubular system penetrated their apical cytoplasm and intermingled with numerous more or less incurvated electron dense bodies (Fig. 9).

After PRL injection, the saltwater α cells were also affected and showed a tendency to display ultrastructural features characteristic of the freshwater α cells from which they are derived (Pisam et al., 1987). They were slightly reduced in size (Table 1). Their apical cavity was shallower and the apical cytoplasm contained a variable number of dense tubular structures or lightly stained cupule-like bodies similar to those observed in freshwater α cells. The tubular system, absent from some cytoplasmic areas, was less extensively developed and consisted of more loosely anastomosed membranous structures (Fig. 8). These cells, however, were still associated with accessory cells but the apical junction between both cell types, although still shallow, was less contorted than in 20‰ salt water (1.78 ± 1.11 shallow junctions per apical cavity instead of 3.35 ± 2.08).

DISCUSSION

As previously shown in the freshwater adapted guppy (Pisam et al., 1987) or in stenohaline freshwater fishes (Pisam et al., 1990), the gill epithelium of the freshwater water tilapia *Oreochromis niloticus* contained two types of chloride cells: the pale and relatively large α cells and the smaller and darker β cells. In accordance with observations recently reported by Maina (1991) in the same species as well as in *Oreochromis alcalicus*, the α cells were preferentially found in the immediate vicinity of the pillar capillaries, in close contact with the basement membrane of the gill epithelium whereas the β cells were more superficially located. As in the seawater adapted guppy, the β cells were no longer observed in the gills of the saltwater adapted tilapia, while the α cells gave rise to the voluminous saltwater α cells. The salient observations in the present paper, however, were rather related to the ultrastructural modifications induced by PRL injections in gills of the 20‰ saltwater adapted fishes (Fig. 10).

Ultrastructural Modifications of the Saltwater α Cells

Herndon et al. (1991) recently noted that prolactin injections induce an average size decrease and a patchy distribution of Na^+ , K^+ -ATPase in opercular chloride cells of seawater adapted tilapia. This is in keeping with the results reported here since, after prolactin injection, the gill chloride cells show a tendency to decrease in size, while their tubular system, which is known to contain Na^+ , K^+ -ATPase, becomes more

Figs. 4 and 5. Apical portions of the α and β chloride cells in the primary epithelium of a freshwater fish (FW). $\times 27,000$.

Fig. 4. In a large α cell (α) associated with an accessory cell (A), the apical surface (as) is slightly depressed to form a shallow apical cavity. The filamentous coat (f) which fills the apical cavity sends extensions into the outside medium. Some darkly stained tubular structures (arrowhead) and larger, paler cupule-like bodies (cb) are interspersed throughout the apical portion of the cell. In the underlying cytoplasm, the tubular system (T) consists of loosely and irregularly anastomosed membranous tubules. At upper right, densely stained elements of the vesiculo-tubular system (vt) are observed just below the flattened apical surface of the accessory cell the cytoplasm of which contains numerous glycogen particles (gl). ER, endoplasmic reticulum; m, mitochondrion.

Fig. 5. The apical surface of the β cell (as) is hardly depressed and displays a wavy outline. The apical cytoplasm is filled with numerous electron dense bodies consisting mainly of straight or curved tubules (db) associated with more irregularly shaped structures (arrowhead). Such bodies are intermingled with elements of the tubular system (T) which in the β cell also invades the apical portion of the cytoplasm.

Figs. 6 and 7.

loosely anastomosed and less regularly distributed. As already demonstrated in seawater adapted *Fundulus kansae* (Potts and Fleming, 1971), tilapia (Clarke, 1973), and european eels (Oliverau and Lemoine, 1973), prolactin injections increase plasma electrolytes, namely, Cl^- and Na^+ concentrations (Table 2). Such an increase might result from the lack of Cl^- secretion after prolactin injection as observed in the opercular epithelium of the tilapia (Foskett et al., 1982) or killifish (Mayer-Gostan and Zadunaisky, 1978), as well as from Na^+ efflux inhibition by prolactin as reported by Dharmamba et al. (1973) in *Tilapia mossambica*. In the latter case, prevention of Na^+ efflux is probably due to the shortening of the length of shallow apical junctions between saltwater α chloride cells and accessory cells which are usually thought to provide a paracellular pathway allowing the fish to excrete ions into a hyperosmotic medium (Sardet et al., 1979; Ernst et al., 1980). Thus, as expected from a "freshwater adapting hormone" (Bern, 1975), prolactin induces some kind of dedifferentiation of the voluminous saltwater α cells, which, after injections of the hormone, tend to resume ultrastructural features and functions of the freshwater α cells.

Reappearance of the β Cells

Previous studies suggest that β cells, which are found exclusively in freshwater fishes or freshwater adapted euryhaline fishes (Pisam et al., 1987, 1990), are implicated in freshwater adaptation (Pisam and Rambourg, 1991). The present data indicate that prolactin induces the reappearance of the β cells when injected in saltwater adapted tilapia normally deprived of such cells. Thus, the presence of β cells in the gill epithelium of fresh water fishes and their absence in saltwater adapted animals is probably controlled by prolactin, which is endogeneously secreted at high rate in freshwater and at low rate in seawater (Ensor and Ball, 1972).

Nothing is known about the function of β cells. However, the net Ca^{++} uptake rate may be boosted by 96% by ovine prolactin in freshwater tilapia (Flik et al., 1986) and still more in eel (Flik et al., 1989). Moreover, it has also been shown in a trout isolated head preparation (Payan et al., 1981) or by X-ray microanalysis in the freshwater goldfish (*Carassius aureus*) (Ishihara

and Mugiya, 1987) that the major Ca^{++} influx is associated with chloride cells of the gill epithelium. Such a correlation between high levels of prolactin, increased rates of calcium uptake in gill chloride cells, and the presence of β cells in a freshwater environment would support the hypothesis of a possible involvement of the latter cells in branchial calcium uptake.

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Figs. 6 and 7. Apical portions of an α cell and its associated accessory cell in the gill epithelium of a 20‰ saltwater adapted fish (SW). $\times 27,000$.

Fig. 6. The apical surface (as) of the α cell (α) is deeply invaginated to form the large apical cavity filled with a filamentous coat (f). The apical portion of the cytoplasm contains numerous electron dense more or less incurvated plate-like structures (dps). The apical surface of the accessory cell (A) is involved in the formation of the wall of the apical cavity. The elements of the vesiculo-tubular system (vt) visible in its apical cytoplasm resemble those found in accessory cells of the freshwater fish. m, mitochondrion.

Fig. 7. In this section which bypasses the apical cavity, the accessory cell (A) sends numerous interdigitations (asterisks) into the apical cytoplasm of the α cell (α) which contains electron dense plate-like structures (dps). The tubular system (T) of the α cell, more deeply located, consists of tightly anastomosed membranous tubules. m, mitochondrion; vt, vesiculo-tubular system of the accessory cell.

Figs. 8 and 9.

Figs. 8 and 9. Apical portions of chloride cells in the gill epithelium of a 20‰ saltwater adapted fish injected with PRL (SW + PRL). $\times 27,000$.

Fig. 8. An α cell (α), located at the base of a secondary lamella, in close contact with the pillar capillary (PC) is associated with an accessory cell (A). Its apical portion located just below the deeply invaginated apical surface (as) contains some tubular (arrowhead) and cupule-like (cb) bodies. The apex of the accessory cell with its characteristic vesiculo-tubular elements (vt) sends apical interdigitations (asterisk) within the apex of the α cell. In the inset, note that the apical junction (arrowhead) between the α chloride cell and the accessory cell (A) is significantly shallower than the one (j) located between the latter and a pavement cell. m, mitochondrion. Inset: $\times 46,000$.

Fig. 9. In contrast to that of the α cell, the apical surface (as) of the β cell (β), located in the interlamellar region remains flattened. The apical portion of its cytoplasm is stuffed with dense bodies (db) characteristic of this cell type. m, mitochondrion; T, tubular system.

Fig. 10. Schematic representation of the ultrastructural features of chloride cells in the gill epithelium of *Oreochromis niloticus* as seen in a freshwater (FW) fish or in a 20‰ saltwater (SW) adapted fish injected or not with prolactin (PRL). During saltwater adaptation: 1) the α chloride cells (α) located at the base of the secondary lamellae, in contact with the pillar capillaries (PC), increase in size while their loosely anastomosed tubular system (T) develops into a tightly meshed network. In their apical cytoplasm, the electron-dense cup-like bodies (cb) initially found in freshwater are progressively replaced by numerous incurved plate-like structures (dps). There is an increase in number of shallow apical junctions (arrowheads) between them and their adjacent accessory cells (A). 2) The β chloride cells with their characteristic apical dense bodies (db) disappear from the interlamellar region. When saltwater adapted fishes are injected with PRL (SW + PRL), the α chloride cells display ultrastructural features intermediary between those observed in freshwater and those present in saltwater adapted fishes. The β cells reappear with characteristics similar to those depicted in the gill epithelium of freshwater fishes. P, pavement cells; j, deep narrow junctions; VT, apical vesiculo-tubular system of accessory cells; m, mitochondrion; ER, endoplasmic reticulum.

Fig. 10.

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