Scanning electron microscopic evaluation of effect of cortisol on chloride cells of tilapia, *Oreochromis mossambicus*

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The effect of exogenous administration of cortisol (0.2 μg/g body weight) for 24, 48 and 72 hr on the gill epithelium of Tilapia has been studied. The results clearly revealed that out of the three sub-types of chloride cells viz., shallow basin, wavy convex and deep hole, the shallow basin ones are the most abundant in number. *In vivo* administration of cortisol conspicuously increased the number of the shallow basin chloride cells and caused noticeable changes in the microridges of pavement cells right from 24 hr treatment onwards. The present study confirms heterogeneity of chloride cells in teleosts.

**Keywords:** Chloride cells, Pavement cells, Gill epithelium, Cortisol, Osmoregulation, Tilapia, *Oreochromis mossambicus*

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The fish gill is a multifunctional organ involved in gaseous exchange, acid-base balance, transport of Na⁺, Ca²⁺, Cl⁻ and nitrogenous excretion. The gill epithelium consists of pavement cells (PCs); mitochondria rich chloride cells (CCs) and mucous cells. The branchial mitochondria rich cell in fish is a very good model to elucidate the relations of morphologies and functions of transepithelial cells.

In adult teleosts, CCs in the gills are the major sites of ionic regulation. In embryos and larvae of several teleosts CCs are detected in yolk sac membrane and body skin. These extrabranchial CCs are considered to be the sites of ionic regulation in the early developmental stages without functional gills. In teleosts, mitochondria rich chloride cells are believed to be the principal site of transepithelial Ca²⁺ and Cl⁻ influxes. Though currently debated, there is accruing evidence that the pavement cells are the sites of Na⁺ uptake. Morphological changes in mitochondria rich cells of gills and variation in ion regulation have been well-documented in teleosts on their acclimation to environments with different osmolarities or ion composition. Treatment with cortisol increased chloride cell numbers and Na⁺-K⁺-ATPase density in the plasma membrane of gill in tilapia, *Oreochromis mossambicus*, induced electrophysiological permeability and ion transporting properties in cultured pavement cell epithelia of freshwater rainbow trout and in embryos of *O. mossambicus*. It is established that steroids, particularly cortisol significantly enhanced branchial ATPases' activity both by genomic and non-genomic actions mediated through Ca²⁺ in this fish. Hence an attempt is made to evaluate the surface ultrastructural changes in the gill epithelium of *O. mossambicus*, particularly on chloride cells and pavement cells with the help of scanning electron microscope.

**Materials and Methods**

Adult healthy *O. mossambicus* of 50±2 g body weights were used from laboratory stocks. The fish were divided into 4 groups of 5 each and kept in 4 separate aquarium tanks maintained under conditions identical to those of stock tanks. All individuals were reared in aerated local well water at 26°-28° C with a 12:12 hr. L:D photoperiod. The fish were fed every day with feed prepared in the laboratory.

**Chemicals**—Cortisol and glutaraldehyde were procured from Sigma Chemicals Co, USA. All other chemicals used were of analytical grade and purchased from SRL, Bombay, India.

**Administration of hormone**—Acclimatized fish received ip injections of cortisol (0.2 μg/g body weight /day) between 0630 and 0700 hrs for 24, 48, or 72 hr.
The hormone was dissolved in a little amount of ethyl alcohol and then diluted with saline (1:200). The daily dose of hormone was administered in 0.1 ml of respective vehicle and the first three groups of fish were sacrificed after 24, 48 and 72 hr respectively. The fourth group served as control and received the hormone vehicle alkaline saline instead of hormone.

**Fixation:**—Fish were killed after stipulated period of hormone treatment by spinal concussion and gill arches were excised straightway and fixed overnight in 2.5% glutaraldehyde in 0.1 M PBS at room temperature (30°± 3°C). After dehydration in ethanol series (30-100%), the tissues were treated with isoamyl acetate for 10 min. Drying was done with liquid CO₂ in a critical point drier (HCP-02 Hitachi) and sputter coated with a gold-palladium complex in a gold ion-sputtering unit (E101, Hitachi). The coated specimens placed on a stub were examined in a scanning electron microscope (Hitachi-s-2400) at an accelerating voltage of 15 Kv.

**Results and Discussion**

Scanning electron micrographs of the gill epithelium (Figs 1 and 2) illustrate the surface morphology of control and cortisol treated fish. In gill epithelia, pavement cells (PCs) were the most abundant cell type, while chloride cells were relatively less especially in the control (Figs 1A and 2A.). Pavement cells showed characteristic concentrically arranged microridges on their cell surface, whereas chloride cells demonstrated apical pits. Chloride cells were mainly located on the trailing edge of the filament epithelium and the bases of lamellae. The CCs displayed a characteristic apical surface morphology and was easily distinguishable from neighbouring PCs. Three subtypes of CCs viz. shallow basin (SB, Fig. 1B), wavy convex (WC, Fig. 1C) and deep hole (DH, Fig. 1E) were observed on the gill epithelium and shallow basin chloride cells were in abundance. The apical membranes of wavy-convex CCs gave a convex surface appearance with an aperture of larger size; shallow-basin CCs having medium size ovoid aperture and infrequently decorated with short microvilli. The apical pores of deep-hole CCs were narrow, deep and round to oval with little or no internal structure. Chloride cells were relatively less with characteristic apical area extrusions extending out between PCs. There were obvious qualitative differences in the surface area morphology of CCs and PCs in cortisol treated groups in comparison to the control.

The morphology of CCs and PCs changed starting from 24, 48 and 72 hr of ip administration of cortisol with a maximal effect by 72 hr (Fig. 2 B-D). The CCs number also increased with increase in period of injection of cortisol.

The control and 24 hr treatment of cortisol (0.2 μg/g body weight) did not reveal any characteristic difference in the chloride cell apical membrane area but few more CCs were observed in 24 hr cortisol treated gill and were recessed below the surface of neighbouring PCs (Fig. 2A and B). After 48 and 72 hr (0.4 and 0.6 μg/g body weight) of cortisol treatments
gills had an increase in apical membrane area, density and size of the individual CCs (Fig. 2C and D; white arrow). The size and characteristic “finger print” pattern of PCs exhibited marked difference starting from 24 hr of cortisol treatment itself (Fig. 2 B) and had conspicuous difference in the gills treated with cortisol after 48 and 72 hr (Fig. 2 C and D).

Studies on control of ionic regulation by cortisol in fish gills have mainly focused on gill chloride cell morphology, ion uptake, and expression of Na⁺-K⁺ ATPase, Na⁺-K⁺ ATPase density and activity. Cortisol is involved in hypo-osmoregulation in early developmental stages as well as in adult tilapia. Ultra structural studies done with the help of scanning electron microscope revealed the occurrence of all the three subtypes of CCs viz. shallow basin, wavy convex and deep-hole as reported earlier in this freshwater fish.

The results of the present study show that in vivo cortisol treatment exerted stimulatory effect on the morphology of CCs and PCs of gill epithelium in O. mossambicus. Exogenous administration of cortisol apparently increased the number and size of CCs particularly shallow basin type and the size of PCs. The study of Wong and Chan in the Japanese eel, Anguilla japonica strongly upholds the findings of the present study. Intramuscular administration of cortisol (0.2 μg/g body weight) in A. japonica stimulated the proliferation and differentiation of CCs and demonstrated recessed apical morphology of CCs accompanied by an increase in cell density. Moreover, in absence of cortisol, chloride cell density of freshwater tilapia decreased and was restored by cortisol administration in a dose-dependent manner.

In freshwater teleosts, CCs are normally singular on gill epithelium, lack an apical crypt, have extensive tight junctions with adjacent cells, and usually have their mucosal surface above the bordering PCs. Tilapia seems to be an exception, with an apical crypt below the surface of PCs. These are in agreement.
with the appearance of CCs in the present study. The most common type of CCs were shallow basin type followed by deep-hole cells, while wavy convex type were scarcely observed. This information forced to assume that long-term cortisol treatment stimulated chiefly the proliferation and expression of shallow basin CCs in tilapia. This indicates that the expression of shallow basin CCs may be influenced by stress and serves as an immediate adjustment for ionic disturbances induced by different environmental stimuli in this freshwater fish.

There is no direct evidence to confirm the different functions of CCs. Some of the earlier studies indicated that CCs are the sites for Ca$^{2+}$ and Cl$^{-}$ uptake, while pavement cells are responsible for Na$^{+}$ uptake\textsuperscript{26, 27}. However, Chang et al.\textsuperscript{3} based on their recent work and on their previous studies\textsuperscript{6, 22}, proposed the functions of Na$^{+}$ or Cl$^{-}$ uptake for wavy convex cells and Ca$^{2+}$ uptake for shallow basin cells.

The role of cortisol on Na$^{+}$-K$^{+}$ and Ca$^{2+}$ ATPases' activity was categorically established both in long-term (in vivo) and short term (in vivo and in vitro) experiments on the gill of \textit{O. mossambicus}\textsuperscript{11,12}. The uptake of Na$^{+}$, K$^{+}$ and Ca$^{2+}$ also positively responded after 30 min of cortisol treatment in short-term in vivo study\textsuperscript{13}. A rapid non-genomic action, as early as 5 min for cortisol and corticosterone has recently been reported for the first time on branchial Na$^{+}$-K$^{+}$ ATPase in \textit{O. mossambicus}\textsuperscript{12}. In the present study, \textit{in vivo} cortisol treatment after 48 and 72 hr specifically demonstrated an increase in number of shallow basin CCs and a characteristic difference in the pattern of circular concentric ridges in PCs. Cortisol treatment apparently did not make any drastic modification on wavy CCs or deep-hole CCs. Recently, the receptor gene expression by \textit{in situ} hybridisation and immunocytochemical staining revealed that cortisol may be one of the important factors regulating CCs functions in teleosts\textsuperscript{28}. However, information on its probable effect in the regulation of different CC subtypes is limited. The increased uptake of Na$^{+}$ and Ca$^{2+}$ in the present study by gill epithelium was augmented by the activity of Na$^{+}$-K$^{+}$ ATPase and Ca$^{2+}$ ATPase suggesting an increase in ion pumping and associated metabolic activities. Moreover, cortisol profoundly influenced the proliferation and cell density of shallow basin CCs and the activity of PCs.

A number of hormones appear to control the movement of ions across the gill epithelium, which is critical for the osmoregulation in fish\textsuperscript{24}. Recently, Kelly and Wood\textsuperscript{5} have reported cortisol induced active uptake of Na$^{+}$ and Cl$^{-}$ in the cultured pavement cell epithelia from the gills of freshwater rainbow trout. Hence it is suggested that Ca$^{2+}$ transport across the gill epithelium is facilitated by shallow basin CCs as suggested by Chang et al.\textsuperscript{3} and Na$^{+}$ absorption by PCs as reported by Perry\textsuperscript{1}, and Kelly and Wood\textsuperscript{5}. This is in agreement with the increased level of Na$^{+}$, K$^{+}$ and Ca$^{2+}$ followed by the ATPases' activity in the gill of \textit{O. mossambicus} as reported by Sunny and Oomen\textsuperscript{15}.

In summary, it is evident that cortisol exerted a stimulatory effect on size and density of CCs and PCs, and in the distribution of concentric microridges of PCs, indicating its accelerated activity. It is proposed that shallow basin CCs are responsible for the uptake of Ca$^{2+}$ and PCs for Na$^{+}$ on the gill epithelium of \textit{O. mossambicus}. The results of the present study also confirm the existence of heterogeneity of chloride cells in teleosts.

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References


