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Dual in vitro effects of cortisol on cell turnover in the medaka esophagus via the glucocorticoid receptor

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ABSTRACT

Aims: Cortisol is a glucocorticoid in mammals, but has both gluco- and mineralocorticoid activities in teleost fish. Our previous in vivo studies on osmoregulatory esophagi of euryhaline fish showed that epithelial apoptosis for the simple epithelium in seawater and cell proliferation for the stratified epithelium in fresh water are both induced by cortisol. The aim of the present study was to examine the mechanism of these dual cortisol effects on esophageal cell turnover.
Main methods: We developed a tissue culture method for the esophagus from euryhaline medaka (*Oryzias latipes*) and assessed cell proliferation and apoptosis in vitro in response to cortisol and 11-deoxycorticosterone (DOC), a recently identified agonist of the teleostean mineralocorticoid receptor.
Key findings: Epithelial apoptosis, a well-established glucocorticoid function, was stimulated by treatment of the esophagus culture with 10 nM cortisol for 8 days, but no effects were seen at higher doses (100 and 1000 nM). In contrast, cell proliferation was induced by 1000 nM cortisol treatment for 8 days and this response was dose-dependent. Both effects were blocked by RU-486, a glucocorticoid receptor antagonist. DOC showed no significant effects at 10–1000 nM.
Significance: In the esophageal epithelium in euryhaline fish, cortisol induces either apoptosis or cell proliferation via the glucocorticoid receptor, depending on the cortisol concentration. The glucocorticoid signaling may play a more important role than mineralocorticoid signaling in differentiation of the osmoregulatory esophagus in euryhaline fishes.

Introduction

Corticosteroids are divided into glucocorticoids and mineralocorticoids. Teleost fish do not have aldosterone, a tetrapod mineralocorticoid, but have cortisol, which has both gluco- and mineralocorticoid activities in these fish (Mommsen et al. 1999; Bury and Sturm 2007). Indeed, dual (opposite) effects of cortisol have been shown in teleosts (Stolte et al. 2006). In the branchial epithelia, cortisol appears to promote the differentiation of both ion-secretory mitochondrion-rich cells and those associated with ion uptake (Sakamoto and McCormick 2006), whereas in the immune system, cortisol induces apoptosis of B cells but inhibits apoptosis of neutrophils (Weyts et al 1998a,b). In our in vivo studies on esophagi from euryhaline fish (mudskipper), cortisol stimulated both apoptosis and cell proliferation in the epithelium (Sakamoto and McCormick 2006; Takahashi et al. 2006c). These effects seem to be related to increased apoptosis for the simple epithelium to give high permeability in seawater, and cell proliferation for the stratified epithelium to reduce permeability in fresh water (Takahashi et al. 2006b, 2007). However, the mechanism of these dual actions of cortisol is unclear. Cortisol may stimulate apoptosis through its well-established glucocorticoid function, and induce cell proliferation as a mineralocorticoid. Since teleostean prolactin secretion is regulated by cortisol (Borski et al. 1991) and prolactin induces esophageal cell proliferation (Sakamoto and McCormick 2006; Takahashi et al. 2006c), in vivo reciprocal effects of cortisol, prolactin and other factors are also possible.

Most teleosts have two glucocorticoid receptor genes (GR1 and GR2) that are expressed in various organs (Bury and Sturm 2007; Stolte et al. 2008b). Each GR requires a different concentration of cortisol to initiate transcription in reporter assays using mammalian cells (e.g., GR2 is 60-fold more sensitive than GR1 in rainbow trout), which may be related to the wide range (1–1000 nM) of cortisol concentration in teleosts (Prunet et al. 2006). However, information on the concentration-dependence of the dual physiological effects of cortisol is limited to those observed in the carp immune system (Stolte et al. 2008b). Cortisol also binds to the mineralocorticoid receptor (MR) (Prunet et al. 2006; Stolte et al. 2008b), and 11-deoxycorticosterone (DOC) has recently been identified as an endogenous agonist that is more potent and specific for the teleost

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MR (Sturm et al. 2005). Therefore, analysis of cell turnover in esophageal epithelia using in vitro preparations may help to clarify the differential actions of corticosteroids in teleosts, especially with respect to the osmoregulatory organs.

In the present study, a culture system for esophageal explants from a euryhaline fish was developed. We chose medaka, which has also been used as a model for teleost osmoregulation (Sakamoto et al. 2001) and for analysis at the molecular level using gene manipulation (Alvarez et al. 2007) and high-quality draft genome/cDNA sequences (Kasahara et al. 2007). We assessed esophageal cell turnover in vitro in response to cortisol and DOC to define the potential dual actions of cortisol on the osmoregulatory esophageal epithelium, and found the first direct evidence of the mode of dual actions on the osmoregulatory organ.

Materials and methods

Animals

Adult medaka (*Oryzias latipes*, 0.1–0.2 g in weight) of both sexes were kept in indoor freshwater tanks at 28 °C and fed on Tetrafin flakes (TetraWerke, Melle, Germany) daily for more than two weeks. Fish were anaesthetized before handling with 0.01% tricaine methane sulfonate (Sigma, Tokyo, Japan) neutralized with sodium bicarbonate. All procedures were conducted in accordance with the Guidelines for Animal Experimentation established by Okayama University.

Tissue culture

Fish were exposed to 0.1% Fungizone (Amphotericin B, Invitrogen, Tokyo, Japan) for two days without food to avoid contamination before isolation of the esophagus. The culture technique was adapted from previously published methods for culturing intestine from salmon and skin from medaka (Veillette and Young 2005; Matsumoto and Sugimoto 2007). Esophagi were gently sliced open along the long axis, cut into halves, and placed in 96-well culture plates containing preincubation medium (MEM with Hanks' salts, 25 mM HEPES, 5 mg/ml BSA, 250 U/ml penicillin G, and 250 μg/ml streptomycin sulfate, adjusted to pH 7.8 at 25 °C). After several hours, the medium was replaced with MEM containing Earle's salts, 4 mg/ml BSA, 292 μg/ml L-glutamine, 50 U/ml penicillin G and 50 μg/ml streptomycin sulfate adjusted to pH 7.8 when saturated with 99% O_2/1% CO_2. The medium osmolarity was adjusted to 300 mOsm/kg with NaCl. Explants were randomly assigned to control and treatment groups, and incubated at 27 °C in an airtight humidified chamber and gassed daily. Cortisol (hydrocortisone sodium succinate; 1, 10, 100 and 1000 nM), DOC (21-hydroxypregn-4-ene-3,20-dione acetate; 10, 100 and 1000 nM; Sigma, Tokyo, Japan), or neither compound was added to the cultures. We chose these concentrations based on the published effective physiological doses, plasma hormone concentrations, and our preliminary studies (Sakamoto et al. 2001; Veillette and Young 2005; Prunet et al. 2006; Takahashi et al. 2006c; Milla et al. 2008; Stolte et al. 2008b). The effect of RU-486 (mifepristone, 14 μM; Sigma, Tokyo, Japan), a GR antagonist, was also tested. This concentration has been used by others to induce GR blockade in fish (Veillette et al. 1995; Bury et al. 2003; Mazon et al. 2004). The culture medium was replaced with freshly prepared medium daily. Although explants were occasionally found to adhere to the bottom of the well, they typically remained unattached during culture. The tissue culture maintained structural integrity for 8 days based on the presence of intact nuclei and cell-to-cell borders (Figs. 1 and 2). The cultured esophageal explants were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) at 4 °C for 4 h for histological analyses ($n=3$–5) or snap-frozen in liquid nitrogen for quantification of apoptosis.

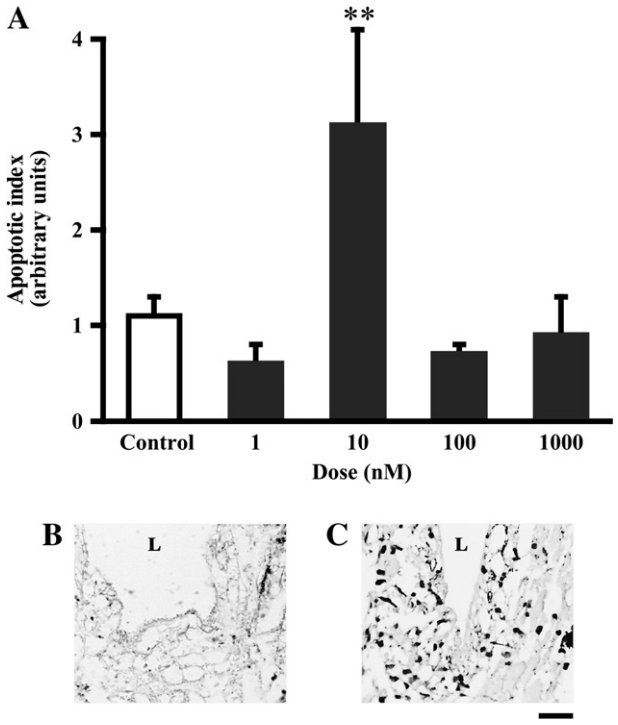

Fig. 1. (A) Effect of cortisol (1, 10, 100 and 1000 nM) on esophageal apoptosis after 8 days of culture. Values are means ± SEM ($n=5$–13) and are expressed in arbitrary units normalized to the initial tissue content. ** $P<0.01$ vs. control. (B and C) Detection of apoptotic cells (dark nuclei) in the esophageal epithelium by TUNEL staining after 8 days in culture. Fewer TUNEL-positive nuclei were observed in the epithelium in control esophagi (B) compared to the epithelium of esophagi treated with 10 nM cortisol (C). *L*: lumen. Representative results are shown. Scale bar = 10 μm.

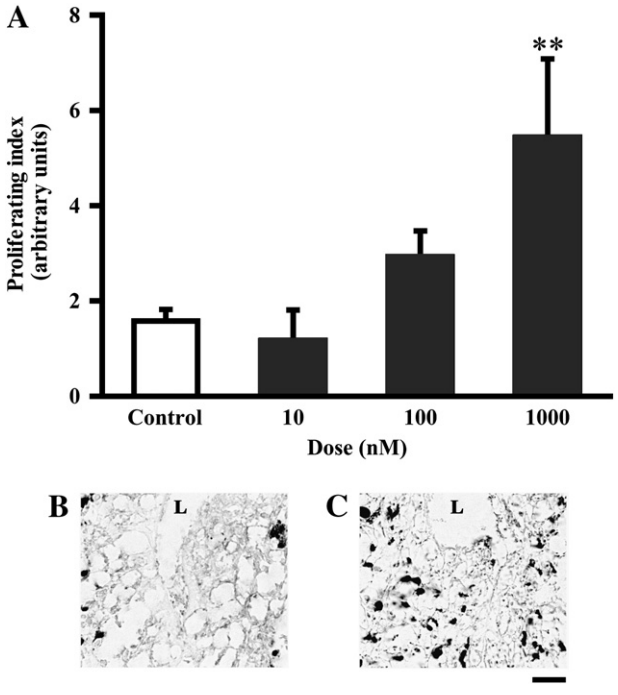

Fig. 2. (A) Effect of cortisol (10, 100 and 1000 nM) on esophageal cell proliferation after 8 days of culture. Values are means ± SEM ($n=5$–12) and are expressed in arbitrary units normalized to the initial tissue content. ** $P<0.01$ vs. control. (B and C) Detection of proliferating cells (dark nuclei) in the esophageal epithelium by PCNA immunocytochemistry after 8 days in culture. A few PCNA-positive nuclei were detected in the epithelium in control esophagi (B), whereas many labeled nuclei appeared in the epithelium in esophagi treated with 1000 nM cortisol (C). *L*: lumen. Representative results are shown. Scale bar = 10 μm.