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In Vitro Effects of Short-Term Cortisol Exposure on Proliferation and Apoptosis in the Skin Epidermis of Rainbow Trout (*Oncorhynchus mykiss* Walbaum)

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INTRODUCTION

The skin epithelium of teleost fish is a complex and usually unkeratinized, multilayered assembly of living cells which are continuous over the body surface and in direct contact with the external environment. The epidermal structure changes in response to a wide variety of stressors, including endocrine factors (Wendelaar Bonga, 1997). Blood levels of the primary stress hormone cortisol are elevated in stressed fish and the effects can be severe, *e.g.* immunosuppression and reduced growth and reproduction (review: Wendelaar Bonga, 1997). In rainbow trout, several typical epidermal stress responses, including increased apoptosis and cell proliferation, are influenced by cortisol administration *in vivo* (Iger *et al.*, 1995).

A 24 hrs *in vitro* organ culture system was used to demonstrate effects of cortisol on chloride cells in the gill of tilapia *Oreochromis mossambicus* (Bury *et al.*, 1998). Here, we used a short-term *in vitro* approach to study mitosis and apoptosis in the trout skin epidermis in response to differing cortisol levels. The glucocorticoid receptor was also demonstrated and localized within the epidermis to see whether it is restricted to particular cell types responding with altered levels of mitosis or apoptosis.

MATERIALS & METHODS

Explants of head skin were sampled from 8 rainbow trout (circa 150 g) and washed 3 times for 5 min in sterile PBS. One piece from each fish was placed in 5 ml of a complete fish skin culture medium (Mothersill *et al.*, 1995). In the same way, replicate skin pieces from each fish were placed into a series of culture media to which cortisol had been added to a final concentration of 50, 500, 1,000 and 2,000 ng/ml (Hydrocortisone; Sigma). After 24 hrs incubation at 20 °C, skin samples were fixed and processed onto polylysine-coated slides. PCNA was studied using conventional immunohistochemistry methods (PC 10, Oncogene, at 1:10,000; anti-glucorticoid receptor, 1:32,000). Apoptosis was visualized with a TUNEL *in situ* kit and a peroxidase-conjugated antibody system (POD) from Boehringer.

Numbers of positive cells were quantified as described by Nolan *et al.* (1999). Data are expressed as means ±S.E.M. for n=8 and differences between groups were tested using the Mann-Whitney U-test. Statistical significance was accepted at $P<0.05$.

RESULTS

Incubation with the highest concentration of cortisol increased the number of cells which stained positively for proliferation by circa 50 % (P<0.01). Apoptotic (TUNEL-positive) cells increased significantly from 500 ng/ml cortisol and higher in a concentration-dependent manner (*i.e.*, increased by 50 to 100%; P<0.01). Immunopositive staining of the cortisol receptor was primarily restricted to the pavement and filament cells of the upper epidermis, plus a small number of migrating cells in the basal layers.

DISCUSSION

This study has shown that low levels of the teleost stress hormone cortisol stimulate apoptosis *in vitro* in the trout epidermis, while high levels stimulate proliferation. These data help to explain anomalies in the literature about the role of cortisol in teleost skin function. Blood cortisol levels rapidly increase during stress (Wendelaar Bonga, 1997) and soon after (typically within hours) increased apoptosis can be observed in the skin. Iger *et al.* (1995) demonstrated the role of cortisol by administering high doses of cortisol to rainbow trout and observed increased apoptosis after 24 hrs and increased mitosis at 4 and 7 days post-cortisol. Cortisol is a pluripotent hormone and high levels stimulate mitosis agreeing with Iger *et al.* (1995). The demonstration of the cortisol receptor in epidermal areas where apoptosis (upper layers) and mitosis (middle layers) are localized, indicates that the bioactivity of this hormone differs depending on the concentration and type of cell expressing the receptor.

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