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Plasma cortisol response to stress in juvenile rainbow trout is influenced by their life history during early development and by egg cortisol content

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ABSTRACT

In this study, the consequences of the exposure of rainbow trout (*Oncorhynchus mykiss*) to a brief stress during early development were explored on the later response of fingerlings to stress. Firstly, we analyzed the ontogeny of cortisol production and that of the initial cortisol response to stress in developing fish. It is only at the eyed stage that the embryos started to produce some basal cortisol. The HPI (hypothalamic–pituitary–interrenal axis) was however not functional before hatching, as exposure of the embryos to a stress did not trigger any cortisol response. A cortisol response to an acute stress was detected 9 days after hatching. In a second set of experiments, we showed that a very brief stress applied at 3 different early stages (eyed, hatching, and yolk resorption) reduced the later cortisol response to stress of 5-month-old fingerlings. This reduction is not likely to be due to alterations in the fish interrenal sensitivity because the 5-month-old fingerlings responded to ACTH treatment (only one dose tested) within the same magnitude as the fish that were not stressed during early development. An experimentally induced increase in egg cortisol just after fertilization also induced a reduction in stress sensitivity of 5 month old fingerlings, which was dose-dependent This study shows for the first time that the responsiveness of the corticotrope axis in 5–6 months old rainbow trout was influenced both by early stress exposure and by initial egg cortisol levels. Whether the HPI was functional or not at the time the initial stress was applied only had a small influence on the later unambiguous effect of early stress exposure.

1. Introduction

The impact of perinatal stress on the cortisol response of young and adult subjects has been extensively explored in mammals. It has been shown that depending on the development stage at which the young are exposed to stress, the opposite regulation of the hypothalamic–pituitary–adrenal (HPA) axis activity operates in adults. Indeed, when a prenatal stress is applied on expectant rats, a prolonged poststress corticosterone secretion is induced in the offspring once they are adults (Vallée et al., 1997), whereas early postnatal handling of the offspring induces a decrease in corticosterone secretion in response to stress (Vallée et al., 1997). In the case of prenatal stress, the observed effect is suggested to be triggered, at least in part, by direct embryo exposure to corticosteroids. Corticosterone in the stressed mother causes a downregulation of fetal glucocorticoid and mineralocorticoid receptors and impairs the feedback regulation of the hypothalamic–pituitary–adrenal axis in infancy and adulthood (Weinstock, 2005).

In adult fish, any alteration in the plasma cortisol concentration has a profound influence on fish homeostasis because cortisol is involved in several major physiological processes, including osmoregulation and intermediate metabolism. Thus, modifications in stress sensitivity, which may influence cortisol response to stress, could have important consequences on the fish throughout its life. However, contrary to mammals, it is not known whether exposure of fish to cortisol variations during the early stages of development can influence the stress sensitivity at later stages. Basal endogenous cortisol changes during development have been studied in several species: Japanese flounder (De Jesus et al., 1991), chum salmon (De Jesus and Hirano, 1992), rainbow trout (Pottinger and Mosuwe, 1994; Barry et al., 1995a,b), Australian seabass (Sampath-Kumar et al., 1995), carp (Flick et al., 2002), and Atlantic cod (King and Berlinsky, 2006). Significant cortisol concentrations of maternal origin were observed in fertilized eggs (De Jesus and Hirano, 1992; Hwang et al., 1992). Thereafter, egg cortisol decreased during embryo development, and endogenous cortisol production began around hatching, as shown from the increase in cortisol concentration. The development of a mature hypothalamic–pituitary–interrenal (HPI) axis able to produce cortisol in response to an external stressor occurred in the first weeks after hatching: cortisol production as an instant response to stress was observed 2 weeks after hatching in turbot and rainbow trout (Stephens et al., 1997; Barry et al., 1995a,b; Pottinger and Mosuwe, 1994), whereas

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it was observed only one week after hatching in yellow perch (Jentoft et al., 2002).

Thus, variations in cortisol exposure of young fish are expected to occur during early development: in eggs, high cortisol concentrations may be present as a consequence of a stress applied to females during vitellogenesis, whereas around hatching, the mature HPI will respond to many abnormal environmental conditions, which may be putative stressors for the fish, by an increase in cortisol concentration. The aim of the present work was therefore to explore whether high egg cortisol concentrations, or early exposure to a stressor, could influence the sensitivity to stress of rainbow trout later on. Firstly, we had to determine the evolution of basal cortisol levels and the appearance of a cortisol response to stress during embryonic development (from fertilization to end of resorbtion), in order to identify, in our rearing conditions with our strain, the different stages of the corticotrope axis development. Based on these characterized stages, we applied a stress at three different key stages; before cortisol production, before and after cortisol responsiveness to stress. We assessed the ensuing stress sensitivity of 5–6 months old fingerlings by measuring their cortisol levels in response to an acute stress. In a last approach, we experimentally modulated egg cortisol concentrations in order to explore whether high cortisol concentrations in eggs would subsequently affect the cortisol response to acute stress in 5–6 months old fingerlings.

2. Materials and methods

Investigations and animal care of rainbow trout (*Oncorhynchus mykiss*) were performed or supervised by authorized persons (authorization given by the French "Direction des Services Vétérinaires") according to the author institution rules. Before experiments, all animals were reared on the experimental fish farm of Le Drennec (Finistère, France) in 28 m^2 circular tanks under natural photoperiod and water temperature. Trout were fed according to the nutrition table given by the food manufacturer (Biomar, Aqualim, France). One month before ovulation, 2 years old females (and several males) were transported to Rennes (France) where they were maintained under natural photoperiod in a recirculated water system at 12 °C.

Three days after ovulation, females were manually stripped under anesthesia (phenoxyethanol, 0.03% vol). Phenoxyethanol has no effect on cortisol secretion in rainbow trout (Auperin et al., 1998). Oocytes were kept in coelomic liquid at 12 °C until fertilization. For each experiment described below, oocytes from at least 2 females were used separately (results from only one female are shown). For oocyte fertilization, a pool of sperm from at least 4 males of the same strain as the females was used. Eggs were incubated at 10 °C in small incubators supplied with running water.

2.1. Experimental protocols

2.1.1. Experiment 1: description of cortisol profiles in embryos and larvae stressed or not during their development

For fertilization, about 2200 oocytes from an autumn spawning strain were mixed with 2 ml of freshly collected sperm and 100 mL of Actifish™ (IMV, France) at 10 °C. After 2 min, eggs were washed with water from the facility. They were incubated for 1 h in recirculated water at 10 °C before being separated into 14 batches (150 eggs each). Each batch was reared in separate incubators in the same recycled water. Sampling of unstressed "fish" was first performed just before the egg distribution into incubators (~1 h postfertilization), and then at 17 h, 1, 2, 3, 6, 10, 14, 17, 20, 27, 34, 44, 48, and 55 days postfertilization (dpf). At each sampling time, 10 pools of 6–7 eggs or 5 anaesthetized larvae (phenoxyethanol, 0.03% vol) were collected. It has been previously demonstrated that phenoxyethanol has no effect on cortisol secretion in rainbow trout (Auperin et al., 1998). Pooled eggs and larvae were immediately frozen in liquid nitrogen and stored at −20 °C before total cortisol extraction. The stressed "fish" were collected from the same incubators as the unstressed "fish". They were exposed to a stress (1 min in 0 °C water, 1 min out of water) immediately after sampling of unstressed "fish" and returned to the incubator for 1 h. Then, embryos and larvae were processed as the unstressed samples. Stressed "fish" were sampled at 14, 17, 20, 27, 34, 44, 48 and 55 dpf.

2.1.2. Experiment 2: influence of stress exposure during development on the stress response of 5–6 months old fingerlings

Oocytes from a spring spawning strain were fertilized as in experiment 1. Eggs were separated into 4 batches. One batch was not stressed and used as a control (CT), whereas the 3 other batches were exposed to a stress (1 min in 0 °C water, 1 min out of water) at one of the three following development stages: eyed stage (17 dpf), hatching (32 dpf), and end of the yolk sac resorbtion (49 dpf). Except during the stress procedures, all 4 batches were reared in the same way. At 50 dpf, fries were transferred into 100 L tanks at 12 °C (recirculated water).

Once the fries reached 20 g (around 5 months postfertilization), they were used to analyze their response to stress. Several days before sampling, fingerlings corresponding to each treatment (CT, eyed stage stress, hatching stage stress, and resorption stage stress) were divided into 5 batches (10 fish each) and left to recover after handling. The day of sampling, fish were exposed to the 5 following treatments:

No stress: blood from all fish was sampled without stress (after the fish were quickly anaesthetized with phenoxyethanol 0.1% vol)

Stress 1 and 3 h: fish were stressed by a 5 min chase with a net in their tank. One or 3 h after the end of the chase, blood was sampled on quickly anaesthetized fish (phenoxyethanol 0.1% vol).

ACTH and NaCl treatment: 10 anaesthetized fish (phenoxyethanol 0.03% vol) were given intra-peritoneal injections of porcine $ACTH_{1-39}$ (A6303, Sigma–Aldrich, St. Louis, MO) at a concentration of 5 IU/ml/kg fish (13.7 nmol/ml/kg) in NaCl 0.9% or with 0.9% NaCl (1 ml/kg fish) and immediately put back into their tank (running water). One hour after injection, blood from anaesthetized fish (phenoxyethanol 0.1% vol) was collected.

2.1.3. Experiment 3: influence of egg cortisol concentration on the stress response of 5–6 months old fingerlings

Oocytes from spring strains were fertilized as described above. After 2 min, eggs were briefly washed and incubated for 1 h in facility water containing different concentrations of cortisol (200 or 1000 ng/ml: F_2 and F_{10}) or in facility water containing only the cortisol vehicle (control batch: CT). Eggs were then washed with facility water and incubated in recirculated facility water (10 °C). To assess the cortisol changes after the egg treatment, several eggs from each of the three conditions (6–7 eggs in each of the 5 sampling replicates) were sampled at different times posttreatment (0, 1, 2, 4, 11, 15, and 25 days after treatment), and total cortisol was extracted. The remaining eggs were incubated up until the end of the yolk sac resorbtion. Fries were then transferred to small rearing tanks. At 20 g (around 6 months), fish from each treatment were divided into two groups (10 fish). Four days later, the blood of one group of fish was sampled readily without stress (anaesthetized with phenoxyethanol 0.1% vol), whereas the blood of the second group was sampled 1 h after the fish being exposed to a challenge (5 min of chase with a net in their tank).