General and Comparative Endocrinology 196 (2014) 8–16

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Social stress modulates the cortisol response to an acute stressor in rainbow trout (*Oncorhynchus mykiss*)

J.D. Jeffrey [2], M.J. Gollock [1,2], K.M. Gilmour *

Department of Biology, University of Ottawa, Ottawa, ON, Canada

ARTICLE INFO

Article history:
Received 10 June 2013
Revised 30 October 2013
Accepted 14 November 2013
Available online 22 November 2013

Keywords:
Rainbow trout
Social stress
Cortisol
HPI axis
Adrenocorticotropic hormone
Interrenal cells

ABSTRACT

In rainbow trout (*Oncorhynchus mykiss*) of subordinate social status, circulating cortisol concentrations were elevated under resting conditions but the plasma cortisol and glucose responses to an acute stressor (confinement in a net) were attenuated relative to those of dominant trout. An *in vitro* head kidney preparation, and analysis of the expression of key genes in the stress axis prior to and following confinement in a net were then used to examine the mechanisms underlying suppression of the acute cortisol stress response in trout experiencing chronic social stress. With porcine adrenocorticotropic hormone (ACTH) as the secretagogue, ACTH-stimulated cortisol production was significantly lower for head kidney preparations from subordinate trout than for those from dominant trout. Dominant and subordinate fish did not, however, differ in the relative mRNA abundance of melanocortin-2 receptor (MC2R), steroidogenic acute regulatory protein (StAR) or cytochrome P450 side chain cleavage enzyme (P450scc) within the head kidney, although the relative mRNA abundance of these genes was significantly higher in both dominant and subordinate fish than in sham trout (trout that did not experience social interactions but were otherwise treated identically to the dominant and subordinate fish). The relative mRNA abundance of all three genes was significantly higher in trout exposed to an acute net stressor than under control conditions. Upstream of cortisol production in the stress axis, plasma ACTH concentrations were not affected by social stress, nor was the relative mRNA abundance of the binding protein for corticotropin releasing factor (CRF-BP). The relative mRNA abundance of CRF in the pre-optic area of subordinate fish was significantly higher than that of dominant or sham fish 1 h after exposure to the stressor. Collectively, the results indicate that chronic social stress modulates cortisol production at the level of the interrenal cells, resulting in an attenuated cortisol response to an acute stressor.

1. Introduction

In teleost fish, as in other vertebrates, the endocrine response to a stressor encompasses both an adrenergic response, the mobilization of catecholamines, and a hypothalamic–pituitary–interrenal (HPI; hypothalamic–pituitary–adrenal, HPA, in other vertebrates) response, the synthesis and secretion of glucocorticoid hormones (reviewed by Wendelaar Bonga, 1997). In teleost fish, cortisol is the main glucocorticoid. Cortisol production in response to a stressor is initiated by activation of melanocortin 2 receptors (MC2R; Aluru and Vijayan, 2008) by adrenocorticotropic hormone (ACTH) released from the pituitary (Wendelaar Bonga, 1997; Bernier et al., 2009). Release of ACTH, in turn, reflects the action of corticotropin-releasing factor (CRF) on the corticotropes of the pituitary (reviewed by Flik et al., 2006; Bernier et al., 2009). Corticotropin-releasing factor is synthesized in the preoptic area (POA) of the brain as well as in several hypothalamic areas (Bernier et al., 2009), and its actions are thought to be regulated at least in part by CRF-binding protein (CRF-BP; Seasholtz et al., 2002; Alderman et al., 2008). Binding of ACTH to MC2R, a G protein-coupled receptor, activates adenylyl cyclase to initiate a cAMP-signalling cascade resulting in the movement of cholesterol to the inner mitochondrial membrane with the help of steroidogenic acute regulatory protein (StAR) (Hagen et al., 2006; Aluru and Vijayan, 2008).

Abbreviations: ACTH, adrenocorticotropic hormone; ANOVA, analysis of variance; BSA, bovine serum albumin; cAMP, 3′-5′-cyclic adenosine monophosphate; cDNA, complementary deoxyribonucleic acid; CRF, corticotropin-releasing factor; CRF-BP, corticotropin-releasing factor-binding protein; P450scc, cytochrome P450 side chain cleavage enzyme; DEPC, diethylpyrocarbonate; EDTA, ethylenediaminetetraacetic acid; HPA, hypothalamic–pituitary–adrenal; HPI, hypothalamic–pituitary–interrenal; L:D, light:dark; MC2R, melanocortin 2 receptor; mRNA, messenger ribonucleic acid; MEM, minimum essential medium; POA, preoptic area; PKA, protein kinase A; RM ANOVA, repeated measures ANOVA; RT-PCR, reverse transcription-polymerase chain reaction; SEM, standard error of the mean; StAR, steroidogenic acute regulatory protein.

* Corresponding author. Address: Department of Biology, University of Ottawa, 30 Marie Curie, Ottawa, ON K1N 6N5, Canada. Fax: +1 613 562 5486.
E-mail address: kgilmour@uottawa.ca (K.M. Gilmour).
[1] Present address: The Zoological Society of London, Regent's Park, London, England.
[2] J.D. Jeffrey and M.J. Gollock contributed equally to this work.

0016-6480/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ygcen.2013.11.010

Subsequent conversion of cholesterol to cortisol begins with the cleavage of cholesterol to pregnenolone by cytochrome P450 side chain cleavage (P450scc) enzyme; this step is the first and rate-limiting step in cortisol biosynthesis (Mommsen et al., 1999; Aluru and Vijayan, 2008).

Although the cortisol response to an acute stressor is thought to benefit the fish by, for example, mobilizing energy reserves, prolonged elevation of circulating cortisol levels during exposure to a chronic stressor may result in negative effects (Wendelaar Bonga, 1997). For example, both chronic stress and the administration of cortisol to mimic chronic stress have been reported to diminish the cortisol response to an acute stressor (Barton et al., 1987; Balm et al., 1994; Øverli et al., 1999; Rotllant et al., 2000a; Ings et al., 2011; Wunderink et al., 2011). The mechanisms underlying attenuation of the acute cortisol response in chronically-stressed fish remain poorly understood. Decreased interrenal cell-sensitivity to ACTH has been documented in several studies (Vijayan and Leatherland, 1990; Rotllant et al., 2000b; Sloman et al., 2002), whereas other studies failed to find an effect of cortisol administration or chronic stress on the response of interrenal cells to ACTH and pointed instead to possible effects upstream in the HPI axis, at the level of the pituitary or brain (Rotllant et al., 2000a). Thus, the objective of the present study was to investigate mechanisms at the level of the brain, pituitary and interrenal cells through which chronic stress could suppress the cortisol response to an acute stressor.

Juvenile salmonid fish confined in pairs form social hierarchies in which subordinate fish experience a sustained elevation of circulating cortisol concentrations characteristic of chronic stress (e.g. Sloman et al., 2001; reviewed by Gilmour et al., 2005). Subordinate rainbow trout (*Oncorhynchus mykiss*) exhibited (unstressed) plasma ACTH concentrations that were significantly lower than control values (Jeffrey et al., 2012), and rates of ACTH-stimulated cortisol production for *in situ* preparations from subordinate trout were lower than those for dominant trout (Sloman et al., 2002). These observations suggest that the cortisol response to an acute stressor will be compromised in subordinate trout. To test this hypothesis, the cortisol response to an acute netting stressor was evaluated in dominant versus subordinate trout. In addition, mechanisms that could attenuate the cortisol response to an acute stressor in fish experiencing chronic social stress were investigated at the level of the brain (*CRF* and *CRF-BP* mRNA abundance), pituitary (circulating ACTH levels), and interrenal cells (cortisol production *in vitro*; *MC2R*, *StAR* and *P450scc* mRNA abundance).

2. Materials and methods

2.1. Experimental animals

Female juvenile rainbow trout (*O. mykiss*; mass = 83.6 ± 1.3 g, fork length = 20.2 ± 0.1 cm, mean ± SEM, $N = 167$) obtained from Linwood Acres Trout Farm (Campbellcroft, Ontario) were transported to the University of Ottawa aquatics facility and housed in 1275 L fibreglass stock tanks. Tanks were supplied with flowing, dechloraminated, aerated city of Ottawa tap water at 13 °C. Fish were held under a 12:12 h L:D photoperiod and were fed every second day to satiation by scattering commercial trout pellets on the surface of the water. Trout were allowed to acclimate to these holding conditions, which tended to minimize hierarchy formation (e.g. use of scatter feeding, homogeneous tanks with a mild current), for at least 2 weeks prior to experimentation. All experiments complied with University of Ottawa institutional guidelines, were approved by the Animal Care Committee (protocol BL-228), and were in accordance with guidelines of the Canadian Council on Animal Care for the use of animals in research and teaching.

Social hierarchies were established in fork-length matched pairs of rainbow trout (fork-length difference averaged 2 ± 0.2 mm or 1.1% of fork length for 71 pairs in total) that were confined together for 3–5 d. Fish in a "sham" treatment group were handled identically but housed individually rather than with a conspecific. Rainbow trout were lightly anesthetized (to the point of losing equilibrium) in a solution of benzocaine (0.05 g L^{-1} ethyl *p*-aminobenzoate) and fish mass, fork length and fin damage were assessed. Fork length-matched fish were placed individually on either side of an opaque divider in a 40 L flow-through observation tank. The divider was removed after an overnight recovery period. Fish were observed twice daily for 5 min and scored for position in the tank, acts of aggression, and feeding. Fin damage was re-assessed at the end of the interaction period, and points awarded for the extent of fin damage accumulated during the interaction period as well as position, aggression and feeding were combined by principal components analysis (SPSS v16.0) to generate an overall behaviour score for each fish. The point system used was similar to that of previous studies (e.g. Metcalfe et al., 1989; Sloman et al., 2001; Jeffrey et al., 2012), and associates higher scores with more dominant traits and behaviours such as less damage to the dorsal and caudal fins (Moutou et al., 1998), occupying the preferred position in the environment, carrying out aggressive acts, and monopolizing food resources (Abbott et al., 1985; Metcalfe, 1986; Abbott and Dill, 1989; Metcalfe et al., 1989; McCarthy et al., 1992). Behavior scores typically ranged from −1 (indicative of subordinate behaviour) to +1 (indicative of dominant behaviour), and the fish within a pair that received the higher behaviour score was assigned dominant social status. Pairs in which behaviour scores differed by less than 0.5 (2% of pairs) were excluded from subsequent experimentation.

2.2. Experimental protocols

Three experimental series were conducted with the objectives of evaluating the cortisol response to an acute stressor *in vivo* (Series 1), measuring cortisol production *in vitro* (Series 2), and investigating the mRNA abundance of key genes in the HPI axis (Series 3).

2.2.1. Series 1: in vivo cortisol response

A set of 28 pairs of trout was used for this experiment (mass = 82.1 ± 2.2 g, fork length = 19.6 ± 0.1 cm, $N = 56$ fish; for pairs, fork-length difference = 0.8 ± 0.1%, behaviour score difference = 1.75 ± 0.13, $N = 28$ pairs). After 3 d of confinement in pairs, trout were lightly anaesthetized (as above) and a 250 μL blood sample was withdrawn by caudal puncture. Fish were then returned to their tank for a 24 h recovery period, following which a second blood sample was withdrawn by caudal puncture but following terminal anaesthesia (0.5 g l^{-1} ethyl *p*-aminobenzoate), either immediately upon removal from the tank ('control' fish), or at the end of 1 h of confinement in a net ('netting stressor'). The members of a pair were always removed from and returned to the tank at the same time, but were confined individually in nets for the netting stressor. Blood samples were collected using a syringe rinsed with 0.5 M Na_2-ethylenediaminetetraacetic acid (Na_2-EDTA) as an anti-coagulant, and were immediately centrifuged (13,200*g* for 3 min) to yield plasma that was flash frozen in liquid N_2 and stored at −80 °C for later analysis of cortisol, ACTH and/or glucose concentrations. Cortisol and ACTH concentrations were measured using commercial radioimmunoassay kits (MP Biomedical) previously validated for analysis of trout plasma samples (Gamperl et al., 1994; Doyon et al., 2006). For cortisol, intra-assay variation (% CV) was 7.3% and inter-assay variation was 6.9%. For