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Is plasma cortisol response to stress in rainbow trout regulated by catecholamine-induced hyperglycemia?

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ABSTRACT

Based on previous studies we hypothesize that under stress conditions catecholamine-induced hyperglycemia contributes to enhance cortisol production in head kidney of rainbow trout. Therefore, treatment with propranolol (β-adrenoceptor blocker) should reduce the hyperglycemia elicited by stress and, therefore, we expected reduced glucosensing response and cortisol production in head kidney. Propranolol treatment was effective in blocking most of the effects of catecholamines in liver energy metabolism resulting in a lower glycemia in stressed fish. The decreased glycemia of stressed fish treated with propranolol was observed along with reduced transcription of genes involved in the cortisol synthetic pathway, which supports our hypothesis. However, changes in putative glucosensing parameters assessed in head kidney were scarce and in general did not follow changes noted in glucose levels in plasma. Furthermore, circulating cortisol levels did not change in parallel with changes in glycemia. As a whole, the present results suggest that glycemia could participate in the regulation of cortisol synthetic pathways but other factors are also likely involved. Propranolol effects on trout stress response were different depending on time passed after stress onset; the direct or indirect involvement of catecholaminergic response in the regulation of cortisol production and release deserves further investigation.

1. Introduction

A well established paradigm in stress physiology is that corticosteroids stimulate glucose production to fuel metabolic processes for reestablishing homeostasis (Wendelaar Bonga, 1997). However, an allostatic control of cortisol levels requires adjustment of set points for cortisol production and release. Levels of circulating metabolites like glucose could be involved in such a mechanism through either inhibitory or stimulatory effects. In a previous study, in order to elucidate the effects of circulating glucose on cortisol production, we incubated head kidney of rainbow trout at different glucose concentrations and monitored cortisol production under ACTH stimulation (Conde-Sieira et al., 2013). We observed that glucose levels modulate the release of cortisol from head kidney in the presence of ACTH. An increased release occurred under high glucose levels, and a decreased release occurred in the presence of cytochalasin B, an inhibitor of transmembrane glucose transport. The relationship between circulating glucose levels and cortisol production suggests that under stress conditions glucose is important for the regulation of cortisol synthesis and release.

The initial step of the stress response in fish results in a surge of plasma catecholamines adrenaline and noradrenaline (Wendelaar Bonga, 1997). The rise in circulating catecholamines results in hyperglycemia due primarily to the activation of hepatic β-adrenoceptors and glycogenolysis (Fabbri et al., 1998). As commented above, based on a previous *in vitro* study (Conde-Sieira et al., 2013), we hypothesize that this catecholamine-induced hyperglycemia contributes to enhance cortisol production in head kidney interrenal cells under stress conditions. To test this hypothesis, rainbow trout were treated with propranolol, an effective β-adrenoceptor blocker in this species (Wright et al., 1989; Dugan and Moon, 1998; Weber and Shanghavi, 2000; Dugan et al., 2003) and exposed to acute stress (chasing for 5 min). Our hypothesis predicts that propranolol would reduce the hyperglycemia elicited by stress and, therefore, a reduced glucosensing response and cortisol production should occur in head kidney. Since cortisol response to stress is usually fully developed in no less than 15 min (Mommsen et al., 1999; Gesto et al., 2013; López-Patiño et al., 2014), and the cortisol elevation could also influence plasma glucose levels (Wendelaar Bonga, 1997), we carried out a second

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experiment applying the same stress protocol, but allowing the fish recover for 10 additional minutes before sacrifice. Also, in this second experiment, we included two additional treatment groups (glucose; propranolol + glucose) in order to corroborate if glucose treatment could counteract the effects induced by propranolol. In both experiments, we evaluated several stress markers in plasma (levels of cortisol, adrenaline, noradrenaline, glucose, and lactate) and liver (glycogen levels, and the activities and/or mRNA levels of fructose 1,6-bisphosphatase (FBPase), glucose 6-phosphatase (G6Pase), glucokinase (GK), pyruvate kinase (PK), glycogen phosphorylase (GPase), and glycogen synthase (GSase)). In head kidney we monitored parameters related to glucosensing (Polakof et al., 2011), such as glycogen levels, activities of GK, PK, FBPase, and GSase, and mRNA abundance of GK, and components of ATP-dependent inward rectifier potassium channel (K^+_{ATP}) such as pore type 6.x-like (Kir6.x-like), and sulfonylurea receptor-like (SUR-like). Finally, we also monitored in head kidney parameters related to cortisol synthesis including mRNA abundance of 3β-hydroxysteroid dehydrogenase (3βHSD), 11β-hydroxylase (11βH), cytochrome P450 cholesterol side chain cleavage (P450scc), and steroidogenic acute regulatory protein (StAR).

2. Materials and methods

2.1. Fish

Rainbow trout (*Oncorhynchus mykiss* Walbaum) were obtained from a local fish farm (A Estrada, Spain). Fish were maintained for 15 days under laboratory conditions on a 12:12 L:D photoperiod in dechlorinated tap water at 15 °C. Fish mass was 101 ± 4 g at the beginning of the experiment. Fish were fed once daily (10:00 h) to satiety with commercial dry fish pellets (Dibaq-Diproteg; proximate food analysis was 48% crude protein, 14% carbohydrates, 25% crude fat, and 11.5% ash; 20.2 MJ/kg of feed). The experiments described comply with the Guidelines of the European Union Council (2010/63/EU), and of the Government of Spain (RD55/2013) for the use of animals in research. Animal protocols were approved by the animal care committee of the University of Vigo.

2.2. Experimental design

Fish were randomly assigned to 100 L experimental tanks (two tank replicates per treatment), and acclimated for 15 days. Following acclimation, fish were fasted for 24 h before treatment to ensure basal hormone and metabolite levels were achieved. In experiment 1, fish were anesthesized within tanks with 2-phenoxyethanol (0.2% v/v), and IP injected once with 5 μL g^{-1} body mass of saline alone (control and stress groups), or saline containing propranolol (1 mg kg^{-1} body mass, propranolol from Sigma Chemical Co.). In experiment 2, two additional groups were added, one injected with glucose (200 mg kg^{-1}) and another injected with glucose and propranolol (at the same final concentrations mentioned above). The concentration of propranolol was similar to that effectively used in previous studies in the same species (Wright et al., 1989; Dugan and Moon, 1998), and we evaluated in preliminary experiments that treatment was effective in blocking hyperglycemic effects of stress (data not shown). The concentration of glucose used in experiment 2 was evaluated in preliminary experiments (data not shown) to induce increased glucose levels in the range typically observed under acute stress conditions. Immediately after injection, fish were returned to their tanks. All fish remained undisturbed for 1 h. After 1 h, in experiment 1 stressed groups were disturbed by chasing for 5 min whereas non-stressed groups in separate tanks remained undisturbed. Therefore, the 4 experimental groups (2 tank replicates/treatment) used in experiment 1 were: (1) control (non-stressed) fish (C); (2) non-stressed fish treated with propranolol (P); (3) fish stressed for 5 min (S); and, (4) fish treated with propranolol and stressed for 5 min (S + P). In experiment 2 stressed groups were also disturbed by chasing for 5 min and followed by 10 min of resting whereas non-stressed groups were undisturbed. Therefore, the 6 experimental groups (2 tank replicates/treatment) used in experiment 2 were: (1) control (non-stressed) fish (C); (2) non-stressed fish treated with propranolol (P); (3) fish stressed for 5 min that rested for 10 min (S); (4) fish treated with propranolol, stressed for 5 min, and rested for 10 min (S + P); (5) fish treated with glucose, stressed for 5 min, and rested for 10 min (S + G); and, (6) fish treated with glucose and propranolol, stressed for 5 min, and rested for 10 min (S + G + P).

For each sampling, fish were anesthesized in tanks with 2-phenoxyethanol (0.2% v/v) and sampled. Blood was collected by caudal puncture with an ammonium-heparinized syringe, and plasma samples were obtained after blood centrifugation, and divided into two aliquots. One aliquot was immediately frozen on liquid nitrogen for the assessment of plasma cortisol levels, whereas the other aliquot, for the assessment of plasma metabolites was deproteinized immediately (0.6 M perchloric acid) and neutralized (with 1 M potassium bicarbonate) before freezing on liquid nitrogen. Fish were rapidly sacrificed by decapitation, and liver and head kidney were taken, and snap-frozen in liquid nitrogen. All samples were stored at −80 °C until assayed. In each group, 10 fish were used to assess enzyme activities and metabolite levels whereas 6 fish were used for the assessment of mRNA levels by qRT-PCR.

2.3. Analytical procedures

2.3.1. Assessment of hormone and metabolite levels in plasma

Levels of glucose and lactate in plasma were determined enzymatically using commercial kits (Biomérieux, Grenoble, France, for glucose; Spinreact, Barcelona, Spain for lactate) adapted to a microplate format. Plasma cortisol levels were assessed by ELISA using a commercially available kit (Cayman, Ann Arbor, MI, USA).

Levels of adrenaline and noradrenaline were assessed in plasma by HPLC with electrochemical detection after plasma deproteinization followed by solid-phase extraction (SPE) as previously described (Gesto et al., 2013). A deproteinized plasma aliquot was diluted to 1 mL in ultrapure water to be used for the SPE procedure. The SPE cartridges (1 mL–100 mg tubes; Discovery® DSC-WCX, Supelco) were conditioned with 1.5 mL of ultrapure water at a flow rate of 5 mL/min. The samples were then placed in the conditioned columns at 1 mL/min, and the columns washed twice with 1 mL of ultrapure water (5 mL/min). Finally, the catecholamines were eluted from the columns with two 400 μL aliquots of 0.3 M $HClO_4$ at 1 mL/min. Recoveries for noradrenaline and adrenaline exceeded 97%. Aliquots of 20 μL of these eluates were directly injected into the HPLC system, which was equipped with a Jasco PU-2080 Plus pump, a 5 μm analytical column (Phenomenex, Nucleosil C18, 150 mm length × 4.6 mm diameter) and a ESA Coulochem II detector. The detection system included a double analytical cell (M5011) with oxidation potentials set at +40 mV (first electrode) and +400 mV (second electrode). The mobile phase consisted of 25 mM citric acid, 25 mM Na_2HPO_4, 25 μM Na_2EDTA, 0.21 mM sodium 1-octanesulfonate, and 1% (v/v) methanol; pH was adjusted to 3.4 with ortho-phosphoric acid (before the addition of methanol), filtered (0.20 μm filter, Millipore, Bedford, USA), and degasified under vacuum before use. Analytical run time was 10 min at an isocratic flow rate of 1.3 mL min^{-1} at room temperature. The sample peaks were quantified by comparing peak areas to those of appropriate external standards. The detection limits for the catecholamines were 3 pg of noradrenaline and 5 pg of

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