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The role of CRH in behavioral responses to acute restraint stress in zebrafish

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ABSTRACT

In teleosts, changes in swimming, exploring, general locomotor activity, and anxious state can be a response to stress mediated by the corticotropin-releasing hormone system activation and its effects on glucocorticoid levels. Zebrafish has been widely used to study neuropharmacology and has become a promising animal model to investigate neurobehavioral mechanisms of stress. In this report the animals were submitted to acute restraint stress for different time lengths (15, 60 and 90 min) for further evaluation of behavioral patterns, whole-body cortisol content, and corticotropin-releasing hormone expression. The results demonstrated an increase in the locomotor activity and an alteration in the swimming pattern during a 5-min trial after the acute restraint stress. Interestingly, all groups of fish tested in the novel tank test exhibited signs of anxiety as evaluated by the time spent in the bottom of the tank. Whole-body cortisol content showed a positive correlation with increased behavioral indices of locomotion in zebrafish whereas molecular analysis of corticotropin-releasing hormone showed a late reduction of mRNA expression (90 min). Altogether, we present a model of acute restraint stress in zebrafish, confirmed by elevated cortisol content, as a valid and reliable model to study the biochemical basis of stress behavior, which seems to be accompanied by a negative feedback of corticotropin-release hormone mRNA expression.

1. Introduction

Zebrafish (*Danio rerio*) has been a popular model organism for developmental biology and genetics studies for more than three decades (Sison et al., 2006; Streisinger et al., 1991). However, in the last decade, zebrafish has become a focus of behavioral neuroscience (Bencan et al., 2009; Egan et al., 2009; Miklosi and Andrew, 2006; Salas et al., 2006; Wong et al., 2010). Zebrafish maintains a typical vertebrate system complexity since it possesses all classical vertebrate neurotransmitters and its neuroendocrine system provides robust physiological responses to stress (Alsop and Vijayan, 2008; Behra et al., 2002; Boehmler et al., 2004; Edwards and Michel, 2002; Kaslin and Panula, 2001; Kim et al., 2004; Kucenas et al., 2003; Rink and Guo, 2004).

In zebrafish the stress system is represented by the hypothalamus–pituitary–interrenal (HPI) axis and similarly, to the mammalian hypothalamus–pituitary–adrenal (HPA) axis, controls the circulating cortisol levels (Alderman and Bernier, 2007, 2009; Alsop and Vijayan, 2008, 2009). Activation of this system initiates at the hypothalamus, which receives inputs transmitted from central and peripheral nervous systems. A stressful signal stimulates secretion of hypothalamic corticotropin-releasing hormone (CRH). In response to CRH, the pituitary releases adrenocorticotropic hormone (ACTH) into the bloodstream, which reaches the head kidney of fish (homologous to the adrenal gland in mammals) and releases cortisol that binds to glucocorticoid receptor (GR). This intricate signaling system resembles the human neuroendocrine system both in complexity and regarding cortisol utilization (as opposed to corticosterone in rodents), reinforcing the contribution of zebrafish to studies on the neurobiology of stress.

Advances in the field of comparative stress physiology suggest that the CRH system plays a prominent role in regulating and integrating neuroendocrine, autonomic, immune, and behavioral responses to stressors in vertebrates (Contarino et al., 1999, 2000; Crespi and Denver,

Abbreviations: CRH, corticotropin-releasing hormone; HPI, hypothalamus-pituitary-interrenal; HPA, hypothalamus-pituitary-adrenal; GR, glucocorticoid receptors; ACTH, adrenocorticotropic hormone; PVN, paraventricular nuclei; CEUA-PUCRS, The Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul; COBEA, Brazilian Collegium of Animal Experimentation; CCAC, Canadian Council for Animal Care; PBS, phosphate buffered saline; RT-PCR, reverse transcription-polymerase chain reaction.

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2004; Heinrichs and Koob, 2004; Smagin et al., 2001). Studies have been designed to describe potential neural mechanisms underlying CRH-mediated behavioral responses in vertebrates, investigating particularly a role for brainstem neurotransmitter systems capable of modulating CRH-induced behavior (Carpenter et al., 2007; Clements et al., 2003; Clements and Schreck, 2004).

A role for CRH in stress-related regulation of HPI axis has been supported by findings that stress increases the CRH mRNA expression or protein within the non-mammalian homologue of the paraventricular nucleus, the preoptic area (Doyon et al., 2003, 2005; Huising et al., 2004). Recently, Alderman & Bernier (2007) showed a remarkably widespread mRNA distribution pattern of CRH in the brain of the zebrafish, with many examples of unique and common expression sites. Although genome duplication events in teleosts are very interesting from the evolutionary and comparative point of view, zebrafish has become a widely popular species in research on the corticosteroid stress axis. The loss of duplicate genes is not a general feature of the zebrafish genome, but zebrafish have lost the duplicate genes for HPI components, CRH (Chandrasekar et al., 2007), ACTH (De Souza et al., 2005) and glucocorticoid receptors (GR) (Alsop and Vijayan, 2008; Schaaf et al., 2008).

Although CRH has been described as promoting increased locomotor behavior in Chinook salmon (Clements et al., 2003; Clements and Schreck, 2004), there are no reports evaluating the relationship between CRH and acute stress effects on distinct behavior patterns in zebrafish. Here we present a new, rapid, and effective acute restraint stress protocol for zebrafish to determine behavioral patterns of swimming in response to acute stressor stimuli. Furthermore, we verified the effect of acute restraint stress on CRH mRNA expression in the zebrafish whole brain and evaluated the effectiveness of the protocol by measuring cortisol levels.

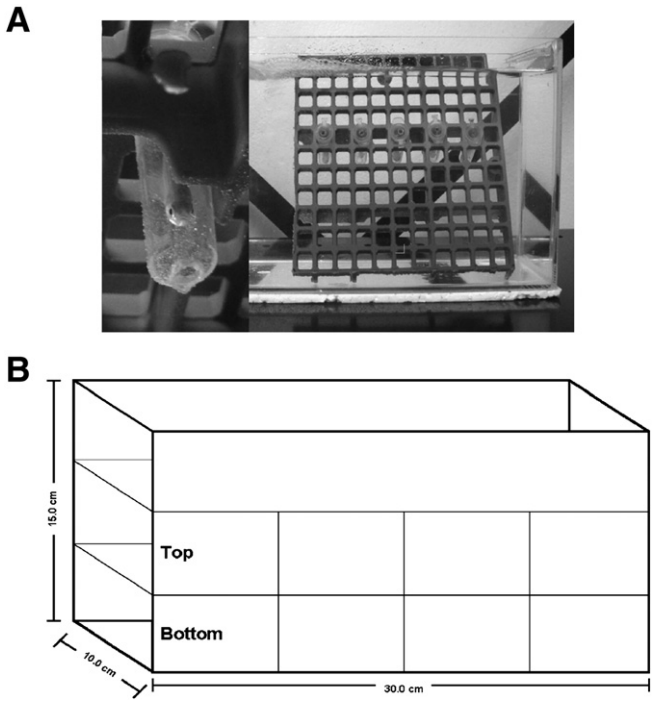

Fig. 1. (A) Picture represents the procedure of the acute restraint stress, showing animals inside the plastic tubes in a stand pipe at home tank; (B) The apparatus consisted in a rectangular glass tank with the specific dimensions described above and virtual divisions were used to evaluation of zebrafish swimming activity in the novel tank test, with two vertical areas (bottom and top) and eight horizontal sections with 4 sections per area.

2. Material and methods

2.1. Animals

The animals were 6 to 9-month-old adult zebrafish (*Danio rerio*) (3–5 cm) of both sexes of heterogeneous wild-type stock (standard short-fin phenotype) obtained from a local commercial supplier (Redfish, RS, Brazil). All fish were acclimated for at least two weeks in the experimental room and housed in groups of 20 fish in 15 l heated (28 ± 2 °C) tanks with constant aerated water. Fish were kept on a 14–10 h day/night cycle and fed three times a day with commercial flakes (TetraMin®) and supplemented with live brine shrimp.

All procedures with animal subjects have been approved by The Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul (09/0126, CEUA-PUCRS) and followed Brazilian legislation, the guidelines of the Brazilian Collegiums of Animal Experimentation (COBEA), and the Canadian Council for Animal Care (CCAC) Guide on the Care and Use of Fish in Research, Teaching, and Testing.

2.2. Acute restraint stress protocol

This experiment consisted in keeping each animal enclosed into microcentrifuge plastic tubes of 2 ml with the cap closed and small openings in both ends to allow free water circulation inside the tube and completely avoid fish locomotion. Fish handling by mesh were carefully placed into plastic tubes containing water and were kept in a stand pipe inside the home tank during the stress protocol (Fig. 1A). Group of 10 animals were submitted to different time lengths of acute restraint stress (15, 60 or 90 min inside the tube) to establish the protocol use. A control group was maintained in the same experimental conditions being handling with the mesh but with no restraint stress. Separated sets of control and restrained animals were used to perform behavioral, biochemical, and molecular analyses. Aeration (8 ppm, Labcom Test® – Camburiú, SC, Brazil) and water temperature (26 ± 2 °C) were controlled throughout the test. Stressed animals were tested immediately after acute restraint stress periods on behavioral task. On each session, control and stressed animals were gently captured from the housing tank using a 6 cm wide fine nylon mesh fish net and those undergoing stress were carefully placed in the behavioral apparatus.

2.3. Apparatus and behavioral testing

Immediately after acute restraint stress, control and stressed animals were carefully placed individually into the novel tank, representing a 1.5 L rectangular tank (30 cm length × 15 cm height × 10 cm width) as previously described for the zebrafish novel tank test (Gerlai et al., 2000) (Fig. 1B). The behavioral test was performed during the same time frame each day (between 10:00 am and 4:00 pm). Animals were first habituated to the apparatus for 30 s and then behavioral activity was recorded over a period of 5 min.

A digital webcam plugged to a computer to record and analyze the videos (Quick cam Pro 9000, LOGITECH) was placed 40 cm from the testing tank to ensure that the apparatus was within the camera vision range and it was used to monitor the location and swimming activity of the fish. The behavioral analysis was performed in a laptop computer using ANY-Maze® recording software (Stoelting CO, Wood Dale, IL, USA) to track the swimming activity of the animals at a rate of 30 frames/s as recently described (Egan et al., 2009; Rosemberg et al., 2011; Seibt et al., 2010). The testing tank was virtually divided into one horizontal and four equally vertical areas in order to evaluate the exploratory activity.

The absolute turn angle represents the sum of all vectors angle of movements created from one position to animal's center point to the next. The anti-clockwise movement was considered negative and clockwise movement positive (−180° to 180 °C). From this measure we calculated the meandering, which is the result of the absolute turn angle divided by the total distance travelled, and the angular velocity, represented by absolute turn angle divided by the test duration. The evaluation

of the exploratory activity of zebrafish was performed by determining the number and the time of transitions between horizontal and vertical areas.

2.4. Cortisol extraction and quantification

Cortisol extraction procedure was modified from Barcellos et al. (2007). Briefly, after the different periods of restraint stress or control condition, fish were immediately frozen in liquid nitrogen and stored at −80 °C until the cortisol extraction. Each zebrafish was weighed, and a pool of three fish were minced and placed into a disposable stomacher bag with 2 ml of ice-cold phosphate buffered saline (PBS pH 7.4) for 6 min. Bag contents were transferred to a 10-ml screw top disposable test tube and 5 ml of laboratory grade ethyl ether was added. This tube was vortexed for 1 min, centrifuged for 10 min at 3000 rpm, and immediately frozen at liquid nitrogen. Unfrozen portion (ethyl ether containing cortisol) was decanted. The ethyl ether was transferred to a new tube and completely evaporated under a gentle stream of nitrogen for 2 h, yielding a lipid extract containing the cortisol. After ether evaporation, the cortisol was reconstituted in l mL of PBS buffer and the extract was stored at −20 °C. To quantify cortisol concentration, ELISA was performed using a high sensitivity human salivary cortisol immunoassay kit commercially available (Salimetrics®, USA). The specificity of the test was evaluated by comparing the parallelism between the standard curve and serial dilutions in PBS (pH 7.4) of the tissue extracts. The standard curve constructed with the human standards ran parallel to that obtained using serial dilutions of zebrafish tissue extracts. High positive correlation ($R^2 = 0.9818$) was found between the curves after linear regression test. The intra-assay coefficient of variation was 3.33–3.65%. The cortisol levels were expressed in $ng.g^{-1}$ of tissue.

2.5. Gene expression analysis by RT-PCR experiments

Immediately after acute restraint stress, groups of animals (control and stressed fish) were cryoanaesthetized and euthanized (Wilson et al., 2009). The brains were removed by dissection and were isolated for analysis of gene expression. Total RNA was extracted with TRIzol reagent (Invitrogen, USA) in accordance with the manufacturer's instructions.

cDNA (160 ng/μl) species were synthesized with SuperScript™ III First-Strand Synthesis SuperMix (Invitrogen, USA) following methods recommended by the supplier. RT reactions were performed for 50 min at 42 °C. cDNA (1 μl) was used as a template for PCR with the specific primer for CRH. β-actin-PCR was performed as a cDNA synthesis control. PCR reactions were performed (total volume of 25 μl) using a concentration of 0.4 μM of each primer indicated below and 1 U platinum Taq DNA polymerase (Invitrogen, USA) in the supplied reaction buffer. Conditions for CRH PCR were as follows: initial 1 min denaturation step at 94 °C, 1 min at 94 °C, 1 min annealing step at 60 °C, 1 min extension step at 72 °C for 25 cycles and a final 10 min extension at 72 °C. Conditions for β-actin PCR were as follows: initial 1 min denaturation step at 94 °C, 1 min at 94 °C, 1 min annealing step at 54 °C, 1 min extension step at 72 °C for 35 cycles and a final 10 min extension at 72 °C. The zebrafish sequence encoding to CRH was retrieved from the GenBank database (NM_001007379) and used for searching specific primers, which were designed using program Oligos 9.6. In order to confirm the primer specificity, each primer was compared with the zebrafish genome and it was able to recognize only its specific target sequence. Thus, the strategy adopted to construct the primers did not allow cross-amplification. The following set of primer was used: for CRH: forward 5′-TCG TCA CCA CGG TGG CTC TGC TCG-3′; and reverse 5′- CAG ATG AAA GGT CAG ATC TAG GGA AAT CG-3′; for β-actin: forward 5′- GTC CCT GTA CGC CTC TGG TCG-3′; and reverse 5′-GCC GGA CTC ATC GTA CTC CTG-3- [43]. The amplification products were: CRH – 383 bp and β-actin 678 bp. PCR products were separated by electrophoresis with a 1% agarose gel. Relative abundance of mRNA versus β-actin was determined by densitometry using freeware ImageJ 1.37 for Windows.

2.6. Statistical analysis

Statistical analyses were performed using Graph Pad InStat 3.00 statistical package. To examine the behavioral effects of acute restraint stress, CRH mRNA expression, and cortisol levels, one-way ANOVA was conducted followed by Tukey's post hoc test, considering time lengths of acute restraint stress as a factor. The data are presented as mean ± S.E.M. and significant difference was attributed to p values less than 0.05.

3. Results

3.1. Behavioral assessment

Distinct parameters of zebrafish swimming activity were evaluated in the 5-min novel tank test. We observed that the time spent in the bottom of the tank across the test is similar for control and stressed animals ($F[3,36] = 0.02$; $p = 0.99$) (Fig. 2A). As indexed by the number of line crossings in the apparatus, there was increased locomotor activity of animals submitted to acute restraint stress during 60 (84%), and 90 min (79%) when compared to the control group (165 ± 19 line crossings; $F[3,36] = 4.12$; $p<0.05$) (Fig. 2B). In Fig. 2C we showed an increase in distance travelled after 90 min (169%) of acute restraint stress compared to the control animals (12 ± 1.6 m; $F[3,36] = 8,02$; $p<0.01$).

We analyzed both the mean and maximum speed that fish could achieve during the behavioral assessment. In Fig. 2D, we demonstrated an enhancement in mean speed just for animals stressed for 90 min (134%) (control group, 0.047 ± 0.006 m/s; $F[3,36] = 5,9$; $p<0.01$). However, there was a significant increase in maximum speed for animals submitted to acute restraint stress during 60 (470%) and 90 min (557%) (control group, 0.3 ± 0.04 m/s; $F[3,36] = 4.8$; $p<0.01$) (Fig. 2E).

The absolute turn angle showed a significant increase in degree turned in fish exposed to 60 (46%) and 90 min (61%) of stress when compared to the control group (52660 ± 5703 degree; $F[3,36] = 6,8$; $p<0.001$) in 5-min of task evaluation (Fig. 2F). Based on the absolute turn angle values, we determined the angular velocity of animals as a method to distinguish routine from escape turns since the angular velocity for normal routines never exceeded degree of magnitude values and meandering (Budick and O'Malley, 2000). The angular velocity measurement followed the increased absolute turn angle, being higher in animals stressed for 60 (46%), and 90 min (61%) when compared to the control group (175 ± 19 degree/s; $F[3,36] = 6,8$; $p<0.001$) (Fig. 2F) and meandering decreased after 60 (61%) and 90 min (60%) compared to the control animals (4731 ± 353 degree/m; $F[3,36] = 6,5$; $p<0.001$) (Fig. 2G).

3.2. Cortisol content and CRH mRNA expression

We verified that whole-body cortisol levels were increased at 15 (9.2 ± 0.6 $ng.g^{-1}$ of tissue), 60 (8.5 ± 0.9 $ng.g^{-1}$ of tissue) and 90 (9.7 ± 0.4 $ng.g^{-1}$ of tissue) min of acute restraint stress compared to the control animals (5.2 ± 1 $ng.g^{-1}$ of tissue; $F[3,36] = 7,6$; $p<0.001$) (Fig. 3). However, the expression patterns of brain CRH mRNA remained unaltered during the first 60 min of restraint stress and decreased at 90 min (71 ± 6 arbitrary units) of restraint stress compared to all other animal groups (154 ± 9.3 arbitrary units control group; $F[3,12] = 28,1$; $p<0.0001$) (Fig. 4).

4. Discussion

Comparative approaches to understanding the relationships between neuropeptides and specific behavioral responses often provide unique opportunities to investigate the neural mechanisms involved

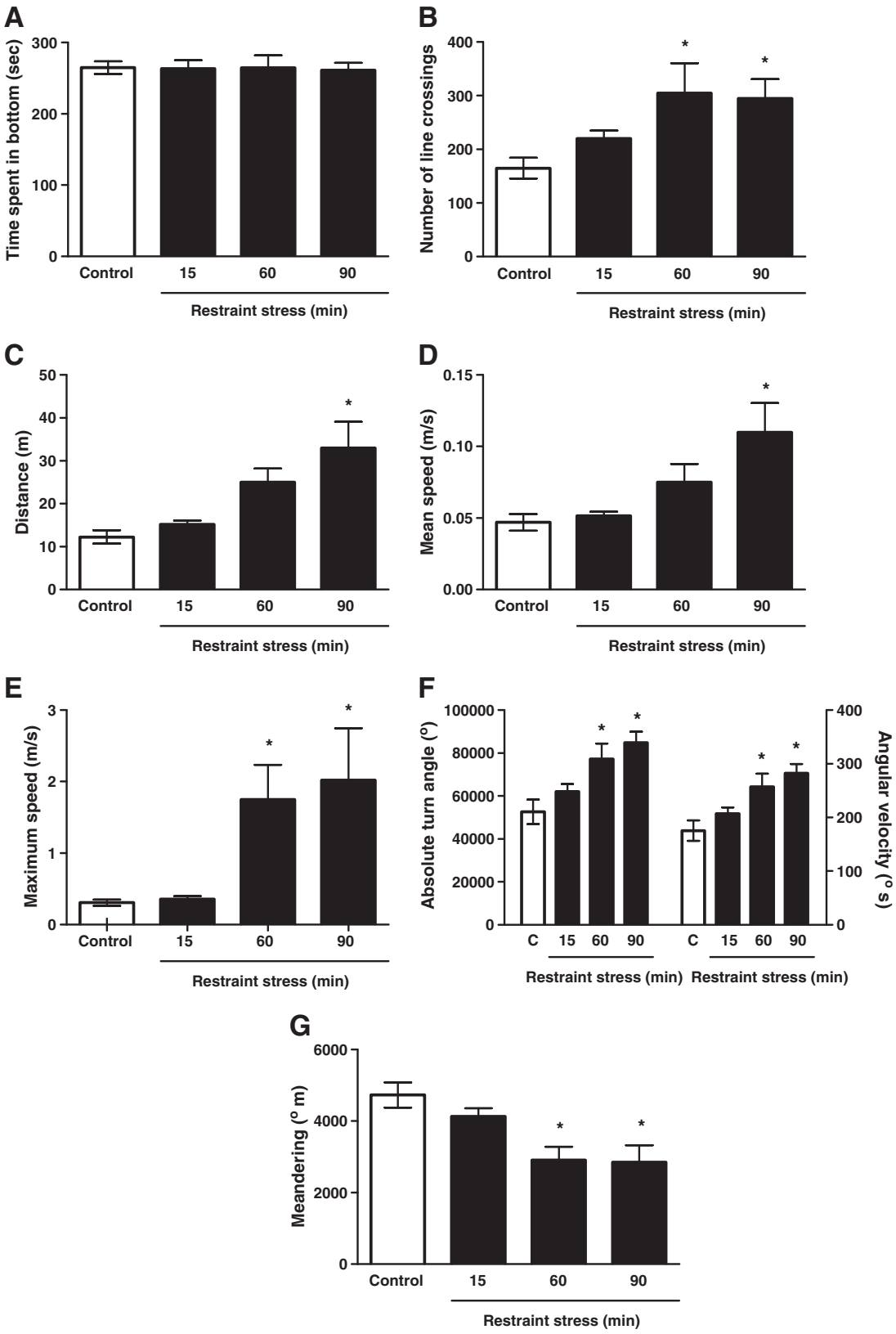

Fig. 2. Effect of different time lengths of exposure (15, 60, and 90 min) to acute restraint stress on time spent in the bottom (A), number of line crossings (B), distance traveled (C), mean speed (D), maximum speed (E), absolute turn angle and angular velocity (F), and meandering (G) during 5 min of video recording. Data are representative of 10 animals per group, presented as mean ± S.E.M. Data were statistically analyzed by One-way ANOVA followed by Tukey's post-hoc test. $^{*}p<0.05$ denotes a significant difference from the control group.

and the ethological relevance of neuropeptide action. Regarding to CRH-induced behavioral responses, there is a remarkable degree of conservation among vertebrates with respect to the effects of CRH on several behaviors that have been studied including, notably, ingestive and reproductive behaviors (Bernier and Peter, 2001; Crespi and Denver, 2004; Dunn and Berridge, 1990; Volkoff et al., 2005).

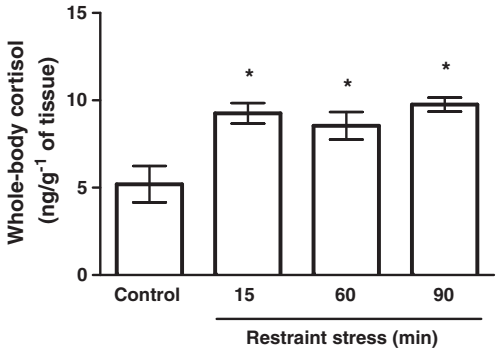

Fig. 3. Whole-body cortisol levels of zebrafish evaluated in samples of control and animal exposure to different time lengths (15, 60, and 90 min) of acute restraint stress. The data were expressed as means ± S.E.M. and analyzed statistically by One-way ANOVA followed by Tukey's post-hoc test. $^{*}p<0.05$ denotes a significant difference from the control group.

One of the most profound and reproducible effects of CRH in vertebrates is the effect on behavioral arousal and locomotor activity (Clements et al., 2002, 2003; Volkoff et al., 2005). Although CRH has been described as promoting increased locomotor behavior, there are no reports evaluating the relationship between CRH and acute stress effects on distinct behavior patterns in fishes. In this work, initially, we exhibited evidence that acute restraint stress in zebrafish is an effective model to promote significant behavior alterations in a time-dependent manner as demonstrated by increased number of line crossings, distance traveled, mean and maximum speed.

Considering the importance of routine turning and its pervasiveness throughout zebrafish life history, we analyzed the absolute turn angle as a measure of routine turns, and showed an increase restraint stress-induced in the routine turns. The turns analyzed here showed that angular velocity of control animals did not exceed 180º in magnitude, but all turns of stressed animals presented angular velocities above this degree. In this context, Budick & O'Malley (2000) stated that in larval zebrafish the best way to distinguish routine from escape turns is to compare angular velocities, since routine turns always had

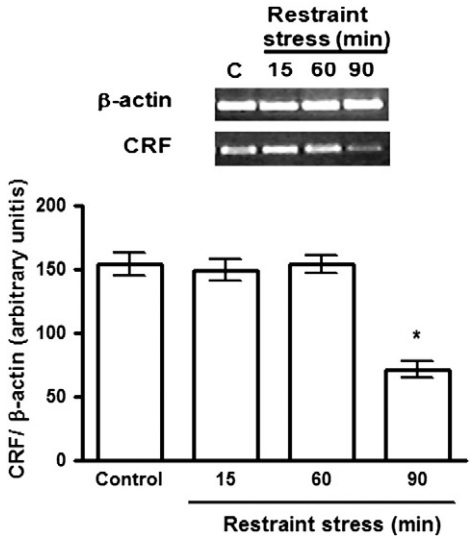

Fig. 4. CRH and β-actin mRNA expression in zebrafish brain. RT-PCR experiments were conducted in samples of control and animal exposure to different time lengths (15, 60, and 90 min) of acute restraint stress. The figure shows a representative gel and the CRH/β-actin mRNA ratio (expressed as arbitrary units) obtained by optical densitometry analysis of four independent experiments, with entirely consistent results. The data were expressed as means ± S.E.M. and analyzed statistically by One-way ANOVA followed by Tukey's post-hoc test. $^{*}p<0.05$ denotes a significant difference from the control group.

angular velocities below 180°. This behavioral parameter emerges to demonstrate a new methodological tool to evaluate stress response, considering that changes in turn angle demonstrate a disorganized pattern of swimming, which could be a response to stress. Indeed, the evaluation of these behavioral alterations in other zebrafish stress models would be relevant to elucidation of such behavioral mechanisms.

Zebrafish in response to novelty display an increase in exploratory behavior, time spent in the top and a decrease in freezing over time (Wong et al., 2010). All these parameters represent behavioral signs of anxiety as evaluated with the anxiolytic and anxiogenic manipulations in a 6-min novel tank test (Cachat et al. 2010; Wong et al., 2010). Although, our results showed an anxious state similar to novelty stress during the 5-min novel tank test evaluated as the time spent in the bottom, the results revealed that control animals exhibit signs of anxiety to the new environment similar to animals submitted to acute restraint stress. These findings support the idea that changes in behavioral activity are not produced by the anxious state, but due to the acute stress model used in this work.

CRH mRNA expression and related peptides are known to be highly expressed in preoptic and tuberal nuclei of the hypothalamus of zebrafish, giving support to the role in regulating hypophysial secretion, while the distribution in other brain regions of the hindbrain and forebrain suggests involvement in the autonomic and behavioral functions (Alderman and Bernier, 2007). The specific site or sites within the central nervous system that mediate CRH-induced increases in locomotor activity are not clear. However, CRH effects on locomotor activity are likely the product of direct and indirect actions on neural systems, including brainstem neuromodulatory systems that together facilitate behavioral response. Here we evaluated the whole brain CRH gene expression, which can underestimate punctual CRH mRNA levels alterations. However, even in this general measure, a pronounced reduction in CRH mRNA expression was observed at 90 min after acute restraint stress. Some studies have showed the involvement of endogenous CRH or CRH-related neuropeptides in behavioral responses to stressors by administration of CRH receptor antagonists (Crespi et al., 2004; Crespi and Denver, 2004; Lowry and Moore, 1991). Moreover, it is not clear which of the endogenous CRH-related neuropeptides are involved in stress-related behavioral activation. Carpenter et al. (2007) suggested that CRH-induced anxiogenic locomotion in rainbow trout was not dependent of cortisol levels but positively correlated with serotonergic and dopaminergic function in specific brain areas. Conversely in rodents, Weninger et al. (1999) propose that CRH was not the neuropeptide involved in stress-induced behavior tested in the open field environment. Likewise, Overli et al. (2002) demonstrated that increased locomotor activity in teleost fish can be mediated in a time and context-dependent way by cortisol and not by CRH.

The secretion of glucocorticoids is a classic endocrine response to stress. In this way, we showed high level of whole-body content cortisol in all stressed animals as a physiological marker of stress and anxiety, confirming the acute restraint stress protocol presented here as a valid model of stress in zebrafish. Stressful stimuli activate a brain stress network and stimulate the sequential release of hypothalamic CRH, ACTH and thence glucocorticoids. After stimulation, glucocorticoids begin to rise in plasma between 3 and 20 min until concentrations attaining maximal values (Dallman, 2005; Sapolsky et al., 2000).

Considering this close relationship between CRH system and cortisol levels we evaluated the CRH mRNA expression from the whole brain of stressed and control zebrafish. Interestingly, our results showed that just at 90 min of acute restraint stress an alteration in CRH mRNA expression was detected. In fact, a decrease of 71% was registered suggesting that CRH mRNA levels, in the longest period of acute restraint stress, could be regulated by the increased glucocorticoid levels, probably by a genomic feedback. Results from goldfish with high levels of circulating cortisol, either by cortisol implant or cortisol secretion after stress mediated by lesion showed reduction of CRH mRNA expression in the telencephalon-preoptic region (Bernier et al., 1999).

Conveniently, the acute effects of glucocorticoids on the main glucocorticoid feedback target sites tend predominantly toward the rapid suppression of HPA axis activation, as would be predicted for a negative feedback regulation mediated by binding to intracellular receptors. In the short term, glucocorticoids suppress CRH-induced ACTH secretion within minutes via a rapid, transcription-independent mechanism (Bernier et al., 1999; Dallman, 2005; Dallman et al., 1994; Hinz and Kirschelmann, 2000). On the other hand, delayed feedback elicited by glucocorticoids in neurons of the paraventricular nuclei (PVN) occurs through a genomic mechanism in which these hormones act to inhibit CRH transcription (Dallman et al., 1994; Schulkin et al., 1998). Thus, the stress axis, or HPI axis in fish, controls circulating cortisol levels and is highly conserved across vertebrates. Similarly to mammals, the detection of a stressful signal in fishes stimulates the hypothalamic nerves as the first track of HPI axis to secrete CRH, which subsequently acts to release ACTH to the blood stream (Alsop and Vijayan, 2008). Doyon et al. (2005) demonstrated that one chasing event with the net led to a significant increase in plasma cortisol levels without changing CRH mRNA in the preoptica area when evaluated in rainbow trout, although repeated physical disturbance was able to increase both cortisol and CRH mRNA levels in relation to the control animals. We could suggest that control animals can be primed by repeated chasing with the net during the behavioral test, explaining the absente correlation with acute restraint stress and high CRH mRNA content. Thus, the expression of CRH mRNA could occur by a stress-immediate response independent of the degree of stimulation whereas high levels of cortisol are released by more severe stress as the model stipulated here.

5. Conclusion

The present work reveals that zebrafish display a complex set of behaviors in response to the acute restraint stress model outlined here. According to our data, we conclude that rigorous acute stressor stimuli are able to induce behavioral changes, accompanied by an increase of cortisol levels with a delayed control of CRH mRNA expression. All these factors provide important reasons to emphasize acute restraint stress as an adequate tool to understand the mechanisms involved in response to stressor events. Besides, the current report supports the idea that zebrafish is undoubtedly a potential animal model for design neuropharmacological and translational research. Finally, more investigation could clarify the potential target involved in zebrafish behavioral patterns in response to acute stress.

Disclosure/conflict of interest

The authors report no conflicts of interest.

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