Effects of cortisol on food intake, growth, and forebrain neuropeptide Y and corticotropin-releasing factor gene expression in goldfish

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Abstract

Although elevated plasma cortisol levels and a reduction in food intake are common features of the response to stress in fish, the potential role of cortisol in the regulation of food intake in these animals is poorly understood. In this study, goldfish (Carassius auratus) were fed ad libitum for 21 days diets prepared to contain 0 (Control), 50 (Low) or 500 (High) μg cortisol/g of food. While feeding remained unchanged in controls and in fish fed the High cortisol diet, daily food intake gradually increased in the Low cortisol diet group and was significantly elevated between days 9 and 21. At the end of the feeding trial, specific growth rate was lowest in fish fed the High cortisol diet, intermediate in those fed the Low cortisol diet, and highest in the controls. Feed conversion efficiency, on the other hand, was significantly reduced in both groups of fish fed the cortisol diets. After 3 weeks on the diets and relative to controls, the Low cortisol diet group was characterized by a 34% increase in neuropeptide Y (NPY) and a 22% decrease in corticotropin-releasing factor (CRF) mRNA levels in the telencephalon-preoptic brain region. In contrast, the High cortisol diet group was characterized by a 46% decrease in CRF mRNA levels and no significant change in NPY gene expression. In a separate experiment, intraperitoneal implants of cortisol (150 and 300 μg cortisol/g body weight) elicited a dose-dependent increase in NPY and decrease in CRF mRNA levels in the telencephalon-preoptic region at 72 h post-treatment. These results show that while moderate increases in plasma cortisol can stimulate food intake slowly over days, larger catabolic doses of glucocorticoids may mask the appetite-stimulatory effects of cortisol. Therefore, excess cortisol in goldfish can be associated with poor growth despite normal food intake. Furthermore, our results indicate that forebrain NPY and CRF may play a role in mediating the effects of cortisol on food intake in goldfish.

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1. Introduction

Elevated plasma cortisol is a key feature of the endocrine response to stress in fish and results from a stimulation of the hypothalamic–pituitary–interrenal (HPI) axis (Wendelaar Bonga, 1997). In response to a variety of stressors, cortisol contributes to the mechanism involved in maintaining homeostasis primarily by mobilizing energy and making fuels available to meet the increased metabolic demand (Mommsen et al., 1999). Through negative feedback loops at every level of the HPI axis (Sumpter, 1997), cortisol also plays an important role in preventing the adaptive features of the endocrine stress response from overshooting and threatening homeostasis. However, despite the regulatory role of cortisol in limiting the size of the stress response, chronic stress can be detrimental to fish and negatively impact various aspects of performance, including growth (Barton and Iwama, 1991).

Although the effects of chronic stress on growth are not always paralleled by sustained increases in plasma cortisol levels (McCormick et al., 1998; Pickering and * Corresponding author. Fax: +1-519-767-1656.
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towards these goals, two different approaches for the growth-suppressing effects of chronic cortisol elevation are the actions of cortisol on intermediary metabolism (Mommsen et al., 1999). Through the mobilization of stored energy and an increase in gluconeogenesis, cortisol may divert energy away from anabolic processes (De Boeck et al., 2001; Vijayan et al., 1991, 1997). Similarly, there is some evidence suggesting that cortisol may suppress growth by reducing the absorption of food through the intestine (Barton et al., 1987; Mommsen et al., 1999). In contrast, although a reduction in appetite is a characteristic feature of the behavioral response to stress in fish (Schreck et al., 1997), whether the growth-suppressing effects of elevated cortisol are also due to a cortisol-mediated decrease in food intake is not clear (Bernier and Peter, 2001b). For example, in rainbow trout (Oncorhynchus mykiss), while cortisol implants may reduce appetite under some experimental conditions (Gregory and Wood, 1999) they can also be associated with poor growth despite normal food intake (De Boeck et al., 2001).

The regulation of food intake in fish, as in other vertebrates, appears to be achieved via a complex hypothalamic neuronal circuitry that integrates multiple orexigenic (stimulatory) and anorexigenic (inhibitory) neuroendocrine signals of central and peripheral origin (De Pedro and Bjornsson, 2001; Lin et al., 2000). One way in which cortisol might interact with the appetite-regulating pathways of the brain in fish is through its negative feedback effect on the forebrain expression of corticotropin-releasing factor (CRF; Bernier et al., 1999). In addition to being a key hypothalamic regulator of the HPI axis (Lederis et al., 1994), CRF is a potent anorexigenic agent in goldfish (Bernier and Peter, 2001a; De Pedro et al., 1993, 1997). Neuropeptide Y (NPY), a powerful stimulant of eating behavior in many species, appears to be a cortisol-mediated decrease in food intake is not clear (Bernier and Peter, 2001b). For example, in rainbow trout (Oncorhynchus mykiss), while cortisol implants may reduce appetite under some experimental conditions (Gregory and Wood, 1999) they can also be associated with poor growth despite normal food intake (De Boeck et al., 2001).

The purpose of this study was twofold: (1) Determine whether the suppression in growth associated with chronic elevations in plasma cortisol involves changes in daily food intake, and (2) investigate the potential mechanisms mediating the effects of cortisol on food intake. Towards these goals, two different approaches were used to chronically elevate plasma cortisol in goldfish. First, we investigated the effects of a 21-day feeding trial with cortisol-treated diets on daily food intake, specific growth rate (SGR), food conversion efficiency (FCE), and forebrain gene expression levels of NPY and CRF. In a second experiment, we investigated the effects of cortisol cocoa butter intraperitoneal (ip) implants on forebrain gene expression levels of NPY and CRF. The two experimental approaches were selected to try to differentiate between the potential effects of chronic elevations in plasma cortisol on forebrain NPY and CRF gene expression with (experiment 2) or without (experiment 1) accompanying stressful stimuli such as anesthesia and handling. Despite their growth-suppressing effects, our results show that moderate chronic elevations in plasma cortisol may promote food intake via interactions with the forebrain neuropeptide neuronal system involved in the regulation of appetite. In contrast, the catabolic effects of large chronic elevations in plasma cortisol may supersede the appetite-stimulatory effects of cortisol.

2. Materials and methods

2.1. Animals

Female and male goldfish of the common or comet varieties weighing 25–46 g (average weight 30.3 ± 0.4 g; n = 108) in experiment 1 and 33–58 g (average weight 48.8 ± 1.1 g; n = 48) in experiment 2 were obtained from Mount Parnell Fisheries (Mercersburg, PA, USA). Fish were maintained in 800-L flow-through fiberglass tanks at 17°C under a simulated natural photoperiod (Edmonton, AB, Canada) and fed ad libitum once daily with commercially prepared Unifeed Nu-Way food pellets (United Grain Growers, Okotoks, AB, Canada).

3. Experimental procedures

3.1. Diet preparation

The experimental diets were prepared by spraying cortisol (hydrocortisone; Sigma Chemicals, St. Louis, MO) dissolved in 100% ethanol onto the surface of the food pellets to produce concentrations of 0 (ethanol only; Control), 50 (Low), and 500 (High) µg cortisol per gram of feed. The diets were air-dried overnight in a ventilated 4°C chamber and then refrozen at −20°C until needed.

3.2. Assessment food intake

Ten days prior to experimentation, individual fish were removed from the holding tanks and placed in
separate 65-L glass aquaria. During this acclimation period, goldfish were fed the Control ethanol-treated diet. Thereafter, goldfish were either fed the Control, Low or High diet for 21 days. During both the acclimation period and the 3-week feeding trial, each goldfish received a daily excess of pre-weighted feed at 10 AM. Uneaten food was collected 2 h later, dessicated at 100 °C for 1 h and weighed. Food intake was calculated as the difference between the initial dry food weight and the adjusted uneaten dry food weight. The uneaten dry food weight was adjusted as per Bernier and Peter (2001a) in order to account for the effects of pellet dissolution during the feeding interval (<4% pellet weight), for potential day-to-day differences in desiccation efficiency and for loss in initial food moisture content due to the drying process.

3.3. Implants

Cortisol was mixed with melted cocoa butter (30 °C) and injected intraperitoneally (i.p.; 4 μl/g body weight, BW) with a 250 μl 18-gauge microsyringe in fish placed on ice to promote solidification of the implants (see Section 3.8 below for dosage). The implantations were on ice to promote solidification of the implants (see Section 3.8 below for dosage). The implantations were carried out on fish anesthetized in a buffered (NaHCO3, Section 3.8 below for dosage). The implantations were on ice to promote solidification of the implants (see Section 3.8 below for dosage). The implantations were carried out on fish anesthetized in a buffered (NaHCO3, 0.4 g/L) solution of tricaine methanesulfonate (0.2 g/L; MS-222; Syndel, Vancouver, BC, Canada) and the incision in the body wall was closed prior to returning the fish to their original tanks.

3.4. RNA extraction

Fish were anesthetized in a buffered solution of MS-222 (1 g/L) prior to decapitation and excision of discrete brain areas: the telencephalon-preoptic and hypothalamic regions in experiment 1 and the telencephalon-preoptic region in experiment 2. Tissue samples were placed in microcentrifuge tubes previously cooled on dry ice. Total RNA was extracted using Trizol Reagent (Life Technologies, Gaithersburg, MD) based on the acid guanidinium thiocyanate–phenol–chloroform extraction method. Total RNA concentrations were determined by ultraviolet spectrophotometry at 260 nm and samples were stored at −80 °C until used.

3.5. DNA probes

Reverse transcription-polymerase chain reaction (RT-PCR) was used to prepare goldfish NPY, CRF, and β-actin DNA probes. In brief, total RNA extracted as above was reverse transcribed to cDNA using Superscript RNase H− reverse transcriptase (Invitrogen, Carlsbad, CA, USA). PCR amplification of the NPY probe was performed using a forward (5′-GCA AGA AGT TCA ATC AAG ACC-3′) and reverse primer sequence (5′-GGG ATC ACC ACA CCA ACT-3′) based on goldfish NPY (GenBank Accession No. M87297). Amplification of the CRF and β-actin probes was performed as described by Bernier et al. (1999). The PCR products were separated by agarose gel, and the bands of desired size were excised and purified using a Gene-clean II kit (Bio 101, La Jolla, CA). The purified DNA fragments were ligated into the plasmid pGEM-T Easy (Promega, Madison, WI) and transformed into the Escherichia coli strain XL1 blue (Stratagene, La Jolla, CA) for cloning. Recombinant plasmid DNA containing the cDNA inserts were purified by an alkaline lysis method (Birnboim, 1983) and both strands of cloned DNA were sequenced in opposite directions to confirm the identity of the PCR fragments. The cloned NPY (329 bp), CRF (579 bp), and β-actin (578 bp) DNA fragments were labeled using a random priming kit (T7 QuickPrime kit, Pharmacia Biotech, Baie d’Urfé, QC, Canada) with [α-32P]dCTP (3000 Ci/mmol, Amersham) and used as hybridization probes.

3.6. Quantification of mRNA

NPY and CRF mRNA levels in the telencephalon-preoptic and hypothalamic brain regions of goldfish were quantified by slot-blot analysis. Five (telencephalon-preoptic region) or ten (hypothalamus) μg of total RNA from individual fish (initially diluted with sterile water to 10 μl) were added to 30 μl of denaturing solution [19.7 μl formamide, 6.4 μl formaldehyde (37%), and 3.9 μl 10× MOPS] and incubated at 65 °C for 15 min. The samples were immediately placed on ice, diluted further with 60 μl of ice-cold 20× SSC, and slotted directly onto Hybond-N membranes (Amersham Life Sciences, Buckinghamshire, England) using a Bio-Dot SF manifold apparatus (Bio-Rad, Richmond, CA). The RNA was fixed by baking the membranes at 80 °C for 2 h and cross-linked by UV irradiation for 30 s. Hybridization was performed using the methods of Church and Gilbert (1984). In brief, the membranes were pre-hybridized in hybridization solution (0.5 M Na2HPO4 pH 7.2, 7% SDS, 1 mM EDTA, and 1% BSA) for 3 h at 65 °C. The hybridization solution was then changed and the labeled NPY or CRF DNA probe was added. After overnight hybridization at 65 °C, the membranes were washed four times (2 × 1 and 2 × 10 min) with washing solution (40 mM Na2HPO4 pH 7.2, 1 mM EDTA, and 1% SDS). Signal detection was achieved by exposing the NPY and CRF membranes to a PhosphorImager screen (Molecular Dynamics, Sunnyvale, CA) for 3 days and quantified by ImageQuant software (Molecular Dynamics). To serve as an internal control, the membranes were stripped and re-probed with the β-actin DNA probe. The NPY and CRF mRNA levels are expressed as a ratio to the hybridization signal for β-actin mRNA and normalized as a percentage of the control value for each individual sampling time. The linearity of the
mRNA signals obtained by slot blot analysis was assessed by determining the NPY, CRF, and β-actin signals from blotting dilutions of total RNA from the telencephalon-preoptic region and the hypothalamus.

3.7. Plasma cortisol determination

Plasma cortisol concentrations were measured in duplicate from unextracted samples with a commercial radioimmunoassay kit (ImmuChem Coated Tube Cortisol 125I RIA Kit; ICN Biochemicals, Costa Mesa, CA). The validity of the RIA for measuring cortisol titers in goldfish plasma was previously determined (Bernier et al., 1999).

3.8. Experimental design

3.8.1. Experiment 1: effects of cortisol-treated feed on food intake, growth and forebrain NPY, and CRF gene expression

Individual fish acclimated to separate glass aquaria and fed the Control ethanol-sprayed diet for 10 days were randomly assigned to one of nine experimental groups (n = 12 per group). Three groups each were fed the Control, Low, or High cortisol diets for periods of 3, 7, or 21 days. On day 0, once daily food intake was assessed as described above, fish were anesthetized in a buffered solution of MS-222 (0.2 g/L), gently blotted with tissue paper, and both initial BW (wt_i) and fork length were measured. At the end of a given trial, once food intake was assessed, fish were terminally anesthetized (1 g/L MS-222), final BW (wt_f) and fork length were measured as above, and blood and brain tissues were sampled. Blood was collected by caudal puncture, centrifuged at 10,000 g for 5 min, and the separated plasma stored at −20 °C for later analysis of cortisol. Brains were obtained by decapitation, regionally dissected to determine levels of NPY and CRF mRNAs in the hypothalamus and telencephalon-preoptic region. SGR (SGR = (ln wt_f − ln wt_i)/days fed) × 100 in %/day), initial and final condition factor (CF = [mass/fork length³] × 100), and FCE (FCE = (wt_f − wt_i)/[mean food intake × days fed] × 100 in%) were calculated for each fish.

3.8.2. Experiment 2: effects of cortisol i.p. implants on forebrain NPY and CRF gene expression

Four groups of 12 fish each were acclimated for a 3-week period to individual 150-L fiberglass tanks. After this acclimation period, one group received a blank cocoa butter implant (sham treatment), two groups received cortisol implants, and one group was left undisturbed. Fish implanted with cortisol received a dose of either 150 or 300 μg/g BW. Seventy-two hour after receiving the implants, all four groups were sampled as above. Blood was sampled to measure plasma cortisol and the telencephalon-preoptic brain region was collected to determine the expression levels of NPY and CRF mRNAs.

3.9. Statistical analysis

All data are presented as mean ± SE. Differences among treatments were assessed by a one-way analysis of variance (ANOVA) followed by a pairwise Student–Newman–Keuls multiple comparison test. Within a given treatment, the effects of a diet on CF were assessed using a paired t test. The statistical significance of the observed effects of a diet on daily food intake within a treatment was tested using a repeated measures one-way ANOVA followed by Dunnett’s multiple comparison test to compare the Day #0 control data point with values at subsequent times. The significance level for all statistical tests was P < 0.05.

4. Results

4.1. Experiment 1: effects of cortisol-treated feed on food intake, growth and forebrain NPY and CRF gene expression

Relative to the Control diet, the Low, and High cortisol diets elicited a dose-dependent increase in plasma cortisol (Fig. 1). The differences in plasma cortisol between the three treatments were clearly established after 3 days on the experimental diets and sustained throughout the 21-day experiment. Overall, plasma cortisol in fish fed the Low and High cortisol diets were, respectively, 7.5 and 42 times higher than the cortisol levels of the control fish (~6.7 ng/ml).

![Fig. 1. Plasma cortisol in goldfish fed either control (0 μg) or cortisol-treated feed (50 or 500 μg cortisol/g food) for 3, 7, or 21 days. Treatments that do not share a common letter for a given time are significantly different from each other as determined by one-way ANOVA and by pairwise Student–Newman–Keuls test (P < 0.05). Values are means ± SE (n = 12).](image-url)
While the Control and High cortisol diets had no significant effect on daily food intake throughout the 3-week feeding trial, the Low cortisol diet elicited a sustained increase in food intake between days 9 and 21 (Fig. 2). Cumulative food intake was almost identical between the three treatments during the first 9 days of the feeding period (Fig. 3). From day 10 onward, however, cumulative food intake was greatest in fish fed the Low cortisol diet, intermediate in the control group and lowest in the High cortisol diet-fed fish. Over the 21-day experiment, fish fed the Low cortisol diet ate 20 and 36% more food than the fish fed the Control and High cortisol diets, respectively.

Irrespective of the diet, CF increased in all the experimental groups after 7 or 21 days of treatment (Table 1). However, when compared with either the 7- or 21-day control values, the increase in CF was lower in fish fed the High cortisol diet and intermediate in fish fed the Low cortisol diet. Similarly, at the end of the 21-day experiment, SGR was lowest in fish fed the High cortisol diet, intermediate in fish fed the Low cortisol diet, and highest in the control group (Fig. 4A). While the 3- and 7-day cortisol treatments did not significantly affect FCE, 3-week exposure to either cortisol diet reduced the efficiency with which food is converted to flesh when compared with controls (Fig. 4B).

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**Fig. 2.** Daily food intake in goldfish fed either control (0 μg) or cortisol-treated feed (50 or 500 μg cortisol/g food) for 21 days. * Significant difference from day 0 for a given treatment as determined by repeated measures one-way ANOVA and by Dunnett’s multiple comparison test (P < 0.05). Values are means ± SE (n = 12).

**Fig. 3.** Cumulative food intake in goldfish fed either control (0 μg) or cortisol-treated feed (50 or 500 μg cortisol/g food) for 21 days. Treatments that do not share a common letter for a given time are significantly different from each other as determined by one-way ANOVA and by pairwise Student–Newman–Keuls test (P < 0.05). Values are means ± SE (n = 12).

<table>
<thead>
<tr>
<th>Condition factor (mass/fork length³) × 100</th>
<th>Initial</th>
<th>Final</th>
<th>Delta</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-day treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 μg cortisol/g food</td>
<td>2.56 ± 0.08</td>
<td>2.68 ± 0.08</td>
<td>0.12 ± 0.05*</td>
</tr>
<tr>
<td>50 μg cortisol/g food</td>
<td>2.40 ± 0.08</td>
<td>2.51 ± 0.09</td>
<td>0.11 ± 0.05*</td>
</tr>
<tr>
<td>500 μg cortisol/g food</td>
<td>2.45 ± 0.11</td>
<td>2.56 ± 0.10</td>
<td>0.11 ± 0.05*</td>
</tr>
<tr>
<td>7-day treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 μg cortisol/g food</td>
<td>2.64 ± 0.08</td>
<td>2.90 ± 0.09*</td>
<td>0.26 ± 0.05*</td>
</tr>
<tr>
<td>50 μg cortisol/g food</td>
<td>2.57 ± 0.10</td>
<td>2.77 ± 0.10*</td>
<td>0.23 ± 0.04b</td>
</tr>
<tr>
<td>500 μg cortisol/g food</td>
<td>2.51 ± 0.08</td>
<td>2.61 ± 0.06*</td>
<td>0.10 ± 0.04a</td>
</tr>
<tr>
<td>21-day treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 μg cortisol/g food</td>
<td>2.38 ± 0.08</td>
<td>2.79 ± 0.09*</td>
<td>0.41 ± 0.04*</td>
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<tr>
<td>50 μg cortisol/g food</td>
<td>2.37 ± 0.07</td>
<td>2.66 ± 0.07*</td>
<td>0.30 ± 0.06ab</td>
</tr>
<tr>
<td>500 μg cortisol/g food</td>
<td>2.60 ± 0.07</td>
<td>2.81 ± 0.08*</td>
<td>0.21 ± 0.06</td>
</tr>
</tbody>
</table>

*Significant difference from Initial value for a given treatment and diet as determined by paired Student’s t test. Values that do not share a common letter for a given treatment are significantly different from each other as determined by one-way ANOVA and by pairwise Student–Newman–Keuls test (P < 0.05). Values are means ± SE (n = 12).

In the telencephalon-preoptic brain region, 3- or 7-day exposure to the cortisol diets had no effects on NPY and CRF mRNA levels relative to controls (Fig. 5). In contrast, after 21 days on the experimental diets, fish fed the Low cortisol diet had higher NPY mRNA levels than controls (Fig. 5A). Three-week exposure to the cortisol diets was also associated with a dose-dependent decrease in CRF mRNA levels (Fig. 5B). In the hypothalamus, NPY and CRF mRNA levels were not significantly different among the three experimental diets at all sampling times (data not shown).
4.2. Experiment 2: effects of cortisol i.p. implants on forebrain NPY and CRF gene expression

Plasma cortisol concentration in the sham treatment was similar to the resting value of the control group 72 h after the implant procedure (Fig. 6). Fish treated with the cortisol implants were characterized by a dose-dependent increase in circulating plasma cortisol levels.

Relative to the control and sham treatments, fish injected with the 300 µg cortisol/g BW implants were characterized by an increase in NPY (Fig. 7A) and a decrease in CRF (Fig. 7B) mRNA levels in the telencephalon-preoptic brain region.

5. Discussion

Results from this study provide the first evidence that cortisol can stimulate food intake in fish and suggest that the orexigenic effects of cortisol may be mediated, at least in part, through regulatory interactions with some of the peptidergic neuronal populations of the forebrain involved in the regulation of food intake, namely NPY and CRF expressing neurons. Our findings also demonstrate that the effects of cortisol on food intake in goldfish are dose-specific. While moderate daily elevations in plasma cortisol increase food intake over a
period of several days, larger catabolic doses have no effect on food consumption over the same time interval. Therefore, we propose that the overall actions of cortisol on food intake in goldfish depend on the summation of the anabolic and catabolic effects of glucocorticoids on both central and peripheral targets.

Two different techniques were used to elevate plasma cortisol in this study. Relative to the basal resting levels (5–10 ng/ml) of the control group, the Low and High cortisol diets elicited a dose-dependent increase in plasma cortisol of approximately, 50–60 and 275–300 ng/ml, respectively. Although the plasma cortisol profile post-feeding was not characterized, based on the results from studies using a similar approach (Barton et al., 1987; Consten et al., 2001), the cortisol values obtained 2 h post-feeding at 17°C in this study likely reflect peak post-feeding values. For example, plasma cortisol peaked 1 h after feeding in common carp (C. carpio) kept at 25°C (Consten et al., 2001) and 3–6 h after cortisol feeding in rainbow trout reared at 12°C (Barton et al., 1987). In contrast, the 72 h post-implant plasma cortisol levels in experiment 2 do not reflect the peak plasma value delivered by the implants. In fact, based on the results from a previous study where goldfish were also given a 300 μg cortisol/g BW implant (Bernier et al., 1999) and from a detailed evaluation of the steroid release profile from cocoa butter implants in goldfish (Pankhurst et al., 1986), the peak plasma cortisol levels in response to the implants in this study were probably one order of magnitude higher than our reported values.

Although several studies have documented growth suppression in cortisol-fed and cortisol-implanted fish (for example, see De Boeck et al., 2001; Gregory and Wood, 1999; Van Weerd and Komen, 1998), few have assessed the effects of these treatments on food intake and the available data is mostly qualitative and equivocal. For example, while Davis et al. (1985) observed no differences in food acceptability between groups of sham and cortisol-fed channel catfish (Ictalurus punctatus), Barton et al. (1987) reported a loss of appetite and aggressive feeding behavior in cortisol-fed rainbow trout, even though all feed pellets presented were eaten. Gregory and Wood (1999) also found that cortisol-implanted rainbow trout had significantly reduced individual food intake, but the interpretation of their results was confounded by handling stressors, social hierarchies within tanks, and high mortality rates. In this study, we examined for the first time the effects of chronic cortisol elevations on daily individual food intake in the absence of physical or social stressors. Under these conditions, moderate elevations in plasma cortisol slowly promoted food consumption over a period of days and high plasma cortisol levels had no effect. Similarly, although glucocorticoids have been shown to stimulate food intake in several bird and mammalian species (for example, see Koch et al., 2002; Sapolsky et al., 2000), the modulatory effects of glucocorticoids on feeding activity in these animals are both dose- and situation-dependent.

The addition of cortisol on to the surface of the Low and High diets has the potential of affecting the taste of the feed. However, given that the High cortisol diet, like the Control diet, had no immediate or long term affect on daily food intake, it seems unlikely that the orexigenic affects of the Low cortisol diet result from changes in the gustatory system of goldfish. To our knowledge, cortisol does not stimulate the taste system in fish. Moreover, neither stress or cortisol injections affect the gustatory response of channel catfish (Ictalurus punctatus; Harpaz and Katz, 1992).

While the potential mechanisms mediating the effects of cortisol on food intake in fish are not known (Bernier and Peter, 2001b), there is good evidence from studies performed in other animals that the orexigenic effects of glucocorticoids are centrally mediated (Tempel and Leibowitz, 1994). In mammals, glucocorticoids promote food consumption in part by stimulating NPY and inhibiting CRF expression and release (Cavagnini et al., 2000). Similarly, the parallel increase in food intake and the changes in telencephalon-preoptic NPY and CRF gene expression in fish fed the Low cortisol diet suggest...
a possible role for NPY and CRF as mediators of the orexigenic effects of cortisol in goldfish. This possibility is strengthened by the results of experiment 2, which show that cortisol implants can increase NPY and decrease CRF gene expression in a dose-dependent manner. In contrast, we observed no change in daily food intake in goldfish fed the High cortisol diet despite a 46% decrease in CRF mRNA levels by the end of the 21-day treatment. Thus, although these results do not exclude the possibility that CRF influences cortisol-induced feeding, they suggest that other factors are more important than CRF in regulating appetite under the current experimental conditions. Moreover, while changes in gene expression do not necessarily predict peptide secretion and we cannot exclude the possibility that changes in food intake, NPY and CRF gene expression happen in association but without a cause and effect relationship, there is increasing evidence that changes in NPY and CRF gene expression in the goldfish brain play a role in the regulation of food intake (Bernier and Peter, 2001a; Narnaware and Peter, 2001; Narnaware et al., 2000).

Interestingly, the cortisol-mediated changes in telencephalon-preoptic NPY and CRF gene expression in experiment 1 were much slower than those in experiment 2. Whereas after 7 days into the feeding experiment the cortisol diets had no discernable effects on the expression pattern of either gene, cortisol implants in experiment 2, as previously observed (Bernier et al., 1999), elicited significant changes in gene expression after only a few days. Although this differential response in gene expression between the 2 experiments may be the result of higher peak plasma cortisol levels in experiment 2 (see above), it is also important to note that while the daily increase in plasma cortisol took place in the absence of concurrent stress in experiment 1, the implant-mediated increase in plasma cortisol in experiment 2 was accompanied by both handling and anesthetic stressors. Therefore, an activation of stress sensitive brain neuronal pathways during the implantation procedure in experiment 2 may have affected the regulatory effects of cortisol on CRF and NPY gene expression, a consideration worth addressing in future experiments.

Our findings also suggest that while the CRF- and NPY-expressing neurons of the telencephalon-preoptic brain region are affected by elevations in plasma cortisol, those of the hypothalamus are not. Similarly, manipulations of glucocorticoid status in goldfish and macro-dissection techniques have previously shown that cortisol only exerts negative feedback action in the telencephalon-preoptic region (Bernier and Peter, 2001a; Bernier et al., 1999) that may be specifically restricted to the CRF neurons of the nucleus preopticus (NPO: Fryer and Boudreault-Châteauvert, 1981). Although the effects of cortisol on central NPY gene expression have not been previously investigated in fish, it is interesting to note that the stimulatory effects of gonadal steroids on NPY gene expression in goldfish also appear to be restricted to the NPO (Peng et al., 1994). In contrast, chronic systemic administration of glucocorticoids in mammals increase hypothalamic NPY mRNA levels in the arcuate nucleus and NPY release from the nerve terminals in the paraventricular nucleus (Larsen et al., 1994; Wilding et al., 1993), the mammalian equivalent to the NPO. While both NPY and CRF have been implicated in the control of food intake in fish, exactly which NPY- and CRF-expressing neurons of the forebrain or the hypothalamus are part of the neural circuits involved in the control of feeding remains to be established (Lin et al., 2000).

Besides NPY and CRF neurons, other central neuronal pathways may also be involved in mediating the effects of cortisol on food intake. For example, the orexigenic effects of NPY in goldfish are, in part, dependent on the orexin A peptidergic system (Volkoff and Peter, 2001). Also, urotensin I, a CRF-related peptide, is a potent anorexigenic factor in goldfish and while the effects of cortisol on urotensin I mRNA levels have not been directly assessed, there is pharmacological evidence suggesting that cortisol inhibits hypothalamic urotensin I gene expression (Bernier and Peter, 2001a). Lastly, studies in mammals suggest that glucocorticoids may enhance food intake by increasing the expression of specific NPY receptor subtypes (Larsen et al., 1994) and the mRNA levels of melanin-concentrating hormone, a potent orexigenic factor (Presse et al., 1992).

Despite having stimulatory effects on food intake, the Low cortisol diet did not enhance SGR and significantly suppressed FCE. Therefore, even under conditions of moderate daily increases in plasma cortisol (~50–60 ng/ml), our results suggest that some of the physiological actions of cortisol counteract the anabolic effects of an increase in energy intake. For example, cortisol may have interfered with the digestive process by altering the morphology of the cardiac stomach epithelial lining (Barton et al., 1987). Similarly, although little is known about the specific actions of cortisol on food absorption (Collie and Stevens, 1985), a cortisol-dependent decrease in plasma triiodothyronine (T3) may have reduced nutrient absorption from the intestine (Mommsen et al., 1999; Pickering, 1993). Given the stimulatory effects of cortisol on energy expenditure (Barton and Schreek, 1987; Chan and Woo, 1978; De Boeck et al., 2001; Morgan and Iwama, 1996), it is possible that the increase in energy intake in the Low cortisol diet-fed fish may have been spent to fuel a cortisol-mediated increase in energy metabolism (De Boeck et al., 2001). Also, there is evidence that cortisol can interfere with the primary hormonal system involved in the regulation of growth, i.e., the growth hormone and insulin-like growth factor axis (Kelley et al., 2001; Pickering, 1993). Clearly, more work is needed to determine the fate of...
the cortisol-mediated increase in energy intake observed in this study and to assess whether lower doses of cortisol can stimulate an increase in energy intake that surpasses their effects on energy expenditure and therefore result in a positive energy balance.

Exposed to significantly higher daily increases in plasma cortisol (~275–300 ng/ml), fish fed the High cortisol diet were characterized by reduced SGR, FCE, and a smaller increase in CF. Similar results have previously been observed in both cortisol-fed and cortisol-implanted fish and the primary cause for the suppression in somatic growth under such conditions has been ascribed to the effects of cortisol on intermediate metabolism (Barton et al., 1987; Davis et al., 1985; De Boeck et al., 2001; Gregory and Wood, 1999). Specifically, available evidence suggests that catabolic doses of cortisol suppress growth by stimulating energy-consuming processes such as increases in glucose, lactate and glycogen synthesis, and through the mobilization of lipid stores (De Boeck et al., 2001; Momsen et al., 1999).

In addition to increasing energy expenditure and suppressing growth, the catabolic actions of cortisol in the goldfish fed the High cortisol diet may have prevented the orexigenic effects of cortisol. In mammals, when plasma glucocorticoids reach catabolic levels, their stimulatory effects on food intake are antagonized by insulin and leptin. While high doses of glucocorticoids raise insulin concentrations via their gluconeogenic actions, they also act directly on adipocytes and increase leptin synthesis and secretion (Leal-Cerro et al., 2001). In return, both leptin and insulin inhibit hypothalamic NPY synthesis and release (Sahu et al., 1995; Stephens et al., 1995) and affect several other orexigenic and anorexigenic pathways (Kalra et al., 1999; Nandi et al., 2002). In fish, the extent to which the metabolic actions of cortisol can stimulate an increase in plasma insulin is not clear (Momsen et al., 1999) and so far there is no evidence that insulin contributes to the regulation of food intake (Silverstein and Plosetskaya, 2000). In contrast, although the search for a leptin-like molecule in fish is ongoing (Johnson et al., 2000) and it is not known whether the lipolytic effects of cortisol result in an increase in plasma leptin, there is evidence that leptin may be involved in the regulation of food intake (Volkoff et al., 2003). In goldfish, injections of murine leptin inhibit food intake and reduce the mRNA levels of NPY in the telencephalon-preoptic and hypothalamic brain regions (Volkoff et al., 2003). Therefore, future studies are needed in fish to determine whether the lipolytic effects of high cortisol levels mediate an increase in a leptin-like molecule and whether this catabolic response plays a role in counteracting the orexigenic effects of cortisol.

In summary, the impact of cortisol on both food intake and growth in goldfish varies markedly with their circulating levels. Whereas chronic daily moderate elevations in plasma cortisol (~50–60 ng/ml) can stimulate food intake without promoting growth, daily increases to higher levels (~275–300 ng/ml) have no impact on food intake and inhibit growth. Thus, cortisol exhibits both anabolic and catabolic actions at moderate levels and primarily catabolic actions when levels are chronically high. Overall, while common features of the response to stress in fish are an increase in plasma cortisol and reductions in both food intake and growth, our results show that the growth-suppressing effects of cortisol in goldfish do not appear to be mediated by a reduction in food intake. In contrast, the actions of cortisol on the mechanisms involved in the regulation of food intake have the potential to suppress and aid the recovery from stress-induced anorexia.

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References


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