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METABOLIC EFFECTS OF CORTISOL TREATMENT IN A MARINE TELEOST, THE SEA RAVEN

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Summary

Sea raven (*Hemitripterus americanus*) given intraperitoneal implants of coconut oil containing cortisol (50 mg kg^{-1}) and sampled 5 days later had plasma cortisol, glucose and urea concentrations higher than in a sham-implanted group. No differences in plasma ammonia, free amino acid or fatty acid concentrations were apparent between the cortisol- and sham-treated groups. There was no change in hepatic glycogen content, whereas glutamine synthetase, allantoicase, arginase, aspartate aminotransferase, tyrosine aminotransferase, alanine aminotransferase, glutamate dehydrogenase, phosphoenolpyruvate carboxykinase and 3-hydroxyacyl-coenzyme A dehydrogenase activities were higher in the cortisol-treated fish liver compared with the sham-implanted fish. On the basis of these general increases in enzyme activities, our results suggest that cortisol stimulates nitrogen metabolism in the sea raven. Amino acid catabolism may be a major source of substrate for gluconeogenesis and/or oxidation, while fatty acid mobilization may provide the fuel for endogenous use by the liver in cortisol-treated sea raven. These results further support the hypothesis that cortisol plays a role in the regulation of glucose production in stressed fish.

Key words: *Hemitripterus americanus*, sea raven, cortisol, nitrogen metabolism, teleost, gluconeogenesis.

Introduction

Cortisol is a metabolic hormone in fish (Vijayan *et al.* 1991, 1994) and limited studies have shown that cortisol increases the metabolic rate of fish (Chan and Woo, 1978). However, the role of cortisol in the regulation of substrate mobilization for energy purposes is poorly understood. Several studies postulate that cortisol mobilizes peripheral proteins, with a resultant increase in amino acids utilized for energy metabolism (for references, see Vijayan *et al.* 1994). Indeed, cortisol administration to fish does increase [^{14}C]alanine incorporation into glucose and CO_2 in hepatocytes (Vijayan *et al.* 1993, 1994). In addition, hepatic gluconeogenic and aminotransferase enzyme activities and circulating glucose concentrations are enhanced in cortisol-injected fish (Butler, 1968; Freeman and Idler, 1973; Whiting and Wiggs, 1977; Chan and Woo, 1978; Leach and Taylor, 1982; Davis *et al.* 1985; Foster and Moon, 1986; Barton *et al.* 1987), suggestive of increased gluconeogenesis from amino acids. As a consequence, cortisol-treated fish tend to show higher rates of ammonia excretion (Chan and Woo, 1978), while fates of nitrogen need to be investigated. In addition to amino acids, previous studies have also suggested that cortisol mobilizes lipids for energy purposes in teleostean species (Dave *et al.* 1979; Lidman *et al.* 1979; Sheridan, 1986; Vijayan *et al.* 1991), while very little is known about free fatty acid concentrations in cortisol-treated fish.

The sea raven (*Hemitripterus americanus*) is a marine benthic predatory fish with a sluggish life-style and low metabolic activity (Milligan and Farrell, 1986; Foster and Moon, 1987). Given their habitat and life-style, sea ravens are probably not exposed to the kind of stressors normally encountered by the more active pelagic species such as many salmonids. In fact, they have a higher threshold for the endocrine response to stress (cortisol and catecholamine) and a delayed release of cortisol (Vijayan and Moon, 1994). Cortisol does enhance glycogen breakdown and oxidation of alanine by sea raven hepatocytes (Vijayan *et al.* 1993), indicative of an increase in energy demand. Consequently, the delayed cortisol response to stress noted in this species (Vijayan and Moon, 1994) may be an adaptation to prevent the excessive mobilization of energy stores to suit its sedentary and sluggish life-style. In the present study, we therefore tested the hypothesis that chronic cortisol stimulation increases substrate mobilization and gluconeogenesis in the sea raven in an attempt to explain the altered endocrine response to stress seen

in these animals (Vijayan and Moon, 1994). Specifically, the objectives were as follows: (i) to determine whether chronic cortisol treatment affects plasma free amino acid concentration and increases the capacity of the liver for amino acid catabolism, ureogenesis and gluconeogenesis, and (ii) to examine whether cortisol affects fatty acid mobilization by measuring plasma free fatty acid concentrations and the hepatic capacity for their oxidation.

Materials and methods

Animals

Sea ravens *Hemitripterus americanus* Gmelin were obtained by otter trawl from Passamaquoddy Bay, New Brunswick, Canada, in May and maintained in large (1000 l) outdoor tanks supplied with flowing, unfiltered sea water at 10 °C at the Huntsman Marine Sciences Centre, St Andrews, New Brunswick, Canada. The fish were fed chopped fish once daily to satiation.

Experimental protocol

Groups of six fish (mean body mass 923±119 g) were given intraperitoneal injections (1 ml kg^{-1}) of either coconut oil alone (sham) or coconut oil containing cortisol at 50 mg kg^{-1} body mass. This protocol has been shown to produce a slow release of cortisol into the circulation (Vijayan *et al.* 1994). Sham- and cortisol-treated fish were held in the same tank after injection and identified by means of fin clips. The fish were fed as described above and feeding was stopped 24 h prior to sampling.

Six fish from each treatment group were sampled 5 days after injection. Sampling consisted of quickly netting and killing the fish with an overdose of anaesthetic (2-phenoxyethanol, 1:1000) and removing blood from the caudal vasculature by syringe. Plasma was obtained by centrifugation (Fisher microfuge; 13 000 ***g***, 1 min) and frozen (−70 °C) for hormone and metabolite determinations (see below). Livers were quickly removed, weighed and pressed between dry ice pieces and stored at −70 °C for the measurement of liver glycogen content and enzyme activities (see below).

Liver glycogen and enzyme measurements

Liver glycogen content was measured after amyloglucosidase hydrolysis according to Keppler and Decker (1974). For enzyme measurements, the liver was homogenized (Ultra Turrax, IKA Works, NC, USA) followed by sonication (Microson, NY, USA) in a 1:10 (w/v) buffer containing 20 mmol l^{-1} Na_2HPO_4, 5 mmol l^{-1} 2-mercaptoethanol, 0.5 mmol l^{-1} EDTA, 0.2 % bovine serum albumin, 50 % (v/v) glycerol and adjusted to pH 7.4 with HCl (Henriksson *et al.* 1986). Aprotinin was added at a final concentration of 10 μg ml^{-1}. This homogenate can be frozen for later enzyme measurements (Henriksson *et al.* 1986). Enzyme activities were measured at 22 °C in a final volume of 250 μl by continuous spectrophotometry (UVMax, Molecular Devices, Inc.) according to Mommsen and Walsh (1989), Foster and Moon (1990) and Vijayan *et al.* (1991). Arginase (at pH 8.0; Mommsen *et al.* 1983), ornithine carbamoyl transferase (Mommsen *et al.* 1983), glutamine synthetase (Webb and Brown, 1980) and tyrosine aminotransferase (Canellakis and Cohen, 1956) were determined colorimetrically at timed intervals. The activities are given as micromoles of substrate consumed or product liberated per minute per 100 g body mass [enzyme activity per gram wet mass of liver × hepatosomatic index, where hepatosomatic index is (liver mass/body mass)×100].

Plasma hormone and metabolite concentrations

Plasma cortisol concentration was quantified using a commercial radioimmunoassay kit (ICN Biomedicals) according to Andersen *et al.* (1991); glucose was determined enzymatically as detailed in Keppler and Decker (1974). Plasma free amino acid, ammonia and urea concentrations were measured using Beckman System 6300 (Beckman Instruments, Palto Alto, CA, USA) ion exchange chromatography, while concentrations of plasma free fatty acids (detection limit set at 0.1 μmol l^{-1}) were assessed by gas chromatography according to Ballantyne *et al.* (1993).

Statistical analyses

The cortisol-treated group was compared with the sham-treated group using an unpaired *t*-test (Sigmastat, Jandel Corp., CA, USA). Log-transformed data were used wherever necessary to satisfy the homogeneity of variance; non-transformed data are shown in the tables. A sample size of six fish was used in this study and, because of an outlier, some data points had *P* values between 0.05 and 0.08. These values, however, tended to be higher (approximately 50–150 % compared with the sham group) and, therefore, we used $P<0.1$ as the level of significance.

Results

Cortisol implantation resulted in higher plasma cortisol, glucose and urea concentrations compared with the sham group (Table 1). There were no differences in final body mass, hepatosomatic index, liver glycogen content and plasma ammonia (Table 1), free amino acid (Table 2) or free fatty acid concentrations (Table 3) between the sham- and cortisol-administered groups.

Cortisol treatment resulted in higher hepatic activities of a number of metabolic enzymes compared with the sham group. The activities of the ureogenic enzymes glutamine synthetase (GNS), arginase and allantoicase (a uricolytic enzyme), but not ornithine carbamoyl transferase (OCT), were higher in the liver of cortisol-administered sea raven compared with the sham group (Table 4). Cortisol administration also resulted in higher hepatic aspartate aminotransferase (AspAT), tyrosine aminotransferase (TAT), alanine aminotransferase (AlaAT), glutamate dehydrogenase (GDH), phosphoenolpyruvate

Table 1. *Final body mass, hepatosomatic index (HSI), liver glycogen content and plasma cortisol, glucose, ammonia and urea concentrations in sea ravens (*Hemitripterus americanus*) given coconut oil alone (Sham) or coconut oil containing 50 mg kg^{-1} cortisol (Cortisol) and sampled 5 days later*

	Sham	Cortisol	*P*
Body mass (g)	1025±130	822±203	0.42
HSI[a]	2.27±0.53	3.21±0.48	0.22
Glycogen (μmol g^{-1})	248±71	296±38	0.56
Cortisol (ng ml^{-1})	70.8±25	582.3±177	0.017
Glucose (mmol l^{-1})	1.1±0.2	1.6±0.05	0.015
Ammonia (mmol l^{-1})	0.09±0.01	0.1±0.02	0.51
Urea (mmol l^{-1})	0.47±0.1	1.21±0.3	0.057

Values represent means ± S.E.M. (N = 6); [a]HSI is (liver mass/body mass) × 100; P shows the level of signifcance (P<0.1 was considered significant; unpaired t-test).

carboxykinase (PEPCK) and 3-hydroxyacyl-coenzyme A dehydrogenase (HOAD) activities compared with the sham group (Table 4). There were no effects of cortisol treatment on hepatic pyruvate kinase (PK) or its activity ratio (data not shown), lactate dehydrogenase (LDH), citrate synthase (CS) or malate dehydrogenase (MDH) activities when compared with the sham group (Table 4).

Table 2. *Individual amino acid concentrations in plasma from sea ravens (*Hemitripterus americanus*) given either coconut oil alone (Sham) or coconut oil containing 50 mg kg^{-1} cortisol (Cortisol) and sampled 5 days later*

Amino acid (μmol l^{-1})	Sham	Cortisol
Glycine	252.6±29.5	249.9±24.8
Alanine	131.1±20.4	137.7±20.5
Valine	111.9±11.0	116.2±10.2
Leucine	106.6±12.0	103.5±10.4
Isoleucine	56.7±8.0	54.9±6.4
Serine	59.5±5.3	48.3±6.0
Threonine	59.0±8.8	59.9±12.1
Cysteine	5.2±0.3	5.5±0.6
Methionine	36.5±6.4	23.8±3.0
Tyrosine	40.9±4.9	29.0±4.2(5)
Phenylalanine	41.6±4.6(5)	42.0±4.9(5)
Proline	33.4±18.6(4)	37.6±15.6(5)
Hydroxyproline	23.2±7.9	31.8±11.0
Histidine	32.3±2.8	33.4±2.2
Tryptophan	6.2±2.7(3)	3.8±0.3(4)
Arginine	76.5±17.7	83.0±8.8
Lysine	114.3±20.7	104.7±17.8
Aspartate	14.5±1.6	14.4±0.9
Glutamate	30.7±1.8	31.9±5.0
Taurine	44.8±5.38	86.0±18.1
Total	1487±84	1511±78

Values represent means ± S.E.M. (N=6, unless otherwise noted); P>0.1 (not significant) for treatment differences for all free amino acids measured (unpaired t-test).

Discussion

This study demonstrates that cortisol treatment increased hepatic metabolic enzyme activites, which strongly suggests enhanced amino acid metabolism in the sea raven. Nothing is known concerning nitrogen excretion in the sea raven, but plasma ammonia and urea concentrations found in the present study suggest that these fish are predominantly ammoniotelic; plasma urea concentration in ureogenic teleosts is variable, but tends to exceed 5 mmol l^{-1}. The potential for urea production by the ornithine–urea cycle, however, does exist in the sea raven as indicated by the presence, albeit small, of hepatic ureogenic enzyme activities (Table 4).

Most teleosts are considered to be ammoniotelic, with certain exceptions (see Mommsen and Walsh, 1991; Wood, 1993). Some of the predominantly ammoniotelic teleosts have been shown to produce urea in extreme environmental or physiological situations (see Wood, 1993). The mechanisms for the switch from ammoniotely to ureotely in these animals are still elusive, although several hypotheses have been proposed (Walsh *et al.* 1994). As plasma cortisol concentration increases with chronic stress in the sea raven (Vijayan and Moon, 1994), the higher plasma urea concentration in the cortisol-

Table 3. *Individual non-esterified fatty acid concentrations in plasma from sea ravens (*Hemitripterus americanus*) given either coconut oil alone (Sham) or coconut oil containing 50 mg kg^{-1} cortisol (Cortisol) and sampled* five *days later*

Fatty acid (μmol l^{-1})	Sham	Cortisol
14:0	8.1±4.9[1]	2.5±2.3[1]
16:0	287.0±29.6	258.2±16.7
16:1	60.3±6.0	67.8±6.8
18:0	82.9±6.2	85.8±5.0
18:1n9	164.0±17.6	157.2±20.8
18:3n3	16.3±5.7	10.5±4.7[1]
20:1	ND	6.8±6.2[1]
20:4n6	49.6±4.3	44.9±4.4
20:5n3	213.3±31.6	216.6±22.9
22:6n3	288.1±30.4	261.0±26.7
Total	1169.6±116.7	1111.1±87.2
Total saturates	377.9±37.9	346.5±22.5
Total monoenes	224.3±23.6	231.7±25.2
Total polyenoic	567.4±59.4	532.9±47.3
n3 polyenoic	517.8±57.7	488.0±43.4
n6 polyenoic	49.6±4.3	44.9±4.4
n3/n6	10.7±1.0	11.0±0.5
Monoenes/polyenoic	0.40±0.03	0.44±0.03

Values represent means ± S.E.M. (N = 6); ND, not detectable; [1] signifies three or more values were not detectable and were included as zeros in the mean; P>0.1 (not significant) for treatment differences for all free fatty acids measured (unpaired t-test).

Table 4. *Activities of some hepatic enzymes of the intermediary metabolism in sea ravens (*Hemitripterus americanus*) given either coconut oil alone (Sham) or coconut oil containing 50 mg kg^{-1} cortisol (Cortisol) and sampled 5 days later*

Enzyme	Sham	Cortisol	*P*
Glutamine synthetase	14.3±2.7	30.2±6.3	0.04
Arginase	0.18±0.03	0.36±0.09	0.07
Ornithine carbamoyl transferase	0.034±0.008	0.050±0.006	0.13
Allantoicase	3.5±0.4	4.8±0.4	0.03
Aspartate aminotransferase	437.4±57.1	710.1±75.2	0.016
Alanine aminotransferase	360.1±58.0	538.9±64.2	0.066
Tyrosine aminotransferase	5.0±0.6	9.33±0.7	0.002
Glutamate dehydrogenase	556.4±89.2	784.8±70.5	0.072
Phosphoenolpyruvate carboxykinase	21.2±4.2	33.0±4.3	0.08
3-Hydroxyacyl-coenzyme A dehydrogenase	39.9±6.7	66.2±7.3	0.024
Pyruvate kinase	26.7±2.0	32.7±4.2	0.23
Citrate synthase	13.7±1.9	19.8±3.0	0.12
Lactate dehydrogenase	16.8±6.4	19.7±4.8	0.72
Malate dehydrogenase	2160.8±527.3	3594.6±594.3	0.10

Values represent means ± S.E.M. (N=6); enzyme activity is presented as μmol min^{-1} 100 g^{-1} fish mass; P shows the level of signifcance (P<0.1 was considered significant; unpaired t-test).

administered fish (Table 1) supports the hypothesis that cortisol contributes to the regulation of urea production during stress in fish, as proposed by Wood (1993) and Walsh *et al.* (1994).

Glutamine synthetase (GNS), the feeder enzyme for ammonia nitrogen into the urea cycle in teleost fish (Mommsen and Walsh, 1991), is considered a key regulatory or rate-limiting step for ureogenesis in the toadfish *Opsanus beta* (Walsh *et al.* 1994). Our study is the first to show that cortisol increases hepatic GNS activity in fish *in vivo*, and this suggests a link between hormone status and urea cycle activity. An earlier study did show an increased GNS activity with dexamethasone treatment in toadfish hepatocytes *in vitro* (Mommsen *et al.* 1992), while an increased activity of this enzyme has been reported in stressed toadfish (Walsh *et al.* 1994). These results support a role for cortisol in modulating GNS activity in fish. The higher hepatic allantoicase activity in the cortisol-treated fish suggests that the uricolysis pathway may constitute another route for urea production that is modulated by cortisol in fish. Our results also suggest that hepatic arginase activity may be modulated by cortisol, although it remains to be seen whether this is a direct effect of the steroid on enzyme induction as shown for mammals (Kumar and Kalyankar, 1984).

The higher hepatic aminotransferase activities in the cortisol-treated fish (Table 4) indicate a higher liver capacity for amino acid metabolism. Corticosteroids have been shown to increase aminotransferase activities in fish, and our results support previous studies on eels (*Anguilla rostrata*: Butler, 1968; Foster and Moon, 1986; *Anguilla japonica*: Inui and Yokote, 1975; Chan and Woo, 1978), brook charr (*Salvelinus fontinalis*: Freeman and Idler, 1973; Whiting and Wiggs, 1977; Vijayan *et al.* 1991), channel catfish (*Ictalurus punctatus*: Davis *et al.* 1985), rainbow trout (*Oncorhynchus mykiss*: Freeman and Idler, 1973; Barton *et al.* 1987) and toadfish (*Opsanus beta*: Mommsen *et al.* 1992). The absence of any change in plasma free amino acid concentrations (Table 2) in the cortisol-treated group, despite the increased hepatic capacity for amino acid catabolism (Table 4), suggests an enhanced turnover of amino acids in the cortisol group, as reported for alanine in cortisol-treated dogs (Goldstein *et al.* 1992).

Although the precise reason for increased plasma concentrations of urea in cortisol-treated sea raven is not known, we speculate that it may have to do with the increased proteolysis and the subsequent amino acid catabolism associated with cortisol stimulation. This increased catabolism perhaps results in the production of ammonia levels far in excess of those the tissue normally encounters. The higher hepatic GDH activity in the cortisol-treated group in the present study (Table 4) suggests a role for cortisol in the enhanced hepatic capacity for ammonia production, as proposed earlier in the Japanese eel *Anguilla japonica* (Chan and Woo, 1978). Also, dexamethasone-treated toadfish showed higher GDH activity in hepatocytes (Mommsen *et al.* 1992), supporting the above argument. It is possible, therefore, that the increased tissue ammonia concentration stimulates the ureogenic pathway and may be a temporary mechanism to clear the excess ammonia. Further studies, especially using cortisol antagonists such as RU486, are necessary to elucidate the temporal changes in nitrogen metabolism associated with cortisol stimulation in this species.

In the present study, cortisol administration did not affect liver glycogen content or hepatic PK and LDH activities in the sea raven, suggesting that neither glycogenolysis nor the hepatic glycolytic potential was modified by cortisol. However, the increase in hepatic transaminase activities and the modest increase in hepatic PEPCK activity imply that gluconeogenesis may contribute to the elevated plasma glucose concentrations (Tables 1, 4). These results further support the hypothesis that cortisol-induced gluconeogenesis may be important for the long-term maintenance of glucose levels during stress in fish (Vijayan and Moon, 1994; Vijayan *et al.* 1994). Amino acids are an important substrate for gluconeogenesis in fish (Suarez and Mommsen, 1986) and a recent study showed that cortisol treatment increases alanine gluconeogenesis in rainbow trout hepatocytes (Vijayan *et al.* 1994). As the potential for amino acid catabolism is higher in the cortisol-administered sea raven (Table 4), it is likely that the carbon sources are channelled into gluconeogenesis, and possibly to oxidation (Vijayan *et al.* 1993). Our results also suggest an increased hepatic capacity for fatty acid oxidation (Table 4), while the absence of any change in plasma free fatty acid concentration may imply an increased capacity for fatty acid turnover in the cortisol-treated group. A similar increase in hepatic HOAD activity with cortisol has been shown before in brook charr *Salvelinus fontinalis* (Vijayan *et al.* 1991), suggesting a role for cortisol in

the regulation of lipid metabolism in fish (Sheridan, 1986; Vijayan *et al.* 1991). This enhanced lipid mobilization may also provide the fuel necessary to meet the higher metabolic demand associated with cortisol stimulation in sea raven hepatocytes (Vijayan *et al.* 1993).

In conclusion, our study shows that chronic cortisol stimulation increased hepatic metabolic enzyme activities, which suggests enhanced amino acid metabolism in the sea raven. The excess nitrogen arising from amino acid catabolism is probably channelled into urea synthesis as indicated by the higher plasma urea concentration and the greater hepatic activities of some of the enzymes involved in ureogenesis. This increase in urea production may be a temporary mechanism in the sea raven to re-route the excess ammonia that is produced as a result of cortisol-induced amino acid catabolism. Amino acid catabolism may be a major source of substrate for gluconeogenesis in the cortisol-administered sea raven, while lipid mobilization may be providing the fuel for the enhanced metabolic activity in the cortisol-treated fish liver. The results suggest that cortisol plays a role in the longer-term regulation of glucose production in fish.

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