

**CRF-RELATED PEPTIDES AND THE APPETITE-SUPPRESSING  
EFFECTS OF HYPOXIA IN RAINBOW TROUT**

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**EXTENDED ABSTRACT ONLY- DO NOT CITE**

**Introduction**

Hypoxia inhibits growth in a variety of fish species primarily via a reduction in food intake (e.g. Chabot and Dutil 1999; Pichavant *et al.* 2001). While the appetite-suppressing effects of hypoxia are well recognized, the mechanisms mediating this response are not known. Overall, the regulation of food intake in fish is achieved through a complex hypothalamic neuronal network that integrates stimulatory and inhibitory signals of central and peripheral origin (Lin *et al.* 2000). Among the signals that suppress appetite in fish are the two related neuropeptides corticotropin-releasing factor (CRF) and urotensin I (UI; Bernier and Peter 2001). In addition to being potent appetite-suppressing factors, CRF-related peptides are generally recognized in vertebrates as the key hypothalamic regulators of the hormonal response to stress. In fish, however, there is very little information on the role of CRF and UI in the stress response (Wendelaar Bonga 1997). Therefore, to determine whether CRF and UI maybe involved in mediating the appetite-suppressing effects of hypoxia in fish, we examined the effects of hypoxia on food intake and on the gene expression pattern of CRF and UI in the brain of rainbow trout.

**Materials and Methods**

Mixed sex rainbow trout were obtained from the Alma Aquaculture Research Station (Alma, ON, Canada) and held in the Aqualab at the University of Guelph (Guelph, ON) in 90 L tanks for at least a month prior to experimentation. Fish were tagged with a passive integrated transponder (PIT),

fed daily to satiation with a commercial trout diet, and maintained in flow-through well water (14°C, dissolved O<sub>2</sub> = 8.3±0.2 mg l<sup>-1</sup>). Six groups of fish (n=12) were acclimated to the above conditions and exposed to one of three O<sub>2</sub> regimes [35, 50, and 80% saturation (control)] for either 24 or 72 h. Using X-radiography, food intake was assessed twice on each individual fish: one week prior to the treatments and during the last 30 minutes of the exposure period. Once the treatments completed, fish were immediately terminally anesthetized to collect the brain, pituitary, and blood. The brains were regionally dissected to determine the expression levels of CRF and UI mRNAs in the telencephalon + hypothalamus (tel/hyp) region. The expression levels of POMCa and POMCb mRNAs were determined in the pituitary, and blood samples were analysed for measurement of plasma cortisol and lactate concentrations. The mRNA levels were determined by slot blot analysis using rainbow trout DNA hybridization probes and expressed against the signal for β-actin mRNA. Plasma cortisol and lactate concentrations were measured with an EIA and an enzymatic kit, respectively.

## **Results and Discussion**

After 24 h exposure, food intake decreased by 28 and 48% in the 50 and 35% saturation treatments, respectively. Relative to the control treatment, tel/hyp CRF and UI mRNA levels were elevated in the 35% saturation treatment, but not in the 50% treatment. Similarly, increases in plasma cortisol and lactate were only observed in the 35% saturation treatment.

After 72 h exposure, food intake decreased by 26 and 42% in the 50 and 35% saturation treatments, respectively. In contrast to the 24 h exposure treatments, tel/hyp CRF and UI mRNA levels were elevated in the 50% saturation treatment, but not in the 35% treatment. Plasma cortisol was elevated in both hypoxic treatments and the increase was inversely related to the O<sub>2</sub> levels. An increase in plasma lactate was only observed in the 35% saturation treatment. In either the 24 or 72 h exposure, there was no difference in the pituitary POMCa and POMCb mRNA levels between the two hypoxic treatments and the control treatment.

While the magnitude of the reduction in food intake paralleled the percent decrease in O<sub>2</sub> saturation, an increase in the duration of the hypoxic treatment did not result in a further reduction in appetite. In contrast, the magnitude of the changes in tel/hyp CRF and UI mRNA levels appeared to depend on both the severity of the hypoxic exposure and its duration. Given the negative feedback

action of cortisol on CRF and UI gene expression under normoxic conditions (Bernier and Peter 2001), future investigation are needed to determine whether cortisol plays an important role in regulating the magnitude of the changes in tel/hyp CRF and UI mRNA levels during hypoxia. Overall, the increased expression of the tel/hyp CRF and UI genes in response to the hypoxic treatments and the simultaneous reduction in food intake suggest that CRF-related peptides may play a physiological role in mediating the appetite-suppressing effects of hypoxia in rainbow trout.

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