

**IMPAIRED ADRENAL STEROIDOGENESIS
IN FISH CHRONICALLY EXPOSED
TO ENVIRONMENTAL XENOBIOTICS.**

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Introduction

The increase in plasma cortisol levels in response to acute exposures to abiotic and biotic stressors has been documented in numerous laboratory studies with teleost fish. However, physiological responses to chronic exposures are less well understood, particularly the responses to chronic environmental exposures to pollutants. A reduced capacity to elevate plasma cortisol in response to a confinement stress has been reported in yellow perch, *Perca flavescens* and northern pike, *Esox lucius*, sampled at sites contaminated by mixtures of organic contaminants and metals (Hontela, 1998). The mechanisms through which chronic environmental exposures disrupt cortisol secretion have not been elucidated thus far. Therefore, the objective of the present study was to determine if a dose-response relationship can be established between the environmental exposure to pollutants, the tissue burden of pollutants and effects on the physiological and endocrine status of the fish.

Materials and methods

Fish were captured in six lakes situated along a metal contamination gradient in a mining region in northwestern Québec. Following capture, fish were placed for 24 hrs into floating enclosures in the lake to facilitate recovery from capture and reduce variability due to handling of the fish. Fish were sampled

the next day between 10:00 and 11:00hr. One group was blood sampled following a 1 hr confinement stress and anesthesia (« stress » group). A second group was blood sampled without the confinement stress and the head kidneys were dissected for the *in vitro* tests. To test the functional integrity of the interrenal tissue *in vitro*, individual head kidneys were cut into small fragments and divided among three microplate wells. Following a preincubation period to reach basal levels of cortisol secretion, the head kidney fragments were stimulated with ACTH (2 I.U.), dbcAMP (4 mM) or medium (MEM) only. Cortisol was assayed in the supernatants collected from the *in vitro* tests; cortisol, chloride and glucose were assayed in plasma from fish sampled with or without confinement. Metals (Cd, Zn and Cu) and metallothionein levels were measured in the liver and head kidneys of all the fish.

Results

Levels of Zn, Cu and Cd, as well as metallothionein increased along a gradient, ranking the lakes in the following order of contamination: L. Dufault > L. Osisko > L. Vaudray > L. Bousquet > L. Opasatica > L. Dasserat. Concentrations of metals and metallothioneins were higher in the liver than in the head kidney (Table 1). The capacity to increase plasma cortisol levels in response to a standardized confinement stress decreased in relation to tissue burdens of metals in the liver and head kidney of the yellow perch. Stressed fish from the reference and intermediate lakes were able to increase their plasma glucose while this response was significantly impaired in fish from the most contaminated lake. No effects of contamination on plasma chloride levels were detected but confinement stress decreased significantly plasma chloride in all the fish (Table 2). *In vitro* response to ACTH and dbcAMP was lower in head kidneys from L. Osisko (contaminated lake) compared to L. Dasserat (reference lake). No differences in cortisol production were detected for the unstimulated head kidneys from the two lakes tested (Table 2).

Table 1. Concentrations of Zn, Cu, and Cd (mean±SE, µg.g dry wt), and correlations between concentrations of metallothionein (MT) [nmol metal binding sites (g dry wt)⁻¹] and metal (M), in pooled samples of liver or interrenal tissue of adult yellow perch collected in six lakes from a mining area.

Lakes	Liver			Head kidney		
	[Zn]	[Cu]	[Cd]	[Zn]	[Cu]	[Cd]
OP	92.4±3.6 a	10.4±1.8 a	2.9±0.4 a	104.6±2.3 a	2.3±0.2 a	0.9±0.1 a
DS	98.6±4.2 ab	10.8±0.9 a	5.3±0.6 b	118.8±2.6 b	2.9±0.3 a	1.7±0.2 b
BO	106.5±5.4 ab	20.4±4.5 b	20.3±2.9 c	96.08±1.2 a	2.2±0.1 a	3.8±0.2 c
VA	108.9±1.6 b	12.9±0.7 ab	25.1±1.7 c	127.6±4.7 b	2.7±0.2 a	5.7±0.7 cd
OS	177.2±9.0 c	246.5±29.8 c	45.7±3.2 d	153.6±6.6 c	6.5±1.0 b	8.0±0.5 de
DT	151.1±3.7 c	148.5±11.1 c	61.3±5.3 d	227±10.3 d	6.4±1.5 b	12.6±0.4 e
Correlati on MTI/	0.93 *	0.95 *	0.91 *	0.82 *	0.77 *	0.95 *

Means followed by the same letter are not significantly different, comparison between lakes only ($p < 0.01$, Tukey-Kramer HSD test). Number of replicates for each measure 7-8.* - $P < 0.001$ (Pearson's test).

Table 2. Cortisol *in vivo* post-stress and *in vitro* post-ACTH or dbcAMP, chloride and glucose (change post-stress (means \pm SE) in yellow perch collected in lakes from a mining area.

Lakes	Plasma parameters			Cortisol secretion by head kidney <i>in vitro</i> ng/ml/mg		
	Cortisol (ng/ml plasma)	Glucose (Δ mg.ml plasma)	Chloride (Δ mg.ml plasma)	ACTH	dbcAMP	none
OP	260 \pm 11 ab	+0.12 \pm 0.02* *	-15.0 \pm 4*	2.5 \pm 0.4 a	2.6 \pm 0.2 a	1.5 \pm 0.3 a
DS	340 \pm 16 a					
BO	240 \pm 19 abc					
VA	270 \pm 20 ab	+0.11 \pm 0.7* ab	- 17 \pm 8 *			
OS	177 \pm 14 c	0.00 \pm 0.01	-33.0 \pm 4 *	2.0 \pm 0.3 b	1.8 \pm 0.3 b	1.3 \pm 0.3 b
DT	110 \pm 10 d					

*Significantly different from the no stress group ($p < 0.05$, t-test).

Discussion

The capacity to secrete cortisol was significantly lower in perch from contaminated lakes, compared to reference lakes. The cortisol impairment was detected *in vivo* in fish subjected to a confinement stress, and also *in vitro* following a challenge with ACTH and dbcAMP. The secretory impairment could not be reversed by stimulation with dbcAMP, suggesting that intracellular steps following the cAMP generation may be impaired in corticosteroidogenic cells of fish from the most contaminated lakes. Head kidney and liver burdens in metals, as well as tissue levels of metallothionein followed a gradient similar to the secretory impairment, in a dose-related pattern. Our data provide evidence that accumulation of metals in the head kidney may be responsible for the functional impairment of the steroidogenic cells. Although *in vivo* exposures of rainbow trout to environmental levels of Cd up to 30 days in the laboratory elevates plasma cortisol and increases the responsiveness of the head kidney to ACTH *in vitro* (Brodeur et al., 1998), recent laboratory studies demonstrated a dose-dependant secretory impairment in rainbow trout head kidney cells acutely exposed *in vitro* to Cd, Zn, Cu or o,p'-DDD (Leblond and Hontela, 1999; Benguira and Hontela, 2000). These results suggest that the mechanism of the cortisol impairment in fish chronically subjected to environmental pollutants may be a disruption of adrenal steroidogenesis occurring when critical burdens of the xenobiotics, resulting from the chronic environmental exposures, are reached in the head kidney tissue.

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