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Short Research Note

Endocrine disruption in the pituitary of white sucker (*Catostomus commersoni*) caged in a lake contaminated with iron-ore mine tailings

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Abstract

Immunohistochemical analysis revealed that white suckers caged in Lake Wabush, which receives a high volume of iron ore tailings, had significantly lower levels of growth hormone per pituitary area than fish caged in an adjoining reference lake, Lake Shabogamo. This demonstrates that the pituitary could be a target to consider in endocrine studies. The findings are also novel with respect to the ecotoxicological potential of mining effluents.

Introduction

Over the last two decades the phenomenon of endocrine disruption (ED) has been the subject of numerous research programs, workshops, conferences, and reviews (Kime, 1998). A variety of environmental substances such as persistent organochlorines, pesticides, plastic enhancers, and heavy metals are known or suspected endocrine disrupting chemicals. The majority of environmental studies on ED effects are connected with aquatic systems and concern substances which mimic or antagonize steroid hormones, thereby potentially affecting reproduction (Arcand-Hoy & Benson, 1998). However, other hormones such as peptides and proteins in the pituitary gland which play a critical role in growth and developmental processes, may be directly or indirectly important targets. Changes which are due to hypothalamic and/or pituitary disruption have received little attention, mainly due to the relative inaccessibility of the hypothalamic-pituitary system (Arcand-Hoy & Benson, 1998; Kime, 1998). Lake Wabush is part of a large lake system in Labrador (Newfoundland and Labrador, Canada) and is a disposal site for more than 30 000 tonnes of iron laden mine tailings per day. Iron is an essential trace metal for several biochemical reactions, such as those involved in electron transport, energy production, cell growth and division, and cell mediated immunity (Fox & Rader, 1988; Chang et al., 1996). However, iron in excess in all forms and by all routes of exposure is potentially toxic (Fox & Rader, 1988; Chang et al., 1996). Pathological iron deposition in human tissues (lungs, liver, spleen, pancreas, heart muscle) may be associated with e.g., cancer, siderosis, iron or hematite pneumoconiosis, liver cirrhosis, pancreas fibrosis, diabetes mellitus, sarcomas (Fox & Rader, 1988; Poirer & Littlefield, 1996).

Risks to fish health have been demonstrated in association with iron ore mining in Labrador. Lake trout (*Salvelinus namaycush*) from Lake Wabush displayed bleached fish syndrome and other pathologies, as well as elevated levels of DNA oxidative damage and reduced levels of vitamin A in liver tissues in comparison with trout taken in a downstream reference lake (Payne et al., 1998, 2001). In addition, rat and salmon liver microsomes exposed to Wabush iron-ore standard revealed induced ascorbic acid dependent lipid peroxidation and decreased cytochrome *c* reductase (Hamoutene et al., 2000).

White sucker, a bottom feeding fish restricted to North America, is abundant and widespread in Canadian lakes including those in Labrador.

This study investigated the potential for endocrine disruption at the level of the pituitary in white suckers living in a lake receiving iron-ore mine tailings, which are heavily contaminated with iron as well as lower levels of other metals.

Materials and methods

Field sites

Pituitary analysis was carried out on fish caged in Lake Wabush and Lake Shabogamo. In Lake Wabush a plume of 7–10 km iron coloration is visible, caused by the dumping of iron-ore mine tailings from a plant processing ores composed mainly of magnetite (Fe_3O_4) and specular hematite (Fe_2O_3) (Payne et al., 1998, 2001). The reference lake, Lake Shabogamo, connected to Lake Wabush by a narrow shallow passage and located about 30 km downstream from the mine site, is not visibly contaminated. ICP-MS studies of heavy metal contaminants demonstrated a strong gradient of metals in the lake system with high concentrations in the plume in Lake Wabush and very low concentration in the reference lake, Lake Shabogamo (Payne et al., 1998).

Experimental setup

White Suckers (July, 1998) were electro fished, removed from the major tributary to D' Aigle Bay in Lake Wabush, and randomly placed into two 20 l perforated buckets (15 fish per bucket; 10–13 cm standard length). The buckets were anchored at a depth of 3 m below the surface in Lake Wabush (N 53 00.152′, W 66 52.877′) and Lake Shabogamo (N 53 08.208, W 66 37.880) for 2 weeks. When the fish were recovered, their lengths (standard) were recorded and the heads fixed in 10% neutral buffered formalin for cytological and immunohistochemical analysis of the pituitary.

Tested effects

Cellular analysis

Brains embedded in paraffin wax were sectioned serially at 6 μm and the pituitaries located. Every tenth section containing the pituitary was mounted with up to six sections on gelatin coated slides. These slides were either stained with the following trichrome stains, Heath, Papanicolalou, Lead Hematoxylin PAS (Luna, 1968) or prepared for immunochemical examination.

Immunocytochemistry

A two-step indirect immunofluorescence staining procedure was used (Sheenan & Hrapchak, 1980) . Consecutive sections were incubated with rabbit anti-carp gonadotropin (anti-cGTH; Dr D.R. Idler, OSC, St John's, NL, Canada: RA Carp B (4) 04/03/1972) or with a rabbit anti-chum salmon growth hormone (anti-sGH; Dr D.R. Idler, OSC, St. John's, NL, Canada: Chum salmon GH Ab # a 30/09/1991) as primary antibody (dilutions 1:500 and 1:1000). Goat anti-rabbit IgG (whole molecule) conjugated to fluorescein isothiocyanate (FITC) (dilution 1:20; Sigma) was used as secondary antibody. Negative controls were run by substituting the primary antibody with the diluent (0.01 M phosphate saline buffer; pH: 7.4) used for the primary antibody.

Evaluation and statistical analysis

The amounts of the fluorescence in the immunoreactive cells (somatotrophs and gonadotrophs) were analyzed by computerized image analysis (Mocha). Differences between fish from Lake Wabush and Lake Shabogamo were determined by one way ANOVA. Significance was set at $\alpha = 0.05$.

Results and discussion

The average length of fish caged in Lake Wabush was slightly lower than those caged in Lake Shabogamo (11.13 ± 0.56, 13.53 ± 0.51 cm, respectively, $F = 10.16$, $p < 0.004$).

The pituitary of teleosts, located on the ventral side of the brain just behind the optic chiasma, is composed of two parts: the neurohypohysis and the adenohypophysis. The morphology and staining characteristic of the leptobasic pituitary of white suckers appear to be similar to those of other teleost species, such as *Danio rerio* (Van Ree, 1977). With the applied trichrome staining methods (Heath, Papanicolalou) three cell types can be distinguished in the proximal pars distalis (meso-adenohypophysis): acidophils and at least two basophils (Fig. 1A and B). The large basophils can be identified as gonadotrophs (GTH cells) with rabbit anti-carp gonadotropin (Fig. 1C). The acidophils dispersed within the gonadotrophs, can be identified as somatotrophs (STH cells) with rabbit anti-chum growth hormone (Fig. 1D). Immunochemical reactions as measured by amount of fluorescence was significantly different ($p < 0.02$) in the acidophilic somatotrophs of fish from Lake Shabogamo (1.26 ± 0.02 area/pituitary area; Figs. 1D and 2) in comparison with Lake Wabush (0.01 ± 0.01 area/pituitary area; Figs. 1E and 2). No significant differences were obtained for the basophilic gonadotrophs. The control reactions were negative (Fig. 1F).

Growth hormone has multifaceted functions in teleosts, such as stimulating growth, and affecting metabolism, reproduction, smoltification, osmoregulation in salmonids and antifreeze synthesis in winter flounder (reviewed in Le Gac et al., 1993; Mommsen, 2001).

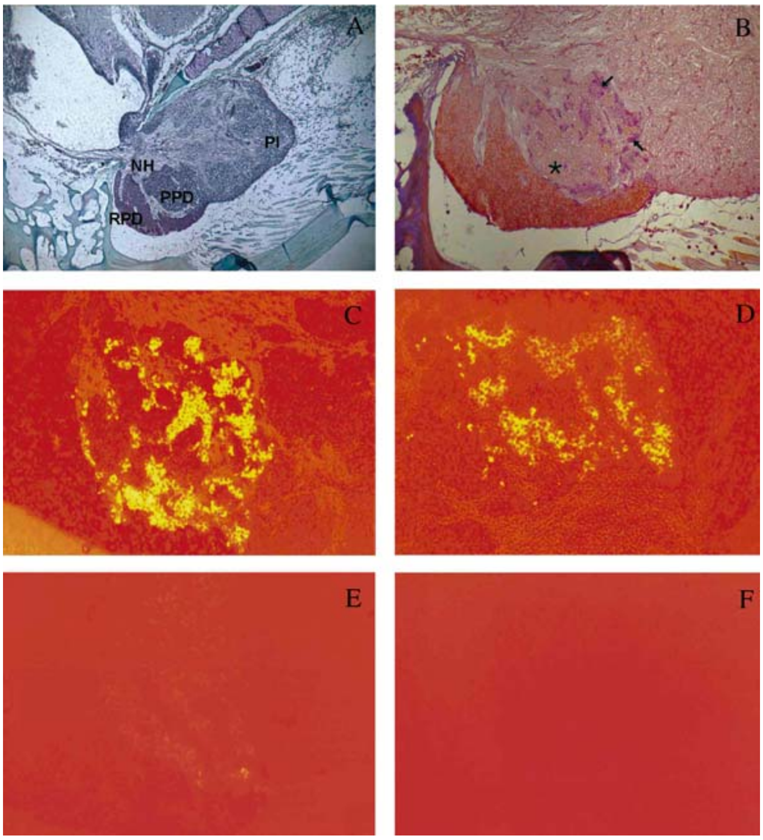

Figure 1. Sagittal histological sections of pituitary glands of white sucker. (A) Fish from Lake Shabogamo. NH: neurohypophysis; RPD: rostral pars distalis; PPD: proximal pars distalis; PI: pars intermedia. Lead hematoxylin stain. ×48. (B) Fish from Lake Shabogamo. Heath's stain, showing the basophils (PAS positive,→) and acidophils (Orange G positive, ∗) ×120. (C) Fish from Lake Shabogamo. Immunofluorescence of gonadotropic hormone in the proximal pars distalis. Dilution of rabbit anti-carp gonadotropin: 1:500.×150. (D) Fish from Lake Shabogamo. Intense immunofluorescence of growth hormone in the proximal pars distalis. Dilution of rabbit anti-chum salmon growth hormone: 1:500. ×150. (E) Fish from Lake Wabush. Little immunofluorescence of growth hormone in the proximal pars distalis. Dilution of rabbit anti-chum salmon growth hormone: 1:500. ×150. (F) Negative control reaction of rabbit anti-chum salmon growth hormone.

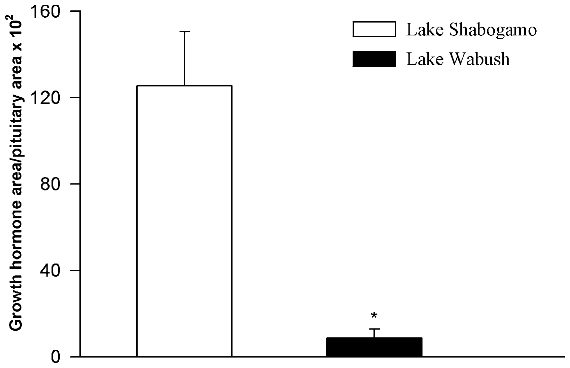

Figure 2. Relative growth hormone area in the pituitary of white suckers at study sites in Lake Shabogamo and Lake Wabush (*significant different: $F = 20.95$, $p = 0.0038$).

It has been shown that stress such as surgery and fasting for long periods can result in hypertrophied and degranulated somatotrophs in some teleosts, indicating increased growth hormone release to adjust metabolism (reviewed in Holmes & Ball, 1974). The decreased level of GH in the somatotrophs in the present study might be caused by chemical stress resulting in decreased synthesis and/or increased release of the hormone due to interruption of negative feedback pathways, or to toxic action on the hypothalamus and/or pituitary.

Conclusions

The pituitary regulates and coordinates many important body functions through synthesis and release of various peptide hormones and could be an important target to consider in endocrine disruption studies. This is supported by novel immunohistochemical observations on lower levels of growth hormone found in the pituitary of fish in association with mining effluents.

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