ELSEVIER

Aquatic Toxicology 42 (1998) 103–113

AQUATIC TOXICOLOGY

In vitro response to ACTH of the interrenal tissue of rainbow trout (*Oncorhynchus mykiss*) exposed to cadmium

Julie C. Brodeur, Claude Daniel, Anne C. Ricard, Alice Hontela *

Département des Sciences Biologiques, TOXEN Research Center, Université du Québec à Montréal, C.P. 8888, Succ. Centre-Ville, Montréal Que. H3C 3P8, Canada

Received 6 January 1997; received in revised form 25 November 1997; accepted 17 December 1997

Abstract

Plasma cortisol levels and responsiveness of the interrenal tissue to ACTH were determined in rainbow trout (*Oncorhynchus mykiss*) subjected to acute and subchronic exposures of cadmium (Cd) in the water. Plasma cortisol levels were significantly increased after 2 days of exposure to 1 μg Cd l^{-1}. They decreased to basal levels after 7 and 14 days of exposure. They significantly increased again after 30 days of exposure. The responsiveness of the interrenal tissue to ACTH was evaluated in vitro by monitoring the secretion of cortisol after stimulation of the head kidneys with 10^{-7} M of ACTH for 10 min in perifusion. The responsiveness to ACTH of the interrenal tissue of fish exposed to 1 μg Cd l^{-1} for 2, 7 and 14 days and 5 μg Cd l^{-1} for 7 days was not significantly different from controls. On the other hand, the interrenal tissue of fish exposed to 1 μg Cd l^{-1} for 30 days secreted significantly larger amounts of cortisol in response to ACTH compared to controls. The significance of these findings to the interrenal dysfunction previously diagnosed in fish from lakes contaminated by heavy metals is discussed.

Keywords: Fish; Cortisol; ACTH; In vitro test; Cadmium

* Corresponding author. Tel.: +1 514 9873000 (ext. 6602); fax: +1 514 9874647; e-mail: hontela.alice@uqam.ca

0166-445X/98/$19.00
PII S0166-445X(98)00042-3

1. Introduction

Cadmium (Cd) is a widely distributed heavy metal, toxic to terrestrial and aquatic organisms (Wright and Welbourn, 1994). In fish, uptake of Cd occurs across the gastrointestinal tract and gills where it involves Ca^{2+} channels (Verbost et al., 1989; Pratap and Wendelaar Bonga, 1993). An exposure of fish to Cd can lead to: (i) a disturbance of ion and water balance; (ii) anemia; (iii) impaired synthesis of sex steroids; and (iv) vertebral deformities (Sangalang and O'Halloran, 1972; Bengtsson et al., 1975; Giles, 1984; Haux and Larsson, 1984; Sjöbeck et al., 1984).

Numerous studies reported an elevation of plasma cortisol levels in fish exposed to Cd (James and Wigham, 1986; Pratap and Wendelaar Bonga, 1990; Fu et al., 1990; Gill et al., 1993; Hontela et al., 1996; Ricard et al., 1997). This increase is a characteristic response of fish to toxicants and to a variety of other stressors such as handling, confinement and variations of pH and dissolved oxygen (Donaldson, 1981; Hontela 1997). The activation of cortisol secretion is part of a general adaptation response to stressors which facilitates—through the osmoregulatory (Flick and Perry, 1989; Laurent and Perry, 1990) and gluconeogenic (Leach and Taylor, 1982) actions of cortisol—maintenance of homeostasis in the presence of an external disturbance. When the doses of the toxicant are acutely lethal, the rise in plasma cortisol is rapid and pronounced. It is maintained until death ensues (Donaldson, 1981; Brown, 1993). At longer laboratory exposures to sublethal doses, the elevation of plasma cortisol is however frequently followed by a decline to basal levels and a secondary rise of plasma cortisol is sometimes observed (Donaldson, 1981; Brown, 1993; Hontela, 1997). In fish chronically exposed in the field to organic pollutants and/or heavy metals, plasma cortisol levels are low and the capacity of the interrenal tissue to respond to adrenocorticotropic hormone (ACTH)—the endogenous stimulus for cortisol secretion by the interrenal tissue—is significantly reduced (Lockhart et al., 1972; Brodeur et al., 1997a,b; Girard et al., 1997). These results suggest that a life-long exposure to sublethal levels of toxicants may lead to an impairment of the normal function of the interrenal tissue.

The mechanisms underlying the interrenal dysfunction in fish from polluted sites have not been elucidated so far. Furthermore, it is not known if functional modifications occur within the interrenal tissue when plasma cortisol levels are increased or decreased during laboratory exposures to Cd. Metals may exert direct toxic effects in the interrenal cells since toxicity of: Cd; mercury; and copper have been demonstrated in the steroidogenic cells of the adrenal gland of mammals, the homologous of fish interrenal tissue (Veltman and Maines, 1986a,b; Mgbonyebi et al., 1994). A chronic elevation of plasma cortisol levels and the synthetic activity of the interrenal cells may however also lead to a functional exhaustion of the steroidogenic capacity of the interrenal cells. To elucidate the mechanisms through which Cd modifies cortisol secretion and to understand how wild fish chronically exposed to heavy metals develop an impairment of the interrenal tissue, the effects of acute (days and weeks) and subchronic (1 month) exposures to sublethal levels of Cd on the responsiveness of trout interrenal tissue to ACTH were assessed in vitro in this laboratory study.

2. Methods

2.1. Fish and maintenance regimens

Immature male and female rainbow trout, *Oncorhynchus mykiss* (mean body weight $\pm$ S.D.; 53 ± 21.4 g) were obtained from a hatchery and maintained for 2 weeks in flow-through (455 l) holding tanks (water hardness 135 mg l^{-1} $CaCO_3$, temperature 10–12°C, photoperiod 12L:12D with onset of the light phase at 07:30 h, dissolved oxygen at saturation, pH 7.2). The water flow rate insured 99% replacement every 2 h, about 4.3 l^{-1} day^{-1}. Fish were fed commercial trout chow daily between 16:00 and 18:00 h at a rate of 1% of the average body weight. Following the 2 weeks, fish were transferred at random into 70-l flow-through experimental tanks (12–14 fish/tank) and were acclimated for 2 weeks before starting the exposure to Cd.

2.2. Experimental treatments

In a first series of acute exposures, fish were exposed through water to 0 and 1 μg Cd l^{-1} as $CdCl_2$ for 2, 7 and 14 days or to 0, 1 and 5 μg Cd l^{-1} for 7 days (1 tank/treatment). A subchronic exposure to 0 and 1 μg Cd l^{-1} for 30 days was conducted in a second series of experiments. The water Cd concentrations were maintained at a constant level with Mariotte bottles (Hontela et al., 1996). The Cd concentrations were measured by atomic absorption by the procedure described in Manca et al. (1992), modified for water samples acidified with ultrapure nitric acid.

2.3. Sampling

Fish were always sampled between 09:00 and 11:00 h to minimize the effects of the diel cycles of cortisol secretion. At the end of the exposure period, all the fish in an experimental tank were removed at once. They were held for 5 s in the net to standardize the handling procedure and they were anesthetized by immersion in a solution of tricaine methanesulfonate (MS-222, 0.1 g l^{-1} of water). Fish were weighed and a blood sample was taken from the caudal vasculature with a heparinized syringe. The plasma was separated by centrifugation (5 min, 13000 rpm), immediately frozen on dry ice and kept frozen at -80°C until cortisol was measured.

2.4. Responsiveness of the interrenal tissue to ACTH—in vitro test

Immediately after fish were blood sampled. The head kidneys containing the interrenal tissue were dissected to evaluate in vitro using perifusion, the functional integrity of the interrenal tissue. The perifusion procedure has been described in detail elsewhere (Brodeur et al., 1997a). Head kidneys are placed in an incubation chamber connected to a peristaltic pump continously supplying the tissues with fresh incubation medium. After perifusion for 110 min to reach the basal rate of

cortisol secretion, the interrenal tissue is stimulated by adding 10^{-7} mol l^{-1} of porcine $ACTH^{1-39}$ to the incubation medium for 10 min. The response of the interrenal tissue is monitored by collecting the medium continously evacuated from the chamber. This is done in 10 min fractions for 120 min and measuring the cortisol concentrations in the fractions by radioimmunoassay. The sum of cortisol measured in all the fractions is used to compare the response to ACTH of fish from different groups. The head kidneys from two animals were pooled in each perifusion chamber.

2.5. Radioimmunoassay of cortisol

Concentrations of cortisol in plasma and in the incubation medium collected from perifusion were determined with a radioimmunoassay kit (ICN Biomedicals No. 07-221102). A standard curve prepared by adding synthetic cortisol (Sigma, St. Louis, MO) to the medium at concentrations used in the kit's standard curve was used to measure cortisol in samples collected from perifusion. The characteristics of the assays done with this standard curve were described previously (Brodeur et al., 1997a).

2.6. Statistical analyses

Differences between groups were tested by either *t*-test or one-way and two-way ANOVA. A Student–Newman–Keuls (SNK) test was used to execute multiple comparisons when the ANOVA detected a significant difference. The data were log-transformed to attain normality and homoscedasticity when necessary.

3. Results

3.1. Cadmium concentrations in water

Exposure was characterized by measuring Cd levels in water of the treatment tanks during the experiments. The average concentrations of Cd (mean $\pm$ S.E.) measured throughout the experiments for the groups treated with 0, 1 and 5 μg Cd l^{-1} were, respectively 0.22 ± 0.6 ($n = 9$), 1.0 ± 0.4 ($n = 8$) and 5.9 ± 1.2 ($n = 2$) μg Cd l^{-1}.

3.2. Exposure to 1 and 5 μg Cd l^{-1}

Plasma cortisol levels of fish exposed to 1 μg Cd l^{-1} for 2 days were significantly higher ($P < 0.05$) than the levels measured in fish from the control group (Fig. 1A). After 7 days, plasma cortisol levels of fish exposed to 1 μg Cd l^{-1}were decreased ($P < 0.05$) compared to the levels measured after 2 days of exposure. There were no significant differences between the control group and groups exposed to 1 or to 5

μg Cd l^{-1}. Fish exposed to 1 μg Cd l^{-1} for 14 days had lower plasma cortisol than the controls which had slightly higher levels ($P < 0.05$) compared to controls on days 2 and 7.

The interrenal tissues of controls and fish exposed to 1 or 5 μg Cd l^{-1} for 2, 7 or 14 days exhibited a similar responsiveness to ACTH since the amounts of cortisol they secreted in response to 10 min of perifusion with 10^{-7} M of ACTH were not significantly different (Fig. 1B). A trend for a decrease in cortisol secretion related to the dose of Cd was however apparent on day 7.

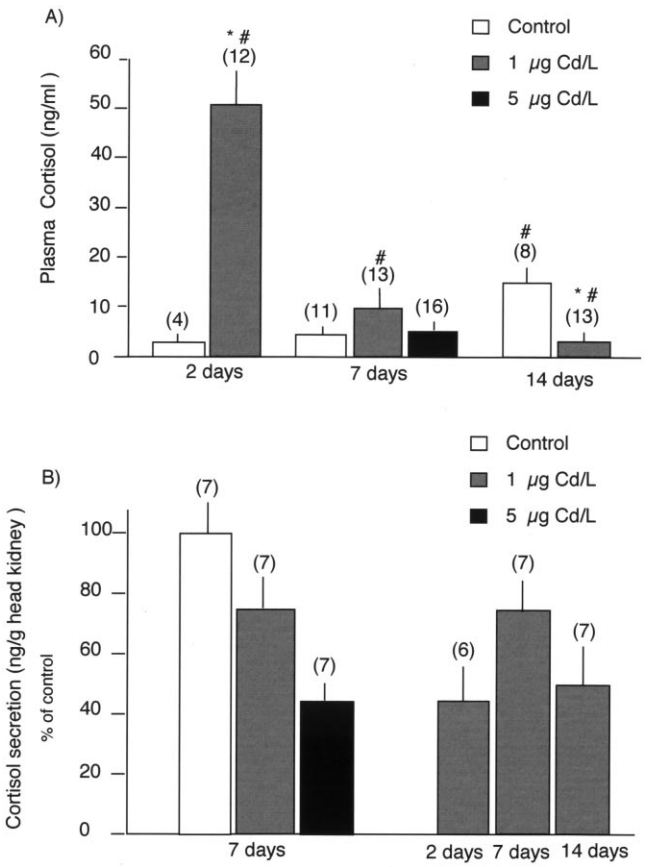

Fig. 1. Exposure of juvenile rainbow trout to 0, 1 and 5 μg Cd l^{-1} for 2, 7 and 14 days. (A) Plasma cortisol levels (mean $\pm$ S.E.). (B) Cortisol (mean $\pm$ S.E.) secreted by the head kidneys after stimulation with 10^{-7} M of ACTH during 10 min in perifusion. Numbers of fish sampled are indicated on top of the error bars. * Significantly different from the control group sampled on the same day and # Significantly different from the two other exposure durations (ANOVA II + SNK test, $P < 0.05$).

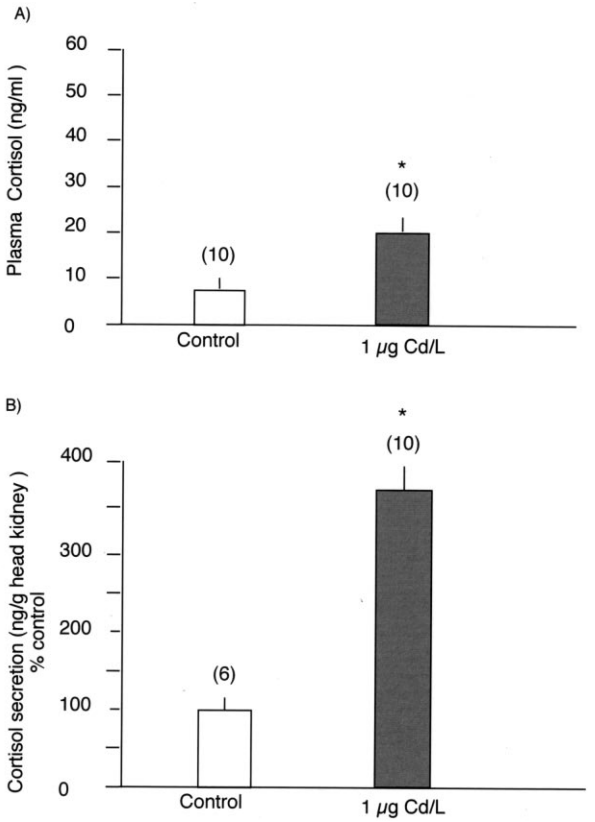

Fig. 2. Exposure of juvenile rainbow trout to 0 and 1 μg Cd l^{-1} for 30 days. (A) Plasma cortisol levels (mean $\pm$ S.E.). (B) Cortisol (mean $\pm$ S.E.) secreted by the head kidneys after stimulation with 10^{-7} M of ACTH during 10 min in perifusion. Numbers of fish sampled are indicated on top of the error bars. * Significantly different from the control group (t-test, $P < 0.05$).

3.3. Exposure to 1 μg Cd l^{-1} for 30 days

Trout exposed to 1 μg Cd l^{-1} for 30 days had significantly higher levels of plasma cortisol compared to fish from the control group (Fig. 2A). The interrenal tissue of fish exposed to Cd (1 μg l^{-1}) for 30 days was also more responsive to ACTH than the interrenal tissue of fish from the control group since it secreted a significantly higher amount of cortisol in response to ACTH (Fig. 2B). The time-course of cortisol secretion by the perifused interrenal tissue of control and treated fish was however similar (Fig. 3).

4. Discussion

The concentrations of Cd used in the present study (1 and 5 μg l^{-1}) are sublethal and represent less than 0.5% of 4 mg Cd l^{-1}, the estimated value of the 48 h LC_{50} of rainbow trout maintained in water at 135 mg $CaCO_3$ l^{-1}. The value of 4 mg Cd l^{-1} was derived from 48 h LC_{50}s reported for rainbow trout by Pascoe et al. (1986), specifically 2.9 mg Cd l^{-1} in water of 70 mg $CaCO_3$ l^{-1} and 5.7 mg Cd l^{-1} in water of 279 mg $CaCO_3$ l^{-1}. No mortality was recorded during the exposures to Cd. Unpolluted lakes have water concentrations of Cd generally lower than 0.1 μg l^{-1} (Tessier et al., 1993; Wright and Welbourn, 1994). Concentrations of Cd of 0.4–0.5 μg l^{-1} are frequently observed in areas where mining and smelting activities occur. Concentrations of 5 μg l^{-1} and more have been reported (Tessier et al., 1993). Fish from polluted environments are also exposed to Cd through their food, unlike fish exposed in the laboratory. The concentrations of Cd used in the present study (1 and 5 μg Cd l^{-1}) can thus be considered as representative of environmental exposures.

The acute (2 days) exposure to 1 μg Cd l^{-1} significantly elevated plasma cortisol levels in the rainbow trout. This increase in response to Cd is a phenomenon that has been previously observed in several fish species (James and Wigham, 1986; Pratap and Wendelaar Bonga, 1990; Fu et al., 1990; Gill et al., 1993; Hontela et al., 1996; Ricard et al., 1997) but not in salmon, *Oncorhynchus kisutch* (Schreck and

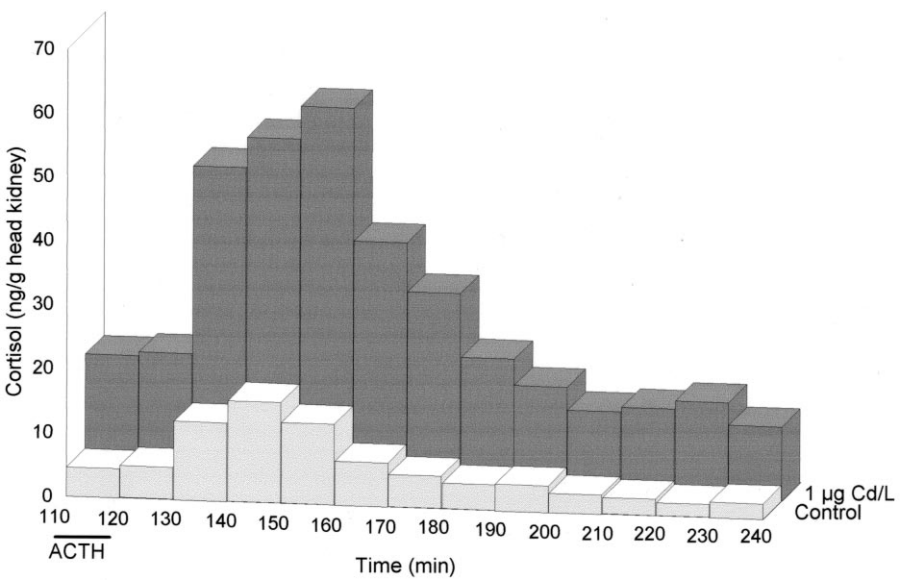

Fig. 3. Cortisol secreted (mean) by head kidneys of juvenile rainbow trout from control group ($n = 6$) and a group exposed to 1 μg Cd l^{-1} for 30 days ($n = 6$) during 10-min intervals after stimulation with ACTH in perifusion. The 10-min interval during which ACTH (10^{-7} M) was administered is indicated by a bar.

Lorz, 1978). The stimulation of cortisol secretion may result from altered osmo–ionic balance since exposure to Cd usually leads to decreased plasma levels of Ca^{2+} and, to a lesser extent, Na^{+} (Haux and Larsson, 1984; Giles 1984; Fu et al., 1990; Ricard et al., 1997). Inhibition of Ca^{2+} uptake and Na^{+}/K^{+}-ATPase in the gills has been demonstrated in fish exposed to Cd (Verbost et al., 1989; Pratap and Wendelaar Bonga, 1993). Changes in osmotic pressure and ion concentrations stimulate cortisol secretion (Decourt and Lahlou, 1986). In turn, cortisol induces proliferation of chloride cells thus increasing influx of Na^{+}, K^{+} and Ca^{2+} (Flick and Perry, 1989; Laurent and Perry, 1990). These actions of cortisol favor the osmo–ionic homeostasis and together with the gluconeogenic action of cortisol, may help the fish to cope with the toxic effects of Cd during acute exposures. An induction of metallothionein synthesis by cortisol, as has been observed in mammals (Hidalgo et al., 1988), would facilitate the process of detoxification.

Despite the continous presence of Cd in the water, plasma cortisol returned to basal levels after 7 to 14 days of exposure to 1 or 5 μg Cd l^{-1} in the present study. The decrease of plasma cortisol levels did not result from an impairment of the functional integrity of the interrenal tissue since the in vitro responsiveness to ACTH of the interrenal tissue of fish exposed to Cd was similar to the one of control fish. Thus, the toxic effects demonstrated for Cd and other heavy metals in the adrenal gland of mammals (Veltman and Maines, 1986a,b; Mgbonyebi et al., 1994) were not observed in the interrenal tissue of trout at the doses of Cd and length of exposure used in the present study. The return of plasma cortisol to basal levels could be mediated by the negative feedback effect (Donaldson, 1981; Bradford et al., 1992) exerted on the hypothalamo–pituitary–interrenal axis (HPI axis) by the elevated plasma cortisol levels observed at the beginning of the exposure. The in vitro responsiveness of the interrenal tissue to ACTH was not significantly reduced after 14 days of exposure to Cd in the present study. The feedback effect most likely reduced the secretion of ACTH by the pituitary gland rather than modifying the sensitivity of the interrenal tissue to ACTH. In a study with tilapia (*Oreochromis mossambicus*), Fu et al. (1990) concluded that the return of plasma cortisol to basal levels illustrated that fish have developed tolerance to Cd. It coincided with a return of plasma Ca^{2+}, Na^{+} and glucose to near normal levels and a rise in the metallothionein-like protein content of gill, kidney and liver. These parameters were not measured in the present study. However the results show that these compensatory mechanisms may not be efficient enough to protect the fish during extended exposures to Cd since plasma cortisol levels increased again after 30 days of exposure to 1 μg Cd l^{-1}.

The elevation of plasma cortisol levels observed after 30 days of exposure to Cd was associated with an increased in vitro responsiveness of the interrenal tissue to ACTH. The subchronic exposure of fish to Cd thus induced functional modifications within the interrenal tissue. This was in contrast to acute exposures which had no major effects on the responsiveness of the interrenal tissue to ACTH. The mechanisms through which changes in the response to ACTH are mediated have not yet been elucidated in fish. Studies with mammals demonstrated that a chronic stimulation of the adrenal gland with ACTH elevates the concentrations of cy-

tochrome P-450. This cytochrome is responsible for the synthesis of glucocorticoids—by enhancing the transcription of their coding genes (Simpson and Waterman, 1988). It is not presently known whether the secretion of ACTH is stimulated in the trout after 30 days of exposure to Cd and the synthesis of steroidogenic cytochrome P-450s is induced, as has been observed in mammals.

The present study provided evidence that a 30 days exposure to 1 μg Cd l^{-1} does not functionally impair the interrenal tissue of trout. The absence of toxic effects after 30 days of exposure is in agreement with the previous observation that the impairment of cortisol secretion detected at field sites contaminated by metals is only evident in fish 4+ and older (Brodeur et al., 1997b). The possibility remains that a laboratory exposure to Cd longer than 30 days will cause toxic effects in the interrenal tissue and studies in progress will test this hypothesis. The results obtained in the present study also demonstrated that rainbow trout does not acclimate to water levels of 1 μg Cd l^{-1} and that cortisol secretion is increased after 30 days of exposure. Sustained stimulation of cortisol secretion and the high metabolic activity of the interrenal tissue may eventually lead to a functional interrenal exhaustion which may not be toxicant-specific. The diagnosis of the interrenal impairment in fish subjected to life-long field exposures to heavy metals (Lockhart et al. 1972; Brodeur et al., 1997a,b; Girard et al., 1997) and also in fish and amphibians tested at sites contaminated by other types of contaminants (McMaster et al., 1994; Hontela et al., 1997; Gendron et al., 1997) supports this hypothesis.

Acknowledgements

Many thanks go to C. Girard and M. Douville for help with sampling and Dr P. Anderson, Concordia University for use of the aquatic facilities. The study was funded by the Canadian Network of Toxicology Centers (Reproductive and Endocrine Toxicology Program). J.C. Brodeur was supported by a scholarship from FCAR.

References

Bengtsson, B.-E., Carlin, C.H., Larsson, A., Svanberg, O., 1975. Vertebral damage in minnows, *Phoxinus phoxinus* L., exposed to cadmium. Ambio 4, 166–168.

Bradford, C.S., Fitzpatrick, M.S., Schreck, C.B., 1992. Evidence for ultra-short-loop feedback in ACTH-induced interrenal steroidogenesis in coho salmon: Acute self-suppression of cortisol secretion in vitro. Gen. Comp. Endocrinol. 87, 292–299.

Brodeur, J.C., Girard, C., Hontela, A., 1997a. Use of perifusion to assess in vitro the functional integrity of interrenal tissue in fish from polluted sites. Environ. Toxicol. Chem. 16, 2171–2178.

Brodeur, J.C., Sherwood, G., Rasmussen, J.B., Hontela, A., 1997b. Impaired cortisol secretion in yellow perch (Perca flavescens) from lakes contaminated by heavy metals: in vivo and in vitro assessment. Can. J. Fish. Aquat. Sci. 54, 2752–2758.

Brown, J.A., 1993. Endocrine responses to environmental pollutants. In: Rankin, J.C., Jensen, F.B. (Eds.), Fish Ecophysiology, Fish and Fisheries Series 9. Chapman and Hall, New York.

Decourt, C., Lahlou, B., 1986. In vitro studies on the release of cortisol from interrenal tissue in trout (*Salmo gairdneri*) II. Action of changes in extracellular electrolytes. Comp. Biochem. Physiol. 85, 747–753.

Donaldson, E.M., 1981. The pituitary–interrenal axis as an indicator of stress in fish. In: Pickering, A.D. (Ed.), Stress and Fish. Academic Press, New York.

Flick, G., Perry, S.F., 1989. Cortisol stimulates whole body calcium uptake and the branchial calcium pump in freshwater trout. J. Endocrinol. 120, 83–88.

Fu, H., Steinebach, O.M., van den Hamer, C.J.A., Balm, P.H.M., Lock, R.A.C., 1990. Involvement of cortisol and metallothionein-like proteins in the physiological responses of tilapia (*Oreochromis mossambicus*) to sublethal cadmium stress. Aquat. Toxicol. 16, 257–269.

Gendron, A., Bishop, C.A., Fortin, R., Hontela, A., 1997. In vivo testing of the functional integrity of the corticosterone-producing axis in mudpuppy (Amphibia) exposed to chlorinated hydrocarbons in the wild. Environ. Toxicol. Chem. 16, 1694–1706.

Giles, M.A., 1984. Electrolyte and water balance in plasma and urine of rainbow trout (*Salmo gairdneri*) during chronic exposure to cadmium. Can. J. Fish. Aquat. Sci. 41, 1678–1685.

Gill, T.S., Leitner, G., Porta, S., Epple, A., 1993. Response of plasma cortisol to environmental cadmium in the eel, *Anguilla rostrata* Lesueur. Comp. Biochem. Physiol. 104C, 489–495.

Girard, C., Brodeur, J.C., Hontela, A., 1998. Responsiveness of the interrenal tissue of yellow perch (*Perca flavescens*) from contaminated sites to an in vivo ACTH challenge test. Can. J. Fish. Aquat. Sci. (In press).

Haux, C., Larsson, A., 1984. Long-term sublethal physiological effects on rainbow trout, *Salmo gairdneri*, during exposure to cadmium and after subsequent recovery. Aquat. Toxicol. 5, 129–142.

Hidalgo, J., Giralt, M., Garvey, J.S., Armario, A., 1988. Physiological role of glucocorticoids and rat serum and liver metallothionein in basal and stress conditions. Am. J. Physiol. 254, 71–78.

Hontela, A., 1997. Endocrine and physiological responses to xenobiotics in fish: Role of glucocorticosteroid hormones. Rev. Toxicol. 1, 1–46, Environmental Toxicology, IOS Press, Amsterdam.

Hontela, A., Daniel, C., Ricard, A.C., 1996. Effects of acute and subacute exposures to cadmium on the interrenal and thyroid function in rainbow trout, *Oncorhychus mykiss*. Aquat. Toxicol. 35, 171–182.

Hontela, A., Daniel, C., Rasmussen, J.B., 1997. Structural and functional impairment of the hypothalamo–pituitary–interrenal axis in fish exposed to bleached kraft mill effluent in the St. Maurice river, Québec. Ecotoxicology 6, 1–12.

James, V.A., Wigham, T., 1986. The effects of cadmium on prolactin cell activity and plasma cortisol levels in the rainbow trout (*Salmo gairdneri*). Aquat. Toxicol. 8, 273–280.

Laurent, P., Perry, S.F., 1990. Effects of cortisol on gill chloride cell morphology and ionic uptake in the freshwater trout, *Salmo gairdneri*. Cell Tissue Res. 259, 429–442.

Leach, G.J., Taylor, M.H., 1982. The effects of cortisol treatment on carbohydrate and protein metabolism in *Fundulus heteroclitus*. Gen. Comp. Endocrinol. 48, 76–83.

Lockhart, W.L., Uthe, J.F., Kinney, A.R., Mehrle, P.M., 1972. Methylmercury in northern pike (*Esox lucius*): distribution, elimination and some biochemical characteristics of contaminated fish. J. Fish. Res. Board Can. 29, 1519–1523.

Manca, D., Lefebvre, M., Trottier, B., LaparÈ, S., Ricard, A.C., van Tra, H., Chevalier, G., 1992. Micro method for the determination of cadmium in tissues and slurried samples by use of flameless atomic absorption spectrometry. Microchem. J. 46, 249–258.

McMaster, M.E., Munkittrick, K.R., Luxon, P.L., van der Kraak, G.J., 1994. Impact of low-level sampling stress on interpretation of physiological responses of white sucker exposed to effluent from a bleached kraft pulp mill. Ecotoxicol. Environ. Saf. 27, 251–264.

Mgbonyebi, O.P., Smothers, C.T., Mrotek, J.J., 1994. Modulation of adrenal cell functions by cadmium salts: 3. Sites affected by $CdCl_2$ during stimulated steroid synthesis. Cell Biol. Toxicol. 10, 35–43.

Pascoe, D., Evans, S., Woodworth, J., 1986. Heavy metal toxicity to fish and the influence of water hardness. Arch. Environ. Contam. Toxicol. 15, 481–487.

Pratap, H.B., Wendelaar Bonga, S.E., 1990. Effects of water-borne cadmium on plasma cortisol and glucose in the cichlid fish *Oreochromis mossambicus*. Comp. Biochem. Physiol. 95C, 313–317.

Pratap, H.B., Wendelaar Bonga, S.E., 1993. Effect of ambient and dietary cadmium on pavement cells, chloride cells and Na^+/K^+-ATPase activity in the gills of the freshwater teleost *Oreochromis mossambicus* at normal and high calcium levels in the ambient water. Aquat. Toxicol. 26, 133–150.

Ricard, A.C., Daniel, C., Anderson, P., Hontela, A., 1988. Effects of subchronic exposure to cadmium chloride on endocrine and metabolic functions in rainbow trout Oncorhynchus mykiss. Arch. Environ. Contam. Toxicol 34, 377–381.

Sangalang, G.R., O'Halloran, M.J., 1972. Cadmium-induced testicular injury and alterations of androgen synthesis in brook trout. Nature 240, 470–471.

Schreck, C.B., Lorz, H.W., 1978. Stress response of coho salmon (*Oncorhynchus kisutch*) elicited by cadmium and copper and potential use of cortisol as an indicator of stress. J. Fish. Res. Board Can. 35, 1124–1129.

Simpson, E.R., Waterman, M.R., 1988. Regulation of the synthesis of steroidogenic enzymes in adrenal cortical cells by ACTH. Ann. Rev. Physiol. 50, 427–440.

Sjöbeck, M.-L., Haux, C., Larsson, A., Lithner, G., 1984. Biochemical and hematological studies on perch, *Perca fluviatilis*, from the cadmium-contaminated river Eman. Ecotoxicol. Environ. Saf. 8, 303–312.

Tessier, A., Couillard, Y., Campbell, P.G.C., Auclair, J.C., 1993. Modeling Cd partitioning in oxic lake sediments and Cd concentrations in the freshwater bivalve *Anodonta grandis*. Limnol. Oceanogr. 38, 1–17.

Veltman, J.C., Maines, M.D., 1986a. Alterations of heme, cytochrome P-450 and steroid metabolism by mercury in rat adrenal. Arch. Biochem. Biophys. 248, 467–478.

Veltman, J.C., Maines, M.D., 1986b. Regulatory effect of copper on rat adrenal cytochrome P-450 and steroid metabolism. Biochem. Pharmacol. 35, 2903–2909.

Verbost, P.M., van Rooij, J., Flick, G., Lock, R.A.C., Wendelaar Bonga, S.E., 1989. The movement of cadmium through freshwater trout branchial epithelium and its interference with calcium transport. J. Exp. Biol. 145, 185–197.

Wright, D.A., Welbourn, P.M., 1994. Cadmium in the aquatic environment: a review of ecological, physiological and toxicological effects on biota. Environ. Rev. 2, 187–214.