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A comparative assessment of the adrenotoxic effects of cadmium in two teleost species, rainbow trout, *Oncorhynchus mykiss*, and yellow perch, *Perca flavescens*

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Abstract

Rainbow trout (*Oncorhynchus mykiss*) and yellow perch (*Perca flavescens)* have a different sensitivity to cadmium (Cd) in vivo (trout < LC50 < perch). Metals and particularly Cd impair cortisol secretion by adrenocortical cells in both species. The purpose of the present study was to assess in vitro the effect of Cd on cortisol secretion by adrenocortical cells of trout and perch, to compare the sensitivity of adrenal steroidogenesis in these two teleosts. Adrenocortical cells were exposed to Cd for 60 min, then stimulated with ACTH, dbcAMP or with pregnenolone, a cortisol precursor. Cd inhibited ACTH-stimulated cortisol secretion in a dose-dependent manner in both fish species, however, the EC50s (concentration resulting in 50% inhibition of cortisol secretion) was significantly lower in trout (EC50 = 0.09 mM) than perch (EC50 = 0.26 mM). To test the specificity of Cd to act as an endocrine disrupter, the LC50 (concentration that kills 50% of the cells) was also evaluated to determine the LC50/EC50 ratio ($LC50/EC50_{O.mykiss} = 175.6 > LC50/EC50_{P.flavescens} = 37.7$). Adrenocortical cells of trout were more sensitive than those of perch and Cd had a higher endocrine-disrupting potential and specificity in trout than in perch. However, in both species, Cd had the same effect on ACTH, dbcAMP and pregnenolone-stimulated cortisol secretion, with pregnenolone maintaining cortisol secretion until cell viability was impaired. These results confirm that for both species, Cd interferes in the signalling pathway of cortisol synthesis in a step prior to the pregnenolone formation. Data provided by the present study revealed important differences in vulnerability of adrenal steroidogenesis between rainbow trout and yellow perch.

Keywords: Fish; Metals; Endocrine toxicity; In vitro; Cortisol; Signalling pathways

1. Introduction

It is well documented that major differences in sensitivity to environmental contaminants exist among species. Hoekstra et al. (1994) predicted the variation in sensitivity of aquatic species, including invertebrates and vertebrates, to toxicants and presented cadmium (Cd) LC50 values (48–96 h exposure) ranging

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from 0.002 to 4600 mg/l. Although toxicological studies use acute or subacute lethality data to compare the susceptibility to environmental contaminants among different species, the cellular mechanisms underlying this difference of sensitivity remain largely unknown.

Rainbow trout (*Oncorhynchus mykiss*) is one of the most sensitive fish to pollutants, particularly to metals including Cd (Hansen et al., 2002). The LC50 values (48 h) for Cd (Table 1) is 4 mg/l at water hardness of 135 mg $CaCO_3$ l^{-1} (Pascoe et al., 1986). Norey et al. (1990) reported that the difference of sensitivity to Cd between rainbow trout, roach (*Rutilus rutilus*) and stone loach (*Nemacheilus barbatulus*), reflected by their LC50s, was correlated with the relative rate of accumulation and the pattern of distribution of Cd within tissues. Their in vivo study suggested that sensitive fish, like rainbow trout, accumulate Cd faster with a major proportion found in the gills. A difference in sensitivity of specific target organs could however also explain the differences of vulnerability to metals. Among fish relatively resistant to Cd exposure is the yellow perch (*Perca flavescens*), a widespread species in metal-contaminated lakes (Sherwood et al., 2002; Eastwood and Couture, 2002). The Department of Environment, UK (1973) published a long-term lethal value for perch (*Perca fluviatilis*) exposed to Cd in hard water as 0.5 mg/l (Table 1) while the value for rainbow trout was 0.01 mg/l (Alabaster and Lloyd, 1982). Although there are no laboratory data on lethal concentration for yellow perch exposed to Cd, there is evidence of accumulation of Cd in the liver, kidney and adrenal tissue of yellow perch from metal-contaminated lakes (Laflamme et al., 2000; Lévesque et al., 2002). Sensitivity to Cd of specific tissues of yellow perch and other teleost species, has not been assessed thus far.

Recent studies provided evidence that the hypothalamo-pituitary–adrenocortical (HPA) axis, crucial for the ability of vertebrates to cope with stressors, is one of the targets of metals in several animal species, including teleost fish (Hontela, 1997; Norris et al., 1999). Cortisol is the main steroid hormone produced in the interrenal tissue of teleosts (homologous to mammalian adrenal tissue). While acute and subchronic in vivo exposures of fish to metals induce an increase of plasma cortisol (Hontela, 1997), chronic in vivo and acute in vitro studies demonstrated that cortisol secretion by adrenocortical cells was significantly compromised by exposure to Cd. Yellow perch inhabiting metal-contaminated lakes exhibit a lower blood cortisol level following confinement stress or i.p. injection of ACTH, compared to fish from reference sites (Brodeur et al., 1997; Laflamme et al., 2000). Although the mechanisms that lead to the impairment of cortisol secretion during chronic exposures to metals are not yet well understood (Hontela, 1997), in vitro studies with rainbow trout adrenocortical cells, demonstrated that ACTH and dbcAMP-stimulated cortisol secretion was significantly compromised by acute in vitro exposures to Cd (Leblond and Hontela, 1999). Exposure-dependant accumulation of Cd in the interrenal organ of yellow perch was documented during long-term, chronic exposure (Lévesque et al., 2002, 2003). Moreover, the uptake of Cd by adrenocortical cells exposed in vitro has been recently characterized in rainbow trout and yellow perch (Raynal et al., submitted). Thus, the in vitro studies may be viewed as a model of the situation of a long term exposure which lead to metal accumulation within the interrenal organ and cause a toxic effect at its site of action in the adrenocortical cells.

Although fish species vary in their sensitivity to metals, it is not known whether differences in the LC50 values in vivo are a manifestation of differences in vulnerability of individual cells, or of the homeostatic mechanisms activated in response to a toxicant. The

Table 1
Comparison of LC50 values of Cd exposure for different teleost species

Species	Term of exposure	Hardness as $CaCO_3$ (mg/ml)	LC50 (mg/l)	Reference
Rainbow trout (*Oncorhynchus mykiss*)	48 h	135	4	Pascoe et al., 1986
Rainbow trout (*Oncorhynchus mykiss*)	50-day	240	0.01	Alabaster and Lloyd, 1982
Stone loach (*Nemacheilus barbatulus*)	60-day	240	2	Alabaster and Lloyd, 1982
Roach (*Rutilus rutilus*)	50-day	250	>9	Alabaster and Lloyd, 1982
Perch (*Perca fluviatilis*)	50-day	250	0.5	Alabaster and Lloyd, 1982

purpose of the present study was to assess in vitro the sensitivity to Cd of adrenal steroidogenesis of two teleost species, rainbow trout and yellow perch, with a different sensitivity to Cd in vivo (Table 1). To test the hypothesis that individual cells mirror the sensitivity to Cd of the whole animal, the effect of in vitro exposures to Cd on cortisol secretion by dispersed adrenocortical cells of rainbow trout and yellow perch were compared in the laboratory. To identify the site of action of Cd in the intracellular pathways leading to cortisol synthesis, the adrenocortical cells of both species were exposed in vitro to Cd, then stimulated with ACTH, dbcAMP or pregnenolone to produce cortisol. The LC50, concentration of Cd that kills 50% of the cells, and EC50, concentration that inhibits 50% of cortisol secretion, were determined in vitro for trout and perch adrenocortical cells, to compare their sensitivity.

2. Materials and methods

2.1. Chemicals

Porcine adrenocorticotropin ($ACTH_{1\text{-}39}$), N^6,2′-*o*-dibutyryladenosine 3′:5′-cyclic monophosphate (dbcAMP), 3β-hydroxy-5-pregnen-20-one (PREG), minimum essential medium (MEM), bovine serum albumin (BSA), hepes, propidium iodide (PI) and cadmium ($CdCl_2$) were purchased from Sigma–Aldrich (Oakville, Ont., Canada). Collagenase/dispase mixture (collagenase from *Achromobacter iophagus* and dispase from *Bacillus polymyxa*) was obtained from Boehringer Mannheim (Laval, Que., Canada). RIA kits for cortisol assays and 3-aminobenzoic acid ethyl ester (MS 222) for anesthesia were purchased from ICN Pharmaceuticals (Orangeburg, NY, USA). Oxytetracycline was obtained from CDMV (St.-Hyacinthe, Que., Canada).

2.2. Experimental animals

Rainbow trout, *O. mykiss* (body weight 100–150 g) were obtained from Labelle Piscultures Inc. (Labelle, Que., Canada). Yellow perch, *P. flavescens* (body weight 50–125 g) were captured by seine in Lake Memphremagog, Quebec, a pristine lake (Brodeur et al., 1997; Girard et al., 1998) approximately 125 km east from Montreal. Upon arrival to the aquatic facilities, fish were maintained in a 600 l freshwater tank at 15 ± 1 °C, supplied with a constant flow rate of 3.8 l/min of filtered and oxygen-saturated water (hardness 70 mg/l $CaCO_3$). Rainbow trout were fed daily with commercial trout food at the rate of 10 g/kg of fish. Wild perch were fed with beef heart, pollock and commercial trout food. Two weeks of acclimation were allowed before the beginning of the experiments. Perch required a 1 week treatment to the antibiotic oxytetracycline (10 mg/l) prior to the acclimation period.

2.3. Preparation of head kidney cell suspension

The method described by Leblond et al. (2001) was used to prepare the cell suspensions. Fish were anaesthetized with MS 222, blood sampled from the caudal vasculature and perfused with a syringe containing saline solution (0.7%), to remove as much blood as possible. Head kidneys were then dissected out and deposited into fresh MEM supplemented with 5 g/l BSA and 2.2 g/l $NaHCO_3$ (complete medium), pH adjusted to 7.4. The tissue was washed and resuspended in 2.5 ml complete medium with 2.0 mg/ml collagenase/dispase, and incubated for 60 min at room temperature with gentle agitation, resuspending the cells every 15 min with a transfer pipette. Following enzymatic digestion, the solution was filtered with a 30 μm mesh cloth and the filtrate was centrifuged at 300 × *g* (1000 rpm) for 5 min. The supernatant was then removed, the pellet resuspended in 1.5 ml complete medium and cellular density adjusted to 75×10^6 cells/ml.

2.4. Determination of the number of steroidogenic cells in the head kidney

The proportion of steroidogenic cells within adrenal tissues of yellow perch and rainbow trout was determined using histochemical detection of 3β-hydroxysteroid dehydrogenase (3βHSD) as described by Shepherd and Holzwarth (1998). In summary, the cell suspensions were plated in a 96-well microplate at 150 μl of 75×10^6 cells/ml per well and fixed for 5 min in 2.6% paraformaldehyde in phosphate buffer (0.067 M, pH 7.4), rinsed in 0.01 M PBS and stained for 3βHSD. Steroidogenic cells, stained blue, were counted under microscope (400×).

2.5. Stimulation of adrenocortical cells

Cells were plated in a 96-well microplate at 150 μl of 75×10^6 cells/ml per well and incubated under optimal conditions as described by Leblond et al. (2001). In summary, a 2 h preincubation with slow agitation was required to reach basal cortisol secretion. Cells were then resuspended in complete medium and the microplate was centrifuged at $300 \times g$ for 3 min. The supernatants were then removed and the cells were resuspended in 150 μl complete medium containing ACTH (0–4 IU/ml), dbcAMP (0–8 mM) and pregnenolone (PREG, 0–50 μM) and stimulated for 120 min. The plate was then centrifuged at $300 \times g$ for 3 min and the supernatants were frozen (−20 °C) until cortisol determination by RIA. All incubations were performed at 15 °C.

2.6. Exposure to $CdCl_2$

Following preincubation, cells were exposed to Cd as $CdCl_2$ (0.01–100 mM) in Ringer solution at 269 mOsm/l, for 60 min at 15 °C. Microplates were then centrifuged ($300 \times g$ for 3 min) and cells were washed once with 150 μl of Ringer solution, centrifuged and the pellets were resuspended in 150 μl of complete medium containing 1 IU/ml ACTH, 2 mM or 10 mM dbcAMP (trout and perch, respectively), or 0.5 μM PREG and incubated as described under *Stimulation of adrenocortical cells*. Cortisol was assayed in the supernatant.

2.7. Viability of head kidney cells

Viability was determined by flow cytometry using the exclusion dye propidium iodide (PI) following the $CdCl_2$ exposure, before the stimulation period. To assess viability, 5 μl samples from each well of the microplate were resuspended in a test tube containing 370 μl of complete medium to which the PI (1 μg/ml) was added. The cells were analyzed by flow cytometry using a FACscan (Becton Dickinson) equipped with an argon laser emitting at 488 nm. For each sample 10 000 events were analysed and the results were expressed as the percentage of live cells. Data analysis were performed with a Consort 32 system and LYSIS-II program.

2.8. Statistical analysis

Statistical significance in each experiment was determined using a one-way analysis of variance (ANOVA) and a Tukey–Kramer Highly Significant Difference (HSD) test ($\alpha = 0.05$). A value of $P < 0.05$ was considered significant. The EC50, LC50 and their respective 95% confidence intervals were determined using non linear regression (curvefit) model using GraphPad Prism. The effective concentration of $CdCl_2$ that decreased the cortisol secretion in response to 1 IU/ml ACTH by 50% (EC50) was determined as well as the lethal concentration that significantly decreased the number of viable cells by 50% (LC50). The ratio LC50/EC50 was calculated to compare the characteristic of Cd to diminish cortisol secretion without inducing a loss in cell viability between trout and perch. A high ratio corresponds to a specific action of the Cd on cortisol secretion, while a low ratio, for example equal to one, could correspond to a non-specific action of the metal, implying that the loss in cortisol secretion is due to cell death.

3. Results

3.1. Proportion of steroidogenic cells

To insure that the potential differences in cortisol secretion between yellow perch and rainbow trout were not caused by differences in the head kidney preparations, the proportion of steroidogenic cells was determined for each species. Steroidogenic cells represented less than 0.02% of all cells present in the head kidney and there were no significant differences in the proportion of steroidogenic cells between trout ($0.014 \pm 0.012\%$) and perch ($0.016 \pm 0.016\%$).

3.2. ACTH, dbcAMP and pregnenolone-stimulated cortisol secretion

Prior to the assessment of adrenal toxicity of cadmium, cortisol secretion by adrenocortical cells of trout and perch in response to ACTH, dbcAMP and PREG were characterized (Table 2). In rainbow trout, 1 IU/ml was chosen as the optimal concentration for subsequent functional tests. Maximal cortisol secretion was obtained with 2 mM dbcAMP (90%

Table 2
Cortisol secretion (% of response to 1 IU ACTH/ml) by adrenocortical cells, from *O. mykiss* and *P. flavescens* stimulated with ACTH, dbcAMP and pregnelonone

	Trout	Perch
ACTH (IU/ml)		
0	3.5 (1.8)	nd
0.05	78.4 (5.7)*	NA
0.1	NA	77.1 (16.6)a
0.5	85.5 (4.5)*	NA
1	*100*	*100*b
4	107.8 (2.3)*	NA
10	NA	111.1 (15.6)c
dbcAMP (mM)		
0	3.5 (1.8)	nd
1	55.8 (2.6)*	NA
2	*90.6* (*7.0*)*	28.7 (20.9)a
4	91.2 (4.8)*	NA
5	NA	48.1 (22.7)b
8	81.9 (3.5)*	NA
10	NA	*75.7* (*20.7*)c
20	NA	87.8 (18.2)d
PREG (μM)		
0	3.5 (1.8)	nd
0.05	11.1 (3.0)*	NA
0.5	*89.4* (*44.7*)*	*243.9* (*225.6*)a
1	NA	533.6 (469.9)a
5	361.1 (125.5)*	1725 (1414)b
50	376.2(144.9)*	NA

The concentration selected for stimulation in subsequent cadmium experiments are in *italic*. Results are expressed as the percentage of response of 1 IU/ml ACTH (mean ± S.D., $n = 11$). * Represents significant difference with control (without stimulation). Different letters represent significant difference among treatments (ANOVA and Tukey–Kramer HSD test $\alpha = 0.05$). NA: non available data. Nd: non detectable.

of 1 IU/ml ACTH response) and no further increase was observed up to 8.0 mM (Table 2). Maximal cortisol secretion was obtained with 50 μM PREG (376% of 1 IU/ml ACTH-stimulated cortisol secretion) and the concentration of 0.5 μM of PREG, inducing a secretory response similar to 1 IU/ml ACTH-stimulated secretion (100%), was chosen. There was no cross-reactivity of pregnenolone within the cortisol assay (data not shown).

In yellow perch, cortisol secretion by adrenocortical cells in vitro was undetectable without stimulation (Table 2). The ACTH-stimulated cortisol secretion increased with the concentration of ACTH, with a maximal secretion at 10 IU/ml. The 1 IU/ml concentration was chosen as reference concentration in subsequent toxicant exposure experiments. The highest dbcAMP concentration tested (20 mM) induced 88% of 1 IU/ml ACTH-stimulated cortisol secretion and 10 mM (75%) was chosen for subsequent exposure experiments. The concentration of 0.5 μM PREG was chosen for toxicant exposure experiments.

3.3. Effect of cadmium on cortisol secretion and viability

The ACTH-, dbcAMP- and PREG-stimulated cortisol secretion and viability of adrenocortical cells, from trout and perch, exposed in vitro to Cd are shown in Figs. 1 and 2. The data are expressed as the percentage of ACTH, dbcAMP or PREG-stimulated controls (not exposed to Cd), to compare the effect of Cd on the responses to these three secretagogues. For both species, stimulated cortisol secretion and viability were impaired in a concentration related manner following in vitro exposure to Cd. Exposure of trout adrenocortical cells to 0.1 mM of Cd significantly decreased ACTH- and dbcAMP-stimulated cortisol secretion (by 45 and 60%, respectively) (Fig. 1), while perch adrenocortical cells were not affected at this concentration of Cd (Fig. 2). In both species, PREG restored cortisol secretion in Cd-exposed cells, until the cytotoxic dose of 10 mM of Cd abolished cortisol secretion and

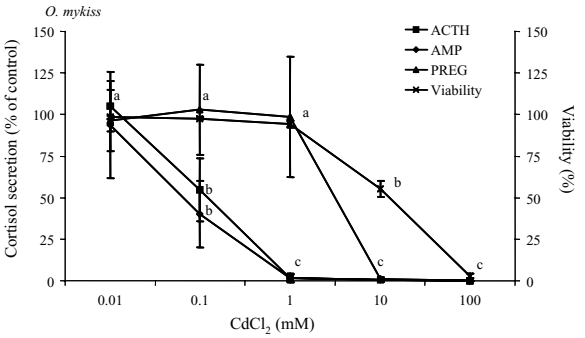

Fig. 1. Effect of acute (60 min) in vitro exposure to cadmium on cortisol production stimulated by ACTH, dbcAMP and pregnenolone, and on viability of adrenocortical cells, from *O. mykiss*. Results are expressed as the mean percentage of unexposed control (0 mM of $CdCl_2$) ± S.D., $n = 11$. Letters represent significant differences among treatments (ANOVA and Tukey–Kramer HSD test $\alpha = 0.05$).

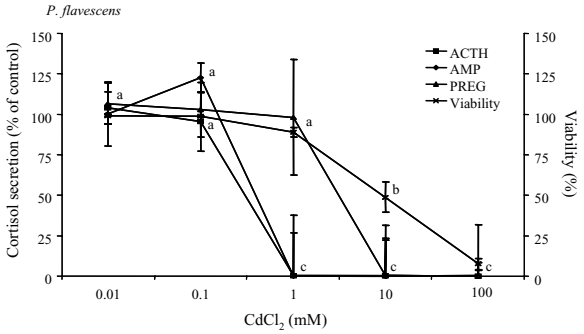

Fig. 2. Effect of acute (60 min) in vitro exposure to cadmium on stimulated cortisol production stimulated by ACTH, dbcAMP and pregnenolone, and on viability of adrenocortical cells, from *P. flavescens*. Results are expressed as the mean percentage of unexposed control (0 mM of $CdCl_2$) ± S.D., $n = 11$. Letters represent significant difference among treatments (ANOVA and Tukey–Kramer HSD test $\alpha = 0.05$).

significantly reduced the viability by 45 and 51% for trout and perch, respectively.

3.4. Comparison of EC50 and LC50

The non linear regression analysis (dose–response curve) of ACTH-stimulated cortisol secretion by adrenocortical cells exposed to Cd determined the EC50 as 0.09 mM (95% confidence intervals 0.06–0.14, $R^2 = 0.93$) and 0.26 mM (95% confidence intervals 0.17–0.39, $R^2 = 0.87$) for trout and perch, respectively (Fig. 3). These results demonstrated

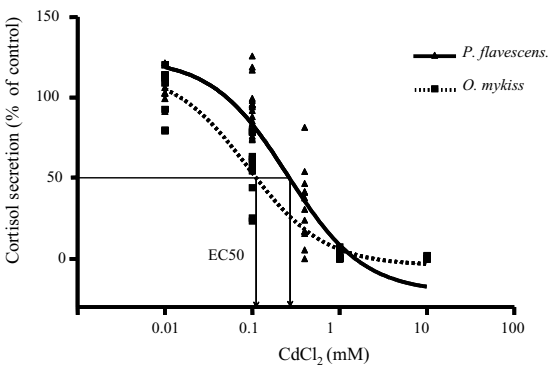

Fig. 3. Comparison of dose–response curve of cortisol secretion of adrenocortical cells, from *P. flavescens* and *O. mykiss*, exposed to $CdCl_2$ and stimulated with ACTH. The differences between EC50s are significant (non linear regression curve fit).

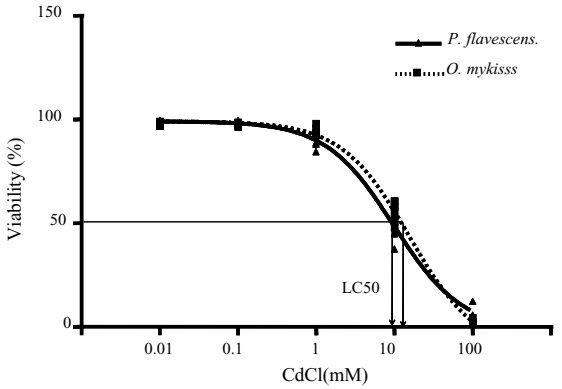

Fig. 4. Comparison of dose–response curve of viability of adrenocortical cells, from *P. flavescens* and *O. mykiss*, exposed to $CdCl_2$ and stimulated with ACTH. The differences between LC50s are significant (non linear regression curve fit).

that rainbow trout is significantly more sensitive to the adrenotoxic effect of in vitro exposure to Cd than yellow perch. The non linear regression analysis of the viability of head kidney cells exposed to Cd determined the LC50 as 15.8 mM (95% confidence intervals 13.3–18.8, $R^2 = 0.99$) and 9.8 mM (95% confidence intervals 7.5–12.8, $R^2 = 0.98$) for trout and perch, respectively (Fig. 4). These results demonstrated a significant difference in the cytotoxic effect of Cd in head kidney cells between these two teleost fish, with the LC50 for perch cells lower than for trout cells. The ratio LC50/EC50 represents the specificity of Cd to impair cortisol secretion. A higher ratio indicates that Cd is more specific to provoke a loss in cortisol secretion without a loss in cell viability. $LC50/EC50_{O.mykiss} = 175.6 > LC50/EC50_{P.flavescens} = 37.7$ indicates that Cd has a higher endocrine-disrupting potential and specificity in rainbow trout than in yellow perch.

4. Discussion

The aim of the present study was to compare in vitro the toxicological responses to Cd of adrenocortical cells from two fresh water teleost species, to determine if differences in whole animal sensitivity to Cd in vivo reflect the vulnerability of individual cells, and to elucidate the mechanisms underlying the difference of sensitivity to metals. Prior to the toxicity testing with

Cd, it was necessary to characterize the optimal cortisol secretion in vitro in both rainbow trout and yellow perch. ACTH, the principal secretagogue for cortisol synthesis by the steroidogenic cells in adrenocortical tissue of teleost fish (Patiño et al., 1986), was used to test the response of the ACTH membrane receptor to Cd. Since cAMP is an important second messenger in the intracellular signal transduction leading to cortisol synthesis, dbcAMP was used as analog of cAMP to test the functional integrity of the post ACTH receptor steps. Pregnenolone, one of the substrates for the biosynthesis of cortisol, was chosen to isolate the effect of Cd on specific enzymatic pathways following the rate limiting step of the P450 cholesterol side chain clivage enzyme. To standardize the cortisol secretion, the concentrations of dbcAMP and pregnenolone were chosen to obtain a secretory response similar to 1 IU/ml ACTH. Perch adrenocortical cells required a higher dbcAMP concentration (10–20 mM) to stimulate cortisol secretion in vitro compared to rainbow trout (2 mM), suggesting a difference in the intracellular signalling pathway at the level of the second messenger, cAMP, between these two teleost species. In both species, pregnenolone induced cortisol secretion without any evidence of substrate limitation.

For both species, stimulated cortisol secretion and cell viability were impaired in a dose related manner following acute (60 min) in vitro exposure to Cd. ACTH- and dbcAMP-stimulated cortisol secretion were significantly decreased at concentrations of Cd between 0.1 and 1 mM (Figs. 2 and 3), while pregnenolone sustained the cortisol secretion until the cytotoxic dose of 10 mM of Cd. These data indicate that in both teleost species, Cd is adrenotoxic and disrupts the signalling pathway in the step prior to pregnenolone synthesis within mitochondria. However, the results demonstrate a significant difference between the EC50s for ACTH-stimulated cortisol secretion determined for the two teleost species, indicating that Cd has a higher endocrine-disrupting potential in rainbow trout than in yellow perch adrenocortical cells (Fig. 3). Our results provided evidence that the vulnerability to Cd of the secretory pathways of the adrenocortical cells of trout and perch mirrors the sensitivity to Cd of the whole animal, with rainbow trout as the more sensitive species. Surprisingly, although the differences in LC50s were not as large as the difference in EC50s, the head kidney cells from the trout appeared more resistant to the cytotoxic effect of Cd than were those from the perch, since the LC50 for trout cells was higher than LC50 for perch cells (Fig. 4). Further assessment of this difference in cell viability is required. However, it must be noted that teleost head kidney tissue is composed of different types of cells and contains chromaffin and immune cells together with steroidogenic cells. Therefore, it is not possible, at this point, to confirm that the difference in the cytotoxic effect of Cd relates exclusively to steroidogenic cells because the viability was evaluated on the whole head kidney cells suspension composed of a small proportion of steroidogenic cells ($<0.02\%$). Our results clearly indicate however that the adrenotoxic action of Cd is more specific for rainbow trout cells than for yellow perch cells, as demonstrated by the ratio LC50/EC50 ($175.8_{\text{O.mykiss}} > 37.7_{\text{P.flavescens}}$). Since there was no difference in the proportion of steroidogenic cells within the head kidney cells suspension of the two species, the difference in sensitivity and specificity to toxicity of Cd can not be attributed to a difference in the number of steroidogenic cells.

The present study provided, for the first time, evidence of differences in vulnerability to Cd of steroidogenesis from two different teleost species. The present results suggest that signal transduction leading to cortisol synthesis is different between perch and trout since dbcAMP-stimulated cortisol synthesis required a higher concentration of dbcAMP for perch adrenocortical cells. Acute and chronic regulation of cAMP in these two species may be different, although cAMP content have not been quantified in adrenocortical cells from perch and trout, following Cd exposure. Nonetheless, in both species, dbcAMP failed to restore the cortisol secretion from cells exposed to Cd, while pregnenolone sustained the secretion, suggesting that for both species the site of action of Cd is located at the same level in the intracellular signalling pathway of cortisol synthesis, prior to pregnenolone synthesis.

The cellular constituents underlying the differences in vulnerability to Cd of the signalling pathways leading to cortisol in different fish species remain to be characterized. Calcium is recognized as an important messenger in steroid synthesis (Van Der Kraak, 1991; Yamazaki et al., 1998) and since Cd competes and/or interferes with calcium ions within the steroidogenic cells (Mathias et al., 1998), the difference in

adrenotoxicity of Cd may be explained by a difference in calcium homeostasis between trout and perch cells. The uptake of Cd through calcium channels has been demonstrated in vitro in adrenal pheochromocytoma cells (Hinkle and Osborne, 1994). Data on Cd transport across membranes of head kidney cells from rainbow trout and yellow perch suggest that the Cd transport is specific and that there is a difference between the two teleost species (Raynal et al., submitted). Although calcium channels have not been characterized in adrenocortical cells of teleost species and the Cd entry may not be specific to steroidogenic cells within head kidney, the difference in Cd transport may contribute to the greater adrenotoxic effect of Cd in rainbow trout steroidogenic cells than in perch.

The intracellular defense mechanisms to metals may also be different between the two teleost species. Metallothionein (MT) levels correlate with metal (Zn, Cu, or Cd) levels in the liver of salmonids and european perch (*Perca fluviatilis*) (Hogstrand et al., 1991; Marr et al., 1995) and Laflamme et al. (2000) also reported an increase of MT levels in adrenal tissue of yellow perch from metal-contaminated lakes. Endogenous levels of MT in adrenocortical cells may be different between teleost species, as there is evidence that the inducibility of MT gene is different between rainbow trout, stone loach and pike (*Esox lucius*) (Olsson and Kille, 1997).

Several studies reported a modulation of antioxidants levels following a Cd exposure in teleost species (Palace et al., 1993; Vaglio and Landriscina, 1999) and differences in antioxidant defense capacity may also contribute to the differences in sensitivity to Cd between trout and perch. Antioxidant enzyme activity levels differ between species (Filho et al., 1993) and exposure-dependant changes in these activities have been recently detected in head kidney cells from rainbow trout exposed in vitro to a pesticide (Dorval et al., 2003). However, mechanisms of oxidative stress have not been characterized in head kidney cells from yellow perch thus far.

The data provided by the present study revealed important differences between rainbow trout and yellow perch in vulnerability of adrenal steroidogenesis to Cd. Rainbow trout, the species more sensitive to the adrenotoxic action of Cd, may be more susceptible to adrenal impairment and exhaustion of cortisol secretion, contributing to compromised survival capacity in environments chronically contaminated by metals. These differences may help to explain the success of different fish species in contaminated aquatic ecosystem.

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