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Short Communication

LOSS OF CAPACITY TO ELEVATE PLASMA CORTISOL IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) TREATED WITH A SINGLE INJECTION OF *o,p′*-DICHLORODIPHENYLDICHLOROETHANE

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Abstract—The organochlorine *o,p′*- dichlorodiphenyldichloroethane (*o,p′*-DDD) is a metabolite of dichlorodiphenyltrichloroethane (DDT), known for its adrenolytic actions in birds and mammals. The effects of *o,p′*-DDD on the cortisol stress response were investigated in rainbow trout, *Oncorhynchus mykiss*, in a dose–response study in vivo. A dose-dependent decrease in plasma cortisol levels was observed on days 7 and 14 after a single i.p. injection of *o,p′*-DDD. Treatment with *o,p′*-DDD had no effect on weight gain, hematocrit, and gonado- or hepatosomatic index but decreased liver glycogen reserves. The results indicate that *o,p′*-DDD is an adrenotoxic compound in rainbow trout and that its effects can be detected even 14 d postinjection.

Keywords—*o,p′*-Dichlorodiphenyldichloroethane Cortisol Adrenotoxicity Disruption Teleost fish

INTRODUCTION

Acute stress conditions activate the teleost hypothalamo-pituitary-adrenal (HPA) axis to release cortisol, the major glucocorticosteroid in fish [1,2]. Cortisol tends to maintain catecholamine-induced hyperglycemia by stimulating protein catabolism and gluconeogenesis, and it promotes lipolysis and has a role in osmoregulation [3]. The capacity to mount the neuroendocrine stress response is a fundamental characteristic of a healthy organism; it enables the animal to cope with acutely stressful situations threatening homeostasis [1]. Plasma cortisol levels have been frequently used to assess the endocrine status of fish exposed to pollutants [2,4], and low plasma cortisol levels have been interpreted as indicators of an unstressed physiological state. However, recent studies have provided evidence that the normal capacity to elevate plasma cortisol in response to acute stress or to stimulation with adrenocorticotropic hormone (ACTH) was impaired in fish chronically field exposed to metals [5], organic contaminants [6], or bleached kraft mill effluent [7–9]. Treatment with β-naphthoflavone or polychlorinated biphenyl (PCB) 126 reduced adrenal sensitivity to ACTH stimulation in vitro and impaired the capacity to respond to acute stress in vivo in tilapia (*Oreochromis mossambicus*) and rainbow trout (*Oncorhynchus mykiss*) [10–12]. These studies demonstrate that low plasma cortisol levels may be a manifestation of a functionally impaired adrenal tissue through endocrine disruption rather than indicating an unstressed physiological status.

The organochlorine DDT is another chemical that interferes with the normal adrenal function. The adrenotoxicity of DDT and its metabolites have been well characterized in mammals and birds [13–16] to reveal major species differences in the type of DDT metabolite that generates the adrenotoxic effects. There is some evidence that DDT and its metabolites are also adrenotoxic in teleost fish. Tilapia injected with *o,p′*-dichlorodiphenyldichloroethane (*o,p*-DDD: 1-[*o*-chlorophenyl]-1-[*p*-chlorophenyl]-2,2-dichloroethane) had lower plasma cortisol and lower response of the adrenal (interrenal) tissue to ACTH in vitro than controls [17,18]. Recently, the effects of DDT compounds on ACTH- and dibutyryl cyclic adenosine 3′,5′monophosphate-stimulated cortisol secretion were characterized in vitro in adrenal tissue of rainbow trout [19,20]. Since DDT and its metabolites remain ubiquitous contaminants in the aquatic environment [21,22] and because there may be species differences in the vulnerability of the adrenal tissue to these organochlorine chemicals, we designed an experiment to determine in vivo the effects of *o,p′*-DDD on the capacity to mount the normal cortisol stress response and the physiological status of rainbow trout, a model teleost species.

MATERIALS AND METHODS

Fish and maintenance

Juvenile rainbow trout (*O. mykiss*), body weight 170 ± 10 g, purchased from a commercial supplier, were maintained in two 600-L, flow-through (3.8 L/min) tanks separated into two compartments of 250 L by a partition permitting circulation of water. They were acclimated to 15°C, 12:12-h light:dark, pH 7.0–7.5, and water hardness of 70 mg/L $CaCO_3$ for three weeks prior to the experiments. Fish were fed commercial trout food (10 mg/kg fish) daily at 07:00–08:00 AM. They were not fed 24 h prior to injection or each blood sampling.

Experimental treatments

Fish were either rapidly removed from the tanks (no confinement) or removed and maintained in a net for 30 s (confined group) before anesthesia with 0.05 g/L MS 222 (ICN Biomedicals, Montreal, PQ, Canada). On day 0, fish were weighed, tagged, blood sampled, and injected i.p. (100 μl/100 g body wt) with 5, 20, or 50 mg/kg *o,p′*-DDD (Aldrich, Milwaukee, WI, USA) dissolved in corn oil (Sigma, St. Louis, MO, USA)

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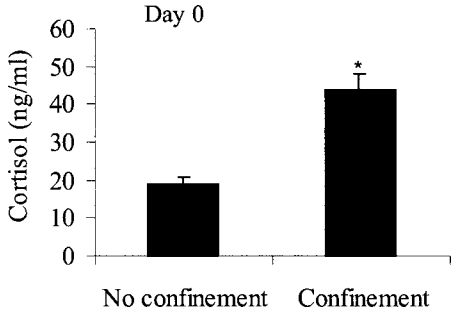

Fig. 1. Plasma cortisol (mean ± standard error of means) of rainbow trout sampled without a confinement stress (no confinement) and following a confinement stress of 30 s (confinement) on day 0. * = statistical significance established by the Dunnett's test ($p < 0.05$); $n = 32$ in each group.

between 08:00 and 10:00 AM. The control group was injected with the vehicle (corn oil) only. The control and 5 mg/kg *o,p′*-DDD-treated fish were kept in one compartment in the first tank and were sampled without confinement. The other two groups (controls and 5 mg/kg *o,p′*-DDD) sampled with confinement were kept in the second compartment of the same tank. Pilot experiments, including selection of the flow-through rate of water (3.8 L/min), validated this experimental design. Fish treated with 20 or 50 mg/kg *o,p′*-DDD were distributed and sampled using the same design in a second tank. All the fish were blood sampled on days 0, 7, and 14 postinjection, and plasma cortisol was assayed by radioimmunoassay (ICN Biomedicals, Montreal, PQ, Canada). Fish were sacrificed on day 28, and liver and gonad weights were determined for the hepato- and gonadosomatic indices (% fresh organ wt/body wt). Blood on day 28 was used for measurements of hematocrit, and liver was used for analysis of glycogen, as described previously [23], and of *o,p′*-DDD.

Analysis of o,p′*-DDD*

Liver *o,p′*-DDD content was measured in 1 g of liver extracted in dichloromethane. The organic fraction was washed with distilled water and filtered on anhydrous sodium sulfate. The organic solvent was then concentrated by evaporation and filtered on Millex HV-13 (Millipore, Nepean, ON, Canada) before purification by gel permeation chromatography. The organochlorine was then purified in a Florisil column (BDH Chemicals, Toronto, ON, Canada). The purified extracts were concentrated and analyzed on a HP-5890 gas chromatograph (Hewlett-Packard, Avondale, PA, USA) equipped with dual capillary columns of 50 m (Ultra-1 and Ultra-2). The detection limit was of 0.07 μg/kg of tissue; the quality control was established with cod liver oil (Standard Reference Material 1588, National Institute of Standards and Technology).

Statistical analyses

Means were compared and statistical significance was established by the Dunnett's test ($p < 0.05$).

RESULTS AND DISCUSSION

Liver *o,p′*-DDD concentrations 28 d postinjection in fish treated with 50 mg/kg *o,p′*-DDD and in controls (corn oil only) were 2.25 ± 0.104 and 0.0097 ± 0.0007 mg/kg, respectively. The levels in the treated fish were about 230-fold higher compared with controls, represent about 5% of the initial dose, and were above concentrations reported for fish sampled in the wild [21]. Although *o,p′*-DDD was not measured in the adrenal tissue in the present study, evidence from a study with tilapia [18] suggests that the toxicant does become bioavailable to the steroidogenic adrenal cells since about 15% of the initial dose was measured in the head kidney as early as 24 h postinjection.

Effect of confinement stress on plasma cortisol

A confinement stress of 30 s on day 0, prior to the injections, significantly increased plasma cortisol levels in all the experimental groups (Fig. 1). The confinement elevated plasma cortisol levels to about 40 ng/ml, similar to other studies where a confinement stress of similar duration (30 s) was used in rainbow trout [24]. Even though higher plasma cortisol levels can be induced by a more intense confinement stress, such treatment can have adverse effects on blood lymphocyte count and weight gain [25]. In the present study, cortisol secretion was stimulated sufficiently to evaluate the capacity of the HPA axis of *o,p′*-DDD treated fish without adversely affecting their weight gain, gonadosomatic index (gonad wt/body wt × 100), hepatosomatic index (liver wt/body wt × 100), or hematocrit (Table 1). However, a dose-dependent decrease in liver glycogen reserves was observed on day 28 in fish sampled following a confinement (at sacrifice, Table 1). Whether the de-

Table 1. Characteristics of rainbow trout treated with 5, 20, 50 mg/kg *o,p′*-DDD or corn oil only (controls, 0 mg/kg)[a]

Group	Weight gain (g)	Gonadosomatic index (%)	Hepatosomatic index (%)	Hematocrit (%)	Glycogen (mg/g liver)
0 mg/kg					
No confinement	43.4 ± 9.4	1.08 ± 0.86	1.93 ± 0.34	41.38 ± 4.47	1.54 ± 0.81A
Confined	52.0 ± 4.3	0.88 ± 0.71	2.10 ± 0.21	41.11 ± 1.64	1.03 ± 0.71A
5 mg/kg					
No confinement	55.8 ± 3.5	3.34 ± 0.96	1.98 ± 0.27	52.5 ± 3.39	1.40 ± 0.79A
Confined	45.7 ± 5.7	1.18 ± 0.97	1.67 ± 0.07	42.15 ± 2.59	1.03 ± 0.61A
20 mg/kg					
No confinement	36.6 ± 10.9	2.14 ± 1.04	1.45 ± 0.17	42.44 ± 3.48	0.61 ± 0.32AB
Confined	45.7 ± 5.7	4.25 ± 1.22	1.94 ± 0.25	47.38 ± 6.88	0.34 ± 0.05BC
50 mg/kg					
No confinement	45.9 ± 6.4	3.69 ± 0.97	1.69 ± 0.13	47.11 ± 3.87	0.49 ± 0.85AB
Confined	46.6 ± 8.8	0.52 ± 0.33	1.98 ± 0.13	40.0 ± 8.18	0.29 ± 0.06C

[a] Data presented as mean ± standard error of the mean (SEM); $n = 10$ per group for all measures except hematocrit ($n = 8$). Means with different letters are statistically different (Dunnett's test, $p < 0.05$). *o,p′*-DDD = 1-[*o*-chlorophenyl]-1-[*p*-chlorophenyl]-2,2-dichloroethane.

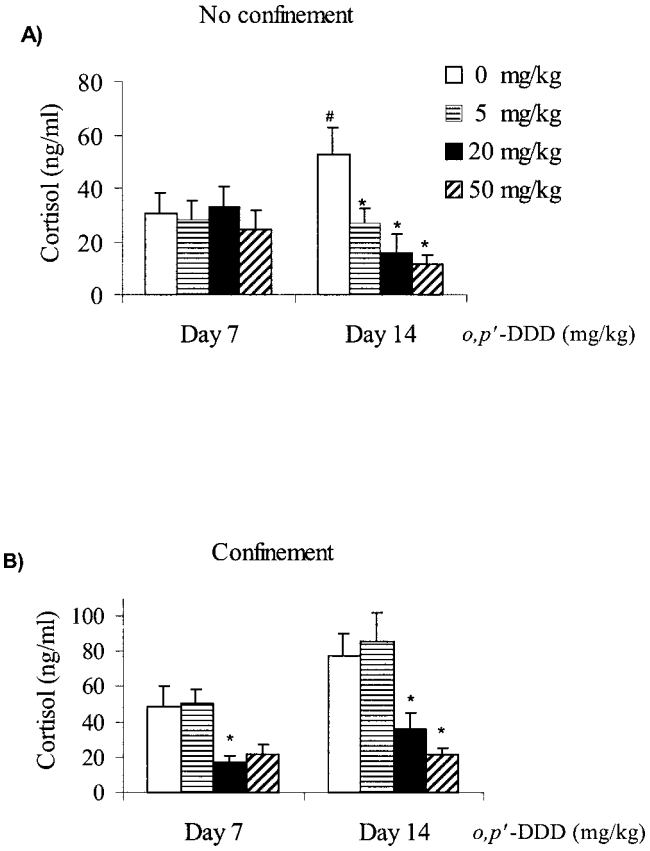

Fig. 2. Plasma cortisol (mean ± standard error of means) in rainbow trout injected on day 0 with corn oil (0 mg/kg) or with 5, 20, or 50 mg/kg *o,p′*-dichlorodiphenyldichloroethane (DDD) and sampled on days 7 and 14 (**A**) without confinement or (**B**) with confinement. Statistical significance was established by the Dunnett's test ($p < 0.05$). * = difference from group treated with 0 mg/kg on same sampling day; # = difference from 0 mg/kg group on day 7; $n = 10$ in each group.

crease in glycogen reserves was caused by a metabolic dysfunction interfering with the synthesis of glycogen or higher energetic demands in *o,p′*-DDD-treated fish remains to be investigated.

Effect of o,p′*-DDD on plasma cortisol*

The temporal dynamics of the adrenotoxic effects of *o,p′*-DDD are shown in Figure 2. In the no-confinement groups, *o,p′*-DDD had no effect on plasma cortisol on day 7 (Fig. 2A). Plasma cortisol increased significantly from day 7 to day 14 in the controls treated with corn oil only (0 mg/kg), probably because of the blood sampling and handling on days 0, 7, and 14. However, a dose-dependent decrease in plasma cortisol is evident on day 14 postinjection (Fig. 2A), clearly demonstrating that, unlike the controls, fish treated with *o,p′*-DDD had a reduced capacity to elevate plasma cortisol levels. The adrenotoxic potential of *o,p′*-DDD is also revealed in the groups sampled following the confinement stress (Fig. 2B). A significant decrease in plasma cortisol levels was observed on day 7 and also on day 14 in the groups that received 20 and 50 mg/kg *o,p′*-DDD. These results provide evidence for adrenotoxic action of *o,p′*-DDD in rainbow trout similar to the effects reported in mammals, birds, and the teleost tilapia [13–15,18]. Interestingly, the doses of 20 and 50 mg/kg did have a significant effect on plasma cortisol on day 7 in groups sampled with a confinement stress while no effect was detected in groups sampled on day 7 without confinement. This suggests that the adrenotoxic effect of *o,p′*-DDD may be more evident during a functional challenge that activates adrenal steroidogenesis.

The effective doses and the mechanisms of action responsible for the decrease in plasma cortisol in fish need to be established because of the ubiquity of this organochlorine in the environment [21,22], its continued use in many countries around the world, and differences in the mechanisms of action of DDT compounds among vertebrates. There is evidence for teleosts that *o,p′*-DDD disrupts the adrenal steroidogenesis at steps upstream from the cAMP generating process within the steroidogenic enzymatic pathways since treatment with dbcAMP, a cyclic adenosine 3′,5′-monophosphate analog, could reverse the inhibition of cortisol synthesis in the presence of *o,p′*-DDD [17,19,20]. In higher vertebrates, *o,p′*-DDD seems to target different intracellular processes. In birds and mammals, *o,p′*-DDD is bioactivated by a cytochrome P450 into a reactive intermediate that covalently binds and inhibits the 11β-hydroxylase, a key enzyme that converts 11-deoxycortisol into cortisol, at a step downstream from cAMP formation [13–16]. Studies in progress in our laboratory aim to identify the specific cellular targets of *o,p′*-DDD in the rainbow trout adrenal steroidogenic cell model.

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