Activation of the hypothalamus–pituitary–interrenal (HPI) axis is characteristic of stress responses, which may result from a variety of environmental challenges. To investigate whether the stress response, and in particular the HPI axis, in tilapia (*Orechromis mossambicus*) is compromised by short-term exposure to PCB 126, fish of both sexes were fed diets containing PCB 126 (50 µg/kg fish • day) for 5 days. In the first approach, which was performed twice, fish were acutely stressed for periods varying between 1 and 30 min at the end of the exposure period; in the second approach fish were sampled at the end of the exposure period either at rest or after 2 h of stress (confinement). After 5 days, the body weights in all experiments were significantly lower in PCB-fed fish than in control fish. There were no changes in basal plasma glucose levels, plasma ion concentrations, or branchial, renal, and intestinal Na,K-ATPase activity following PCB exposure. In the first experimental approach, in which fish experienced acute sampling stress, plasma cortisol levels reached lower levels in PCB-fed fish than in control fish. There were no changes in basal plasma glucose levels, plasma ion concentrations, or branchial, renal, and intestinal Na,K-ATPase activity following PCB exposure. In the first experimental approach, in which fish experienced acute sampling stress, plasma cortisol levels reached lower levels in PCB-fed fish than in control fish. This suggests an impaired ability to acutely activate interrenal steroidogenesis in PCB-treated tilapia. Adrenocorticotropic hormone (ACTH) and cAMP-stimulated in vitro cortisol release from superfused head kidneys was lower in tissues from tilapia exposed to PCB 126 than in tissues from control animals. This effect persisted after 24 h in vitro, which, together with the high PCB 126 concentrations measured in the head kidneys of PCB-fed fish, may indicate direct toxic effects on the interrenal cells. The second experimental approach demonstrated that basal plasma cortisol and ACTH levels were not influenced by PCB treatment, but that the basal ACTH content of the rostral pars distalis (RPD) of the pituitary gland of PCB-fed fish was lower than that of control fish. After 2 h confinement, plasma cortisol levels and ACTH content of the RPD rose to similar values in both groups, whereas plasma ACTH levels were higher in confined PCB-fed fish than in confined controls. PCB-fed fish showed a lower hyperglycemic response to confinement than control fish. Confinement resulted in similarly elevated renal and intestinal Na,K-ATPase activities in both PCB-fed and control fish; branchial enzyme activities were not affected. Since PCB did not affect Na,K-ATPase activities and plasma ion concentrations, it is concluded that the effects of PCB 126 on the HPI axis in tilapia are not secondary to ionoregulatory dysfunction.

**Key Words:** cortisol; adrenocorticotropic hormone; endocrine disruption; pituitary–interrenal axis; stress; teleost fish; PCB 126

**INTRODUCTION**

The stress response of vertebrates involves several endocrine tissues and their targets. This response is essential for responding successfully to environmental changes (Sumpter, 1997; Wendelaar Bonga, 1997). Disruption of this stress-related endocrine system may therefore impair the ability to cope with additional stressors. Such reduced ability may lead to impaired reproduction or may even be fatal (Barton and Iwama, 1991). Recently, toxicants have been shown to interact with several endocrine control mechanisms (Goksøyr
and Förlin, 1992; McKinney and Waller, 1994; Barron et al., 1995).

Polychlorinated biphenyls (PCBs) are highly toxic environmental pollutants with specific modes of action (Safe, 1984, 1990). They interfere with neuroendocrine systems, partly by exerting estrogenic effects (Soontornchat et al., 1994). Studies investigating the mechanisms of action underlying the effects of PCBs on endocrine tissues revealed that PCBs can interfere with steroidogenesis and steroid receptors (McKinney and Waller, 1994) and with the mixed-function oxygenase systems involved in steroid metabolism (Goksøyr and Förlin, 1992), and are able to bind to the Ah receptor (Nebert, 1989). PCBs may interfere with corticosteroidogenesis and corticosteroid action in a similar manner as described for gonadosterooidogenesis (Barron et al., 1995) and thyroid hormone synthesis (McKinney and Waller, 1994; Murk et al., 1994). Little information is available, however, on PCB effects on adrenal function, in particular during stress.

In fish, including freshwater-adapted tilapia (Balm et al., 1986), cortisol, the main end product of the hypothalamic–pituitary–interrenal (HPI) axis, regulates both energy and hydromineral balances [for review, see Sumpter (1997) and Wendelaar Bonga (1997)]. Cortisol release is regulated by many factors including adrenocorticotropic hormone (ACTH) (Young, 1985; Arnold-Reed and Balment, 1994; Balm et al., 1994). A variety of toxic and nontoxic stressors stimulate the HPI axis to increase both ACTH and cortisol levels [for review, see Sumpter (1997) and Wendelaar Bonga (1997)]. Field studies of the influence of water pollutants, including PCBs, on the stress response in fish (Hontela et al., 1992, 1995) showed lower cortisol levels in acutely stressed fish from polluted environments than in acutely stressed fish from reference sites. In a laboratory study, feeding low doses of PCB 169 to rainbow trout (Oncorhynchus mykiss) increased in vitro production of cortisol, cortisone, and other progesterone metabolites by interrenal tissue of these fish compared with controls. At higher doses, however, the in vitro production of these metabolites was impaired (Freeman et al., 1984). In another study on rainbow trout, no influence was observed on in vitro cortisol production 5 days after a single injection with PCB 169 (Miranda et al., 1992), whereas a more recent study (Vijayan et al., 1997a) suggested that the impaired ability to elicit a stress response in trout exposed to PCB 77 might be due to enhanced hepatic cortisol clearance. Therefore, conclusions drawn regarding the effects of PCBs on the HPI axis differ, possibly because only interrenal and not corticotrophic function was examined in these studies.

The objective of this study was first to investigate the influence of stress-free administration of PCB 126, one of the most toxic PCB congeners (Safe, 1990), on the HPI axis in tilapia (Oreochromis mossambicus). Second, we wanted to examine whether possible influences of PCB 126 on the HPI axis were direct (e.g., related to accumulation of PCB 126 in the head kidneys of these fish) or indirect (due to ionoregulatory dysfunction). Such hydromineral disturbances are known to act as stimuli for interrenal cortisol production (Wendelaar Bonga, 1997). It has been shown that organic pollutants (Mallatt, 1985) and also PCBs (Teh et al., 1997) cause functional and structural changes in the gills with negative effects on water and ion balance.

Two different experimental approaches were taken: In the first approach, fish were sampled after acute sampling stress (maximum 30 min). It had been shown earlier in tilapia that over this period, levels of cortisol, but not of ACTH, increase (Balm et al., 1994). This approach allows assessment of the effect of PCB 126 on the acute phase of the interrenal stress response, resembling the acute stress situation in the field studies of Hontela et al. (1992, 1995). In the second approach, fish were sampled both at rest and after prolonged stress (2-h confinement). Under these conditions, plasma ACTH and cortisol levels are elevated (Balm et al., 1994).

MATERIAL AND METHODS

Animals

Tilapia of both sexes from our laboratory stock (kept at 24 ± 1°C) were weight-matched and divided into groups of 8 or 10 fish for experiments investigating the effects of dietary PCB on the acute response to capture (approach I, experiments Ia and Ib) or the response to confinement (approach II). Fish were kept in glass
tanks containing 45 liters of water on a 12-h-light, 12-h-dark regimen. A freshwater flow-through rate of 10 liters per day per tank was achieved by peristaltic pumps. Average body weights at the beginning of the acclimation period were as follows: experiment la—20.9 g (controls, n = 8), 19.4 g (PCB group, n = 8); experiment lb—24.2 g (controls, n = 16), 23.9 g (PCB, n = 16); experiment II—20.8 g (controls, n = 20), 20.6 g (PCB, n = 20). The resulting densities vary from 3.9 to 4.6 kg/m³. Fish were acclimated for at least 3 weeks during which they were fed 2% of their body weight divided over two daily meals. Fish were fed TetraMin flakes enriched with Vitamin C. Food was contaminated with PCB 126 by mixing it with ethanol containing PCB and subsequently evaporating the vehicle. The fish received either PCB 126-containing food (50 µg/kg fish per day in two daily meals of 1% body weight each) or food contacted with vehicle only (controls).

In experiments la and lb (which consisted of duplicate tanks per treatment) fish were caught, after 5 days of the experimental feeding regimen, in alternating order between treatments at 1.5-min intervals (experiment la) and 1-min intervals (experiment lb) starting with a PCB-fed fish. Balm et al. (1994) showed that in tilapia this sampling procedure leads to a rapid increase in plasma cortisol levels, reaching a plateau after approximately 6 min. For calculation of this plateau, plasma cortisol values from the first two animals sampled from each group were omitted.

In experiment II fish were kept in duplicate tanks per treatment and fed either PCB-contaminated or control food. One week before the onset of PCB treatment, a daily “training” procedure was started: In random order, one fish from each tank was netted and subsequently released into the tank from which it was taken. It is known that such a treatment habituates the animals to a sampling-related event resulting in a reduction of the sampling-associated rise in plasma cortisol levels (Schreck et al., 1995; and our unpublished observation). Daily from day 4 until day 7 of the experimental feeding, one animal from each tank was quickly caught and treated as described below. This was performed to obtain more fish sampled as “firsts”, with resting plasma cortisol levels. These animals comprised the groups sampled “at rest” (n = 4 per tank). On day 7 the remaining 6 fish per tank were confined in nets in groups of 3 for 2 h in their original tanks. These fish are further referred to as “confined” fish (n = 12 per treatment).

**Blood**

Immediately after sampling, fish were killed, and blood was taken and prepared as previously described (Balm et al., 1994). Plasma cortisol and ACTH levels were determined by radioimmunoassays (Balm et al., 1994). Plasma glucose levels were determined with the Boehringer UV test kit (Boehringer, Mannheim, Germany). Plasma Na⁺ levels were measured with a flame-photometric Auto Analyser (Model IV, Technion), and Cl⁻ concentrations were determined spectrophotometrically via formation of ferrothiocyanate.

**Pituitary Rostral Pars Distalis**

Pituitary glands were dissected from the brain. The rostral pars distalis (RPD), which contains the ACTH-producing cells, was separated and treated as described earlier (Balm, 1986). The rostral pars distalis (RPD) which contains the ACTH-producing cells, was separated and analyzed as described previously (Balm et al., 1994). The percentage of circulating ACTH, compared with the total amount of ACTH, which was estimated for every fish as [total plasma ACTH/(total plasma ACTH + RPD ACTH content)] × 100%, assuming that the plasma volume constitutes 4% (v/v) of the body weight as calculated for brook trout (Nichols et al., 1985).

**Head Kidneys**

Head kidneys from tilapia used in experiments la and lb were dissected and superfused as described previously (Balm, 1986). The head kidneys were challenged with 15-min pulses of ACTH (1 nmol/liter human ACTH₁-39, Pensinsula) after 130 min of incubation. 15-min pulses of cAMP (1 nmol/liter 8-bromo-cAMP, Sigma) after 400 min and, in experiment II, also with a second ACTH pulse (15 min 1 nmol/liter) after 24 h in vitro. Cortisol release from the head kidneys was determined as described earlier (Balm et al., 1994) and calculated as pg cortisol min⁻¹ g body wt⁻¹. Maximally stimulated values of cortisol release from each chamber were used for statistical analysis.

After superfusion head kidney tissues were pooled from four randomly chosen fish of each group (experiment la), and PCB concentrations were determined as described earlier (de Boer et al., 1993).
**Chloride Cells**

From each fish one operculum was dissected and incubated for 1 h in a 2 μM 2-(dimethylaminostyryl)-1-ethylpyridinium iodine (DASPEI) solution, which stains the mitochondria-rich chloride cells. The total amount of chloride cells was then quantified as described earlier (Verbost et al., 1994).

**Na,K-ATPase Activity**

Gill filaments, whole kidneys, and an approximately 1-cm-long piece of the middle part of the intestine were isolated, placed in 1 ml ice-cold buffer [0.3 M sucrose, 20 mM Na₂EDTA, 0.1 mM imidazole, pH 7.1, with HCl (Zaugg, 1982)], frozen on dry ice, and stored at -70° until assayed. Prior to determination of enzyme activity, tissues were homogenized using a Polytron Ultraturrax for 1 min at full speed and centrifuged in an Eppendorf centrifuge for 5 min at 10,000 g. Protein content of the resulting supernatant was determined with Coomassie brilliant blue (Bradford, 1976). The supernatant was diluted with storage buffer so that the protein content was approximately 1 µg µl⁻¹. Na,K-ATPase activity was determined by a modification of the protocol described earlier (Flik et al., 1983). In brief, 5 µl supernatant (containing 5–10 µg protein, the optimal protein concentration for this assay, compared with 10–20 µg in the original protocol) was transferred into the wells of a 96-well plate (8 wells per sample). One hundred microliters of either ouabain (1 mM, 4 wells) or KCl (13 mM, 4 wells) containing incubation medium (100 mM NaCl, 5 mM MgCl₂, 0.1 mM Na₂EDTA, 30 mM imidazole, pH 7.4) was added and samples were incubated for 30 min at 23°. Incubation temperature was 23° rather than 37° to match the ambient temperature at which the fish were kept. Incubation for 30 min resulted in maximal inorganic phosphate (Pᵢ) production under the conditions applied (data not shown). The reaction was stopped by adding 200 µl of a 1:1 mixture of 8.6% trichloric acid and “color reagent” (0.66 mM H₂SO₄, 9.2 mM ammonium molybdate, 0.33 mM FeSO₄ · 7 H₂O). After 30 min incubation the Pᵢ produced was determined spectrophotometrically at 620 nm using a EAR 400 ELISA reader (SLT Labinstruments, Austria). A combined calcium phosphate standard (4.84 mM Pᵢ, Sigma) was used as standard and the amount of Pᵢ produced was calculated (Flik et al., 1983). The advantages of this modified assay are that (a) it is performed at the aquarium temperature; (b) it is less time consuming because of shorter pipetting and reading times, resulting in lower intra- and interassay variations; and (c) fewer chemicals and tissues are required.

**Statistics**

Results are presented as means ± SEM (n - 1). The Mann-Whitney U test was used to assess differences between treatments; P < 0.05 was accepted for statistical significance. In none of the experiments were tank-related or gender-related differences or differences between sampling days (approach II) within treatments observed (data not shown), and therefore data were pooled within treatments.

**RESULTS**

There was no mortality during the experiments and no differences in behavior were observed between groups. Food was ingested completely within a few minutes in all groups.

**Experiments Ia and Ib: Effect of PCB Treatment in Combination with Acute Stress**

At the end of the experiments the average body weight of PCB-fed fish was lower than the average body weight of control tilapia. In experiment Ia body weights averaged 25.6 ± 3.1 and 21.8 ± 2.8 g for control and PCB-fed fish, respectively (n = 8 each treatment, P = 0.036). In experiment Ib mean body weight of control fish was 28.7 ± 0.9 g, and that of PCB-fed fish, 25.1 ± 0.9 g (n = 16 each group, P = 0.018). For every tank, plasma cortisol levels were higher in the second fish than in the first (Fig. 1). From the third fish onward, plasma cortisol levels reached a plateau. The mean “plateau cortisol levels” were significantly reduced after 5 days of exposure to PCB 126: experiment Ia—237 ± 28 ng/ml (controls) and 147 ±
FIG. 1. Plasma cortisol levels of fish sampled using approach I: experiments la and lb. Closed squares represent controls and open circles represent PCB-fed fish. In experiment la, fish were sampled in alternating order from two tanks (one per treatment) at 1.5-min intervals, starting with a PCB-fed fish. Fish were sampled in alternating order taking into account the influence of catching order on plasma cortisol levels (Balm et al., 1994). In experiment lb, fish were kept in duplicate tanks (two per treatment) and sampled at 1-min intervals in alternating order starting again with a PCB-fed fish, so that the first and the fifth fish sampled were taken from the same tank. The same holds true for the second and sixth fish, etc.
17 ng/ml (PCB-fed fish, $P = 0.038$); experiment Ib—248 ± 32 ng/ml (controls) and 132 ± 16 ng/ml (PCB-fed fish, $P = 0.004$). Figure 2 depicts the time course of the head kidney superfusion experiments. Maximal stimulation, in pg min$^{-1}$ g$^{-1}$, of cortisol secretion occurred in tissues of both PCB-fed and control fish 15 to 30 min after the pulse and was lower in PCB-fed fish than the maximal cortisol released from head kidneys of control tilapia: experiment Ib—first ACTH pulse, 41.1 ± 11.7 (controls), 9.6 ± 2.8 (PCB-fed fish, $P = 0.02$); second ACTH pulse, 7.8 ± 3.2 (controls), 1.7 ± 0.3 (PCB-treated fish, $P = 0.048$). After stimulation with cAMP $\textit{in vitro}$ cortisol release from tissues of PCB-treated fish was also lower than that from control tissues: experiment Ia—49.9 ± 14.4 (controls), 12.1 ± 2.6 (PCB-fed fish, $P = 0.008$); experiment Ib—14.9 ± 2.9 (controls), 6.3 ± 1.2 (PCB-fed tilapia, $P = 0.021$). After superfusion the concentration of PCB 126 in a pool of four head kidneys from each treatment (experiment Ia) was 136 µg/kg tissue (wet wt) for head kidneys from PCB-exposed tilapia and below the level of detection in tissue from control fish. PCB treatment had no influence on the Na,K-ATPase activity of gill, kidney, or intestine (Table 1), and plasma Na$^+$ and Cl$^-$ concentrations were also not affected (Table 1). However, the number of chloride cells in the opercula of PCB-exposed fish was higher than in opercula from control animals (Table 1).

FIG. 2  Effect of PCB 126 on $\textit{in vitro}$ cortisol release from head kidneys stimulated with ACTH and cAMP (approach I, experiments Ia and Ib). The results of two independent superfusion experiments are shown. Symbols represent mean ± SEM cortisol release from eight chambers in each experiment containing the head kidneys of one (experiment Ia) or two (experiment Ib) fish. In both experiments cortisol release from the tissues was stimulated after 130 min preincubation with 1 nM ACTH for 15 min, after 400 min with 1 mM cAMP for 15 min, and in experiment Ib after 24 h again with 1 nM ACTH for 15 min.

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TABLE 1
Effects of PCB Exposure in Combination with Acute Sampling Stress on Tilapia (Approach I, Experiment Ia; n = 8)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PCB-fed</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Opercular chloride cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(No./mm²)</td>
<td>340 ± 21</td>
<td>430 ± 28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Na,K-ATPase (µmol P/j • mg protein)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gill</td>
<td>9.5 ± 1.1</td>
<td>10.2 ± 1.2</td>
<td>n.s.</td>
</tr>
<tr>
<td>Kidney</td>
<td>7.3 ± 1.1</td>
<td>7.9 ± 0.7</td>
<td>n.s.</td>
</tr>
<tr>
<td>Intestine</td>
<td>24.1 ± 6.1</td>
<td>35.3 ± 6.7</td>
<td>n.s.</td>
</tr>
<tr>
<td>Plasma ions (mmol/liter)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>168 ± 3</td>
<td>164 ± 2</td>
<td>n.s.</td>
</tr>
<tr>
<td>Chloride</td>
<td>133 ± 3</td>
<td>130 ± 4</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Note. Results are presented as means ± SEM. P values resulting from a Mann-Whitney U test are given, n.s., not significantly different.

Experiment II: Effect of PCB Treatment in Fish Sampled at Rest or after 2 h Confinement

At sampling, the mean body weight of control fish was 25.9 ± 1.1 g, and that of PCB-fed fish, 23.2 ± 1.1 g (n = 20 each treatment, P = 0.018). Table 2 summarizes the results obtained when PCB-fed and control fish were sampled either at rest or after 2 h confinement. Resting plasma glucose levels were similar between treatments, but confined control fish were more hyperglycemic than confined PCB-fed fish. Basal plasma cortisol were similar in both groups and, after confinement, rose to similar values in PCB-fed and control fish. Although basal plasma ACTH levels were similar in both groups (Fig. 3), after confinement plasma ACTH levels of PCB-fed fish were higher compared with plasma ACTH levels of controls (P = 0.017; Fig. 3). In controls an approximately 50% increase in plasma ACTH levels was observed (P = 0.009; Fig. 3), whereas PCB-fed fish displayed a rise of about 200% (P = 0.025; Fig. 3). Resting ACTH levels in the RPD were significantly lower in PCB-fed fish than in controls (P = 0.021; Fig. 3). Confinement resulted in comparable increases in the ACTH content of the RPD in both groups (P = 0.003 for controls and P = 0.001 for PCB-fed fish; Fig. 3). The percentage of circulating ACTH (inset to Fig. 3) was highest in PCB-fed fish sampled at rest (P = 0.049), and confinement resulted in a significant decrease in the percentage of circulating ACTH in PCB-fed fish only (P = 0.002; inset to Fig. 3). Na,K-ATPase activity in gill, kidney, and intestine of fish sampled at rest was not influenced by PCB treatment (Table 2). After confinement, significant increases in Na,K-ATPase activity in kidney and intestine were observed in both PCB-fed and control tilapia. Gill Na,K-ATPase activity was not affected by the confinement procedure (Table 2). Neither exposure to PCB nor

TABLE 2
Effect of PCB 126 on Tilapia Sampled at Rest or after Prolonged (2 h Confinement) Stress (Approach II)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PCB-fed</th>
<th>Control</th>
<th>PCB-fed</th>
<th>Control</th>
<th>PCB-fed</th>
<th>Control</th>
<th>PCB-fed</th>
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<tr>
<td></td>
<td>at rest</td>
<td>at rest</td>
<td>confined</td>
<td>confined</td>
<td>at rest</td>
<td>at rest</td>
<td>confined</td>
<td>confined</td>
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<tr>
<td>Plasma cortisol (ng/ml)</td>
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<td></td>
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<td></td>
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<tr>
<td>(n = 8)</td>
<td>13 ± 3</td>
<td>19 ± 9</td>
<td>158 ± 26</td>
<td>168 ± 30</td>
<td>n.s.</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>n.s.</td>
</tr>
<tr>
<td>Plasma glucose (mmol/liter)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>(n = 8)</td>
<td>2.5 ± 0.5</td>
<td>2.2 ± 0.3</td>
<td>10.1 ± 0.9</td>
<td>7.1 ± 0.4</td>
<td>n.s.</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.012</td>
</tr>
<tr>
<td>Opercular chloride cells</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>(No./mm²)</td>
<td>159 ± 7</td>
<td>205 ± 4</td>
<td>153 ± 9</td>
<td>176 ± 9</td>
<td>&lt;0.001</td>
<td>n.s.</td>
<td>0.021</td>
<td>n.s.</td>
</tr>
<tr>
<td>Na,K-ATPase (µmol P/j • mg protein)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Gill</td>
<td>16.8 ± 1.8</td>
<td>16.6 ± 1.7</td>
<td>15.4 ± 1.9</td>
<td>16.1 ± 1.0</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Kidney</td>
<td>13.8 ± 2.4</td>
<td>12.8 ± 2.2</td>
<td>21.5 ± 1.4</td>
<td>20.7 ± 1.4</td>
<td>n.s.</td>
<td>0.001</td>
<td>0.009</td>
<td>n.s.</td>
</tr>
<tr>
<td>Intestine</td>
<td>8.9 ± 1.8</td>
<td>8.5 ± 1.5</td>
<td>13.0 ± 1.2</td>
<td>15.2 ± 0.9</td>
<td>n.s.</td>
<td>0.001</td>
<td>0.002</td>
<td>n.s.</td>
</tr>
<tr>
<td>Plasma ions (mmol/liter)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>162 ± 7</td>
<td>161 ± 7</td>
<td>167 ± 2</td>
<td>174 ± 2</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Chloride</td>
<td>133 ± 5</td>
<td>132 ± 5</td>
<td>138 ± 2</td>
<td>138 ± 2</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Note. Results represent means ± SEM of control and PCB-fed fish sampled at rest or after 2 h confinement. P values obtained by means of a Mann–Whitney U test are also indicated. Cont + conf, confined control fish; PCB + conf, confined PCB-fed fish; n.s., not significantly different.
FIG. 3. Effect of PCB 126 on RPD and plasma ACTH levels in tilapia sampled at rest and after 2 h confinement (approach II). Plasma ACTH levels (left Y axis) as well as the ACTH content of the RPD (right Y axis) are shown. Bars represent means ± SEM (n = 8 for control and PCB, and n = 12 for control and PCB-fed fish sampled after confinement). Inset: plasma ACTH circulating as a percentage of total ACTH circulating, calculated as [(circulating ACTH)/(circulating ACTH + RPD ACTH)] × 100%. Statistical differences (P < 0.05) are indicated as follows: * PCB versus controls (either at rest or after confinement); ^ confined versus resting fish (within treatments).

Confinement had any influence on plasma Na⁺ or Cl⁻ concentration (Table 2). PCB treatment increased the number of chloride cells. After confinement the chloride cell numbers were lower in PCB-fed animals but were unaffected in controls (Table 2).

DISCUSSION

Influence on the Hypothalamus–Pituitary–Interrenal Axis

The lower cortisol response to acute sampling in our PCB-fed fish suggests an altered responsiveness of the cortisol-producing interrenal cells to this stressor. A reduced cortisol response to sampling was also observed in fish collected in heavily polluted waters (Hontela et al., 1992, 1995). These waters contained PCBs as well as many other pollutants, and therefore the observations made in these field studies could not be attributed to a specific contaminant. The similarities between the effects observed in these field studies and the present data render it likely that the PCBs were involved in the impaired cortisol response observed by Hontela and co-workers.

To investigate whether PCB treatment influenced the ACTH and cAMP responsiveness of head kidneys, these tissues were stimulated in vitro with ACTH and cAMP. cAMP was used to bypass the ACTH receptor-associated events (Young, 1985; Arnold-Reed and Balment, 1994), as it acts as a second messenger mediating ACTH-stimulated cortisol release (Schimmer, 1995). Since dietary PCB exposure leads to decreased in vitro cortisol release after stimulation with both ACTH and...
cAMP, the data suggest that PCB 126 does not solely exert its effects at the level of the ACTH receptor but also or rather on the intracellular signal transduction cascade, which involves cAMP and protein kinase A activating cytochrome P450 enzymes involved in steroidogenesis (Schimmer, 1995). Consistent with this mechanism of action, PCBs are known to interfere with the P450 enzyme systems in fish (Goksøyr and Förlin, 1992). The PCB data presented here differ from those of an earlier study where ACTH-, but not cAMP-, stimulated in vitro cortisol release was impaired after treatment of the tilapia Sarotherodon aureus with the organochlorine insecticide o,p-DDD (Ilan and Yaron, 1980). Therefore, suppression of ACTH-stimulated cortisol release from the interrenal tissues might be a general response to PCBs and structurally related compounds, but evidently the mechanisms of action differ. This PCB effect might be direct or indirect. Recently Hontela et al. (1997) suggested that the impaired cortisol response in fish exposed to bleached kraft mill effluents is correlated with histopathological changes of the pituitary corticotropes and the steroidogenic interrenal cells, which may indicate direct interference of these substances with the HPI axis. We also obtained evidence for direct actions of PCB 126 on the HPI axis: (a) the PCB effect observed persisted after a 24-h cleansing period in vitro; (b) PCB 126 concentrations in the head kidneys of exposed fish accumulated to similar (high) levels as in the livers of these fish, in which PCB 126 concentrations of 100 μg/kg tissue in PCB-fed animals versus 0.9 μg/kg liver tissue of control fish were detected (Quabius et al., 1998). No further information on PCB concentrations in head kidneys is available. Also, reports of PCB concentrations in the adrenal cortex of mammals are scarce, which is surprising because among the endocrine glands, the adrenal appears to be the most sensitive to toxic substances such as PCBs and o,p-DDD (Colby and Longhurst, 1992). It has been reported that the degree of accumulation of PCBs in endocrine tissues is related to the severity of the endocrine disruptive effects (Soontornchat et al., 1994).

It could be argued that in tissues from both control and PCB-treated fish the decreased in vitro responsiveness to a second ACTH pulse in comparison to the initial ACTH pulse reflects exhaustion of the tissue. However, interrenal tissue of coho salmon remains highly responsive to a first ACTH challenge after 18 h of preincubation (Young, 1988), and therefore alternatively the reduced sensitivity of the interrenal cells to a second ACTH pulse may indicate that ACTH receptors are in a refractory state as demonstrated previously (Balm, 1986).

Since confinement resulted in similar increases in plasma cortisol levels in PCB-fed and control tilapia, it is unlikely that the impaired cortisol response observed in response to the acute stress is due to exhaustion of the interrenal tissue. Exhaustion has been suggested as an explanation for the reduced cortisol response in yellow perch after lifelong exposure to a mixture of toxicants (Hontela et al., 1995). The amplified plasma ACTH response to 2 h confinement observed in the PCB-treated fish might be compensatory for the lower interrenal ACTH sensitivity and thereby explain why the confinement-induced rise in cortisol was similar in both PCB-fed and control fish.

In fish sampled at rest the ACTH content of the RPD was lower in PCB-fed tilapia than in at-rest-sampled controls. There are two explanations for this phenomenon: depletion or exhaustion of the ACTH-producing cells, and a higher rate of turnover of ACTH. The increased ACTH levels in plasma and RPD of PCB-treated fish after 2 h confinement argues against the first explanation. Also, the finding that the plasma ACTH as percentage of total ACTH was highest in at-rest-sampled PCB-fed fish points to a higher rate of turnover of ACTH rather than to depletion of the ACTH-producing cells of the RPD.

**PCB Effects on Targets of the Hypothalamus–Pituitary–Interrenal Axis**

The reduction in body weight observed in PCB-exposed fish was also observed in carp exposed for 42 days to a PCB mixture (Svoboda et al., 1994) or in tilapia after 5 days of cadmium exposure (Lock et al., 1994). This response is a general one to a wide range of environmental stressors (Pickering, 1993) and the mechanisms of action involved may vary. Generally, the catabolic actions of cortisol are considered responsible (Vijayan et al., 1991, 1996), but in our case no evidence for changes in resting cortisol levels were obtained.

Hyperglycemia has been ascribed to catecholamine-mediated increases in glucose release from the liver in
stressed rainbow trout (Vijayan and Moon, 1992) and confined tilapia (Vijayan et al., 1997b). Thus the observed impaired hyperglycemic response to 2 h confinement may indicate that the PCB treatment interferes with confinement-associated catecholamine release. Alternatively, it may reflect a depletion of energy stores in PCB-fed tilapia which took place during the 5-day exposure period. This is supported by electron microscopic analysis of livers from resting PCB-fed and control tilapia showing a decrease in liver glycogen stores (Quabius et al., 1998).

Besides ionoregulation, Na,K-ATPase of kidney and intestine also provides the driving force for several Na-dependent metabolite transporters, such as the Na-dependent glucose transporter, which is expressed in intestine and kidney of mammals, as well as in teleost kidney (Freire et al., 1995) and intestine (Ahearn et al., 1992; Houpe et al., 1996). Possibly the increased intestinal Na,K-ATPase activity after confinement reflects an increased need for metabolites and contributes to hyperglycemic conditions. This interpretation would be consistent with unaltered Na,K-ATPase activity in gills and could relate to the observation by Ellory et al. (1972) that cortisol does not modify mucosal permeability to sodium and their suggestion that cortisol probably mobilizes energy within the mucosa.

In summary it can be said that exposure to PCB 126 disturbs the responsiveness of the HPI axis in tilapia, in the absence of signs of ionoregulatory dysfunction both at rest and under stress. It is therefore concluded that the effects of PCB 126 on the HPI axis are not secondary to ionoregulatory dysfunctions and may be exerted directly at the level of the interrenal or corticotropic cells. Impairment of the HPI axis may have serious consequences for the response of fish to stressful stimuli in PCB-contaminated environments.

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