Effects of acute and prolonged naphthalene exposure on brain monoaminergic neurotransmitters in rainbow trout (*Oncorhynchus mykiss*)

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Abstract

We have shown previously that acute (1 to 6 h) and prolonged (1 to 5 days) exposure of rainbow trout to naphthalene resulted in decreased plasmatic cortisol and 17-β-estradiol levels. In order to elucidate the mechanisms through which naphthalene might disrupt endocrine regulation, the present study investigated whether brain monoaminergic neurotransmitters are altered by the action of this polycyclic aromatic hydrocarbon. In a first experiment, immature rainbow trout were injected with vegetable oil alone or containing naphthalene (10 and 50 mg/kg, i.p.), and sacrificed 1, 3 and 6 h after treatment. In a second experiment, slow-coconut oil implants alone or containing naphthalene (doses of 10 and 50 mg/kg) were i.p. located and fish sacrificed 1, 3 and 5 days after treatment. Levels of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and noradrenaline (NA) were measured in several brain regions by HPLC. The results show that short-term naphthalene increases DA and 5-HT contents in hypothalamus and telencephalon, but differentially alter contents of the acid metabolites. Implants with naphthalene reduced DA content in hypothalamus and preoptic region but increased in telencephalon. 5-HT metabolism was decreased in hypothalamus, preoptic region, pituitary and brain stem after 3 to 6 days of treatment. In addition, the levels of NA were increased in hypothalamus and telencephalon after acute treatment and in hypothalamus and preoptic area after several days of exposure to naphthalene. These data suggest that brain neurotransmitter systems are sensitive to polycyclic aromatic hydrocarbons and could represent a target of the naphthalene-induced neuroendocrine disruption.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are the main toxic and persistent compounds present in most crude oils. As a result of the release of petroleum oils to the sea PAHs are now ubiquitous contaminants of aquatic ecosystems (Leonard and Hellou, 2001). Some of these chemicals have been demonstrated to have mutagenic/carcinogenic (Hendricks et al., 1985; Malins et al., 1987), genotoxic (Pacheco and Santos, 1997, 2001) and cytotoxic (Schirmer et al., 1997) properties. In different vertebrate species, environmental PAHs might act as potent endocrine disruptors (Cooper and Kavlock, 1997; Stahlschmidt-Allner et al., 1997), in particular by binding to and activating nuclear estrogen receptors, resulting in alterations in the transcription rates of estrogen-regulated genes (Hutz et al., 1999; Barron et al., 2004a). In fish, PAHs have been shown to impair cellular steroidogenesis that occurs in gonad and interrenal gland, and also to influence systemic steroid regulation via the hypothalamus–pituitary–gonadal (HPG) and hypothalamus–pituitary–interrenal (HPI) axis (Hontela et al., 1992, 1997; Vijayan et al., 1997; Wilson et al., 1998; Monteiro et al., 2000a,b; Evanson and Van Der Kraak, 2001; Navas et al., 2004). In consequence, PAHs could interfere with reproductive activity and with the ability of fish to respond to stress (Al-Kindi et al., 2000, for a review). In addition, PAHs can affect several physiological functions in fish, such as energy metabolism, growth and immunity (Hontela et al., 1992; Pacheco and Santos, 2001, 2002; Teles et al., 2005; Tintos et al., in press).

Many environmental pollutants have been shown to act as neurotoxicants (Tilson and Kodavanti, 1998; Bemis and Seegal, 1999) and potentially could impair the function of
neurotransmitter systems and then influence pituitary hormonal secretion. Despite of all adverse effects PAHs have on aquatic living animals, there are very few studies about PAHs effects on nervous system. Indeed, PAHs have been shown to accumulate highly in fish brain (Varanasi et al., 1979; Collier et al., 1980). In a pioneer study, Fingerman and Short (1983) showed that naphthalene (a two-ring PAH) alone or in combination with other pollutants like benzo-α-pyrene (a five-ringed-PAH) and Aroclor 1254 (a polychlorinated biphenyl, PCB, mixture) affected brain neurotransmitters levels in Channel catfish (Ictalurus punctatus). In addition, several other environmental pollutants have been shown to alter the levels of biogenic amines in discrete brain regions of channel catfish (Fingerman and Russell, 1980), trout (Rozados et al., 1991; Aldegunde et al., 1999) and the Atlantic croaker (Khan and Thomas, 1997, 2001; Khan et al., 2001).

Naphthalene and naphthalene derivatives like alkyl-naphthalenes are the most abundant PAHs present in most crude oils (Burns et al., 1997; Aas et al., 2000) and are frequently found in polluted environments (Lee and Anderson, 2005). Exposure of fish to naphthalene has been shown to induce a variety of responses at both cellular and systemic level in teleost fish (Thomas and Budiantara, 1995; Navas and Segner, 2000; Evanson and Van der Kraak, 2001). In trout, we have recently shown that naphthalene exposure induced a marked dose- and time-dependent decrease in plasma levels of cortisol and 17β-estradiol, and also affected the energy metabolism in several organs, including brain (Tintos et al., in press). As an underlying mechanism(s) mediating endocrine effects of naphthalene, it is plausible that it might interfere with brain neurotransmitter systems involved in regulating the activity of the hypothalamo–pituitary–hormonal axis. In this complementary report, we determine the effects of naphthalene on the content of dopamine (DA), noradrenaline (NA) and serotonin (5-HT), as well as their relative metabolites in discrete brain regions of rainbow trout (Oncorhynchus mykiss).

2. Materials and methods

2.1. Animals and experiments

Immature rainbow trout (O. mykiss, about 60 g) were obtained from a local hatchery and transferred to the laboratories at Faculty of Marine Sciences in Vigo, Spain. Fish were acclimated for at least two weeks in well-aerated 150 l tanks, with an open water system. During the experiments, fish were maintained under natural photoperiod (October 2004) and temperature (18 °C). Fish were fed daily with 1% body mass commercial dry pellets (Dibaq-Diprot S.A., Segovia, Spain). They were fasted 24 h before sampling. The experiments described comply with the guidelines of the European Union Council (86/609/EU) for the use of laboratory animals.

In a first experiment, trout were caught by netting, anesthetized with MS-222 (50 mg/l) buffered with sodium bicarbonate, weighed, intraperitoneally injected (2 μl/g body mass, bm) with sunflower oil alone (control) or containing naphthalene (10 mg/kg and 50 mg/kg bm) and returned to their tanks. 24 fishes per group were used. Eight fish were sampled from each group 1, 3 and 6 h after injection.

In a second experiment, anesthetized trout were weighed and intraperitoneally implanted (10 μl/g bm) with slow-release coconut oil implants alone (control) or containing naphthalene at doses of 10 mg/kg bm and 50 mg/kg bm, and returned to their tanks. Eight fish from each group were sampled 1, 3 and 5 days after injection.

On each experiment, non-injected fish were included to assess the possible vehicle effect. At each sampling time, fish were quickly caught by netting, anesthetized with MS-222 (50 mg/l) and sacrificed by decapitation. Brains were immediately dissected out and the following regions were sampled: telencephalon (excluding olfactory bulbs), hypothalamus, pituitary gland, preoptic area (including the optic tract) and brain stem (mainly including the medulla oblongata). All tissues were stored without delay at −80 °C until assay.

2.2. Analysis of monoamines and metabolites

The contents of NA, DA, 3,4-dihydroxyphenylacetic acid (DOPAC, a major DA metabolite), 5-HT and 5-hydroxyindole-3-acetic-acid (5-HIAA, a major 5-HT metabolite) were analyzed by high performance liquid chromatography with electrochemical detection (HPLC-EC) according to the method described by Miguez et al. (1999) with modifications. The HPLC system was equipped with a Jasco PU-2080 Plus pump, a 5 μm analytical column (Phenomenex, Nucleosil C18, 150 mm length × 4.6 mm diameter) and a ESA Coulochem II detector. The detection system included a double analytical cell (M5011) with oxidation potentials set at +40 mV (first electrode) and +340 mV (second electrode). The mobile phase was composed of 63.9 mM NaH2PO4 (Merck), 0.1 mM Na2EDTA (Sigma), 1.63 mM sodium 1-octanesulfonate (Fluka) and 14.9% (v/v) methanol. Its pH was adjusted to 2.79 with ortho-phosphoric acid (Merck), and was filtered (0.20 μm filter, Millipore, Bedford, USA) and degassed at vacuum before use. Tissues were homogenized by ultrasonic disruption in 0.1 ml (pituitary), 0.5 ml (hypothalamus, telencephalon, preoptic region) and 1 ml (brain stem) of mobile phase. After extracting an aliquot for protein measurement (Bradford, 1976), homogenates were centrifuged (16,000×g, 10 min) and supernatants were diluted with mobile phase prior to HPLC analysis. Dilutions were 1:1 (supernatant/mobile phase) for telencephalon, medulla oblongata and preoptic region, and 1:2 for hypothalamus. Pituitary supernatant was not diluted and was injected directly into the HPLC system. The levels of NA were not measured in the pituitary gland since they were below the detection limit of our technique and in mesencephalon due to interferences in the analytical assay.

Analytical run time for each sample was 15 min at an isocratic flow rate of 1.1 ml/min at room temperature. Acquisition and integration of chromatograms were performed using the Biocom RP computer-assisted software (Micron Analítica, Madrid, Spain). Quantification of sample peaks was estimated comparing peak areas with those of appropriate external standards. Detection limits for indoles examined were between 0.5 and 3 pg per injection, with a signal-to-noise ratio of 2.
2.3. Data analyses

Data values are presented as mean plus standard error of the mean (mean±S.E.M.) of each experimental group. The ratios DOPAC/DA and 5-HIAA/5-HT contents were obtained by using the respective values of the amine and metabolite content for each sample and data were grouped according to the treatment. The SPSSv14.0 statistical package was used to the statistical analysis. The Levene test was used to ascertain the homogeneity of variances between groups of data. The effects

Fig. 1. Levels of dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) and values of DOPAC/DA ratio in hypothalamus, pituitary, telencephalon, preoptic region and brain stem in vehicle- (sunflower oil; white bars) and naphthalene-treated (10 mg/kg body weight: light grey bars; 50 mg/kg body weight: dark grey bars) juvenile rainbow trout. The X-axis indicates the time (1, 3 and 6 h) after the intraperitoneal injection. Each value represents the mean±S.E.M. of six to eight fish.

⁎: Significantly different (P<0.05) from control group (Student–Newman–Keuls test).
of different doses of naphthalene and time of exposure were analyzed using a two-way ANOVA followed by Student–Newman–Keuls test. In cases of nonhomogenous variances for groups, the Kruskal–Wallis nonparametric one-way ANOVA was used followed by the Mann–Whitney U-test. Differences between means were considered significant when $P<0.05$.

3. Results

No mortality or health disturbances in fish were observed after either the short-term (one to 6 h) or the long-term (1 to 5 days) naphthalene treatment. In the short-term experiments, the intraperitoneal administration of naphthalene induced a

![Graphs showing levels of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) and values of the 5-HIAA/5-HT ratio in hypothalamus, pituitary, telencephalon, preoptic region and brain stem in vehicle- (sunflower oil; white bars) and naphthalene-treated (10 mg/kg body weight: light grey bars; 50 mg/kg body weight: dark grey bars) juvenile rainbow trout. The X-axis indicates the time (1, 3 and 6 h) after intraperitoneal injection. Each value represents the mean±S.E.M. of six to eight fish. *: Significantly different ($P<0.05$) from control group (Student–Newman–Keuls test).]
significant increase in the content of DA in the hypothalamus (10 mg/kg, 6 h after treatment) and telencephalon (50 mg/kg, 3 h after treatment). In the last region, the highest naphthalene dose also increased DOPAC content, as well as decreased DOPAC/DA ratio (Fig. 1). The levels of the DA metabolite were also increased in preoptic region 1 h after the injection of naphthalene 10 mg/kg. At the hypothalamic level, naphthalene increased the content of 5-HT (10 mg/kg, 6 h after treatment) and 5-HIAA (10 and 50 mg/kg, 6 h after treatment), as well as the 5-HIAA/5-HT ratio (both doses, 6 h). In addition, increases in 5-HT and 5-HIAA content occurred in the telencephalon with the higher dose of naphthalene (50 mg/kg, 6 h after treatment),

![Graphs showing changes in DA and DOPAC levels in different brain regions.](image)

Fig. 3. Levels of dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) and values of DOPAC/DA ratio in hypothalamus, pituitary, telencephalon, preoptic region and brain stem in vehicle- (coconut oil; white bars) and naphthalene-treated (10 mg/kg body weight: light grey bars; 50 mg/kg body weight: dark grey bars) juvenile rainbow trout. The X-axis indicates the time (1, 3 and 5 days) after intraperitoneal implantation. Each value represents the mean ± S.E.M. of six to eight fish.

⁎: Significantly different (P<0.05) from control group (Student–Newman–Keuls test).
whereas 5-HIAA content increased with the two doses tested (10 mg/kg after 3 and 6 h of treatment; 50 mg/kg after 6 h of treatment). The 5-HIAA/5-HT ratio decreased in the telencephalon 6 h after treatment, but it was increased in brain stem (50 mg/kg, 1 h) and pituitary gland (10 and 50 mg/kg, 6 h) (Fig. 2).

To investigate longer-term effects of naphthalene, fish were implanted with slow-release coconut oil implants containing the hydrocarbon or adequate vehicle. This procedure has been used to administrate PAHs in fish species, like rainbow trout (Vijayan et al., 1997) and eel (Pacheco and Santos, 1998). After the naphthalene implants, a decrease in DA content was found in
the hypothalamus (50 mg/kg, 5 days) and preoptic region (50 mg/kg, 3 days). This last area also exhibited a significant decrease in DOPAC content in fish exposed for 5 days to both doses of naphthalene (Fig. 3). A different effect of the hydrocarbon was observed in the telencephalon since it induced increases in DA content (10 mg/kg at 5 days; 50 mg/kg at 1 and 5 days of exposure) and decreases in DOPAC levels (10 and 50 mg/kg, 1 day of exposure). The DOPAC/DA ratio was decreased by treatment in telencephalon (1 and 3 days of exposure), but increased in the hypothalamus (after 5 days) and the pituitary (after 1 day of exposure).

Changes observed in indoleamines after chronic naphthalene implants attained all regions, except the telencephalon (Fig. 4). In the hypothalamus, the dose of 50 mg/kg induced a significant decrease in the content of 5-HIAA (3 days of exposure) and 5-HT (5 days of exposure), which resulted in increases (3 days) and decreases (5 days) in the 5-HIAA/5-HT ratio. Also in the preoptic region naphthalene treatment induced a decrease in the content 5-HT (5 days of exposure), which resulted in increases (3 days) and decreases (5 days) in DA content (10 mg/kg at 5 days; 50 mg/kg at 1 and 3 days of exposure), whereas a drop in 5-HIAA level was observed in the pituitary (both naphthalene doses, 3 days of exposure) and brain stem (50 mg/kg, 5 days of exposure). In addition, the 5-HIAA/5-HT ratio was reduced in brain stem with the higher naphthalene dose (3 and 5 days of treatment).

Changes in NA levels after acute or chronic naphthalene treatment are shown in Table 1. We observe a short-term stimulatory effect of naphthalene on the content of NA in the hypothalamus (50 mg/kg, 3 h) and telencephalon (10 and 50 mg/kg, 6 h). The long-term exposure to naphthalene also caused significant increases in the levels of this neurotransmitter in the preoptic region (1 day of exposure) and telencephalon (1 and 3 days of exposure), without significant changes in the hypothalamus.

4. Discussion

The present study describes for the first time detailed changes in catecholaminergic and indoleaminergic neurotransmitters in discrete brain regions of immature rainbow trout exposed from 1 h to several days to naphthalene. In general, changes induced by naphthalene were very dependent upon the type of neurotransmitter and exhibited a marked brain regional selectivity, being the major effects located in the hypothalamus, telencephalon and preoptic area. The effects observed were inconsistent over time of exposure and the dose of naphthalene tested.

Various studies have shown that when fish were exposed to these hydrocarbons brains accumulate relatively high levels of naphthalene and its methyl-substituted derivatives (Varanasi et al., 1979; Collier et al., 1980). However, the effects of environmental pollutants on brain function were poorly studied in fish. Decreased levels of 5-HT and DA and increased levels of their main metabolites were found in Atlantic croaker (Micropogias undulates L.) after exposure to PCBs-mixture Aroclor 1254 indicating that PCBs may affect brain neurotransmitter activity (Fingerman and Russell, 1980; Khan and Thomas, 1997, 2000). The pesticide lindane has also been shown to change brain serotonergic metabolism in rainbow trout O. mykiss (Rozados et al., 1991). With respect to naphthalene, there is only a preliminary report of Fingerman and Short (1983) describing a variety of changes in whole brain monoamine content of channel catfish which varied largely over time of exposure. Our present results confirm the lack of a consistent temporal trend in the effects of naphthalene, as well as a clear dose–response following the two doses of naphthalene examined (10 and 50 mg/kg). These data are in contrast with other studies addressing dose- and time-related effects of naphthalene and related PAHs derivatives on several tissues and cellular processes, i.e., oxidative stress in brain and liver, hepatic intermediary metabolism and steroid secretion from endocrine glands (Hutz et al., 1999; Evanson and Van der Kraak, 2001; Bagchi et al., 2002; Tintos et al., in press).

The time course of the effect of chemical pollutants on brain neurochemistry is probably due to the rate of uptake of the pollutant and their effects on the different mechanisms regulating the content of individual neurotransmitters (i.e., synthesis,
storage, uptake/release and degradation) in discrete regions of the nervous tissue. DAergic neuronal bodies in teleost fish brain are located mainly in hypothalamus, telencephalon and pretectal areas, and in medulla oblongata. Fibres of these neurons are widely distributed in teleostean brain with the highest density of DAergic terminals in ventral telencephalon, and preoptic and hypothalamic regions (Hornby and Piekut, 1990; Meek, 1995). 5-HTergic cells bodies are mainly confined to discrete diencephalic areas and the mesencephalon, whereas 5-HT-containing fibres are particularly dense in the hypothalamus (Frankenhuis-van den Heuvel and Nieuwenhuys, 1984; Rodríguez-Gomez et al., 2000). In our study, the administration of naphthalene produced a short-term increase in the contents of DA and 5-HT in the hypothalamus, but inhibited the level of these compounds after a 5-day exposure. The increases in hypothalamic DA levels took place without any alteration in its main acid metabolite, the DOPAC, suggesting that acute naphthalene treatment increased synthesis rather than catabolism of this neurotransmitter. However, the increases in 5-HT content in the hypothalamus were accompanied by parallel alterations in the levels of 5-HIAA and also in the 5-HIAA/5-HT ratio, which is considered to be a more direct index of the neurotransmitter activity than absolute values of the monoamines (Winberg et al., 1992, 1997). The 5-HIAA/5-HT ratio was increased after 6 h and decreased after 5 days of naphthalene exposure, indicating that short-term naphthalene treatment increased both synthesis and turnover of the neurotransmitter, whereas both processes were inhibited by a several-day exposure. This differential effect of naphthalene over time of exposure agrees with previous studies showing a reversal of the effects of naphthalene and other chemicals pollutants on monoamine levels in whole brain of fish (Taylor and DiStefano, 1976; Fingerman and Russell, 1980; Fingerman and Short, 1983).

Savolainen (1977) has postulated that acute neurotoxic effects of low molecular weight hydrocarbons in mammals are due to accumulation of the parent hydrocarbons in the brain and that chronic effects may be due to the formation of reactive intermediates. Studies of Collier et al. (1980) found that several hours after exposure to naphthalene, trout brain accumulates naphthalene at concentrations similar to those found at the same time in liver, but brain capacity to take up, metabolize and flush them out is very different from liver. In fact, trout brain has been shown to display very little potential for converting aromatic hydrocarbons to oxidized metabolites and a limited capacity to acquire metabolites from blood (Collier et al., 1980). Therefore, it seems that differences between short-term (hours) and long-term (days) effects of naphthalene on hypothalamic monoamines are not due to formation of intermediate naphthalene metabolites, but rather to changes in sensitivity of the neuronal mechanisms regulating the levels of aminergic compounds.

The effects of naphthalene on telencephalic DA metabolism were similar after hours and days of treatment, with increases in DA and decreases in DOPAC contents. In addition, the DOPAC/DA ratio was decreased by treatment, suggesting a low utilization of the neurotransmitter which caused an elevation of the amine content. In the same way, treatment with naphthalene decreased DA content in the preoptic region after 3 days and DOPAC content after a 5-day exposure. Taking together these results and those obtained the hypothalamus, it seems that naphthalene exposure induced a predominant low DAergic activity in diencephalic (hypothalamus and preoptic area) and telencephalic regions of trout brain, which have high density of dopaminergic somas and terminals.

Similarly to that found for DA, the content of 5-HT in the hypothalamus was increased by short-term treatment and decreased after long-term exposure to naphthalene. However, differentially to DA, changes in 5-HT content were accompanied by similar effects on the levels of its main acid metabolite, the 5-HIAA. This suggests that several hours of exposure to naphthalene increases 5-HT synthesis and turnover while the contrary takes place after several days of exposure. Increased 5-HT and 5-HIAA contents were also found in telencephalon after short-term, but not long-term, naphthalene exposure. In addition, in the preoptic region implants of naphthalene inhibited 5-HT and 5-HIAA contents respectively at 3 and 5 days of exposure to the hydrocarbon. These results point to a predominant rise of 5-HT turnover in diencephalic and telencephalic regions after acute naphthalene treatment and a decrease after an exposure to the hydrocarbon for several days.

There are immunohistochemical studies in teleosts that confirmed the presence of fibres containing monoamines and monoamine-synthesizing enzymes in the pituitary (Rodríguez-Gomez et al., 2000). These fibres originate likely in the ventral hypothalamus and belong to the neuronal complex involved in modulating pituitary hormone secretion (Yu et al., 1997). In the present study, we detect very low levels of DA, 5-HT and related acid metabolites in trout pituitary, which agrees with previous neurochemical studies (Saligaut et al., 1990; Hernández-Rauda et al., 2000; Hernández-Rauda and Aldegunde, 2002). The effect of naphthalene on pituitary monoamines evidences inconsistent changes over time with increases in the 5-HIAA/5-HT ratio at 6 h and 1 day, and decreases in 5-HIAA contents 3 days after naphthalene treatment. These changes follow a similar trend as those described for hypothalamic monoamines at these specific times, suggesting that changes observed in the hypothalamus are immediately translated into changes in pituitary monoamine levels.

The mesencephalic brainstem that contains the raphe nuclei is particularly rich in neuronal serotonergic soma (Frankenhuis-van den Heuvel and Nieuwenhuys, 1984; Meek et al., 1989; Aldegunde et al., 2000). In this region, effects of naphthalene modified 5-HT turnover, and changes followed a biphasic trend since the 5-HIAA/5-HT increased at 1 h but decreased at 3 and 6 days of naphthalene treatment. This decrease is explained mainly by decreased 5-HIAA content more than alteration in the levels of the amine, which suggests a lower transformation of 5-HT to 5-HIAA without apparent changes in 5-HT synthesis. Thus, it seems that neural mechanisms mediating naphthalene effects on 5-HT are simpler in brain areas containing predominantly neuronal 5-HT bodies (brain stem) than those having both neuronal somas and terminals (i.e., hypothalamus and telencephalon), in which alterations in both synthesis and turnover of the neurotransmitter coexist.
A great deal of studies have demonstrated that the dopaminergic and serotonergic systems of the telencephalon, preoptic region and hypothalamus are involved in the control of behavior in fish, including feeding, swimming activity, stress response and social interactions, among others (Winberg et al., 1992, 1997; Overli et al., 1998; Höglund et al., 2005). The DAergic and 5-HTergic neurons of the hypothalamic–pituitary complex are also implicated as a major component of the vaste neural regulation affecting pituitary hormone secretion. A previous study of our group has shown that both acute and prolonged treatment with naphthalene to immature trout inhibited drastically the levels of cortisol and 17β-estradiol in blood (Tintos et al., in press). The i.p. administration of naphthalene (10 mg/kg and 50 mg/kg) induced an acute dose-dependent decrease in plasma cortisol levels, which was even more evident after several days of exposure to naphthalene implants. In addition, short-term naphthalene induced a consistent decrease in plasma 17β-estradiol levels, while long-term treatment was followed by a clear dose-dependent reduction in the levels of this steroid. The data of the present study showing parallel naphthalene-induced effects in the hypothalamic aminergic neurotransmitters point to that some of these endocrine effects of naphthalene could be mediated through the hypothalamic–pituitary DAergic and 5-HTergic systems, which are major factors influencing the HPI and HPG axis in fish (Winberg et al., 1997; Yu et al., 1997; Lepage et al., 2002; Höglund et al., 2002). However, there was not a clear correlation among the dose- and time-dependent effects of naphthalene on plasma hormonal levels and those observed in brain neurotransmitters. This is consistent with the fact that a major part of PAHs effects on circulating cortisol and 17β-estradiol might be mediated by directly acting the hydrocarbon on the interrenal glands and gonads, affecting either the sensitivity to regulation by pituitary hormones or the steroidogenesis process (Stahlschmidt-Allner et al., 1997; Vijayan et al., 1997; Wilson et al., 1998; Hutz et al., 1999; Khan et al., 2001; Khan and Thomas, 2001; Monteiro et al., 2000a,b; Evanson and Van der Knaak, 2001; Navas et al., 2004). In addition, it is well known that in teleost fish there is an important steroid feedback on hypothalamo-hypophysical activity (Peter and Yu, 1983). Endogenous steroids participate in the up- and down-regulation of pituitary hormone secretion at least in part by exerting positive and negative effect on brain inputs to the pituitary, including the neurotransmitters DA and 5-HT (Mommsen et al., 1999; Hernández-Rauda and Aldegunde, 2002; DiBattista et al., 2005). Considering that naphthalene-exposed trout displays altered levels of gonadal and interrenal steroids it is not unlikely that the variable effect of naphthalene over time on brain neurotransmitters is due, at least in part, to disturbances in the steroid feedback action on hypothalamic monoaminergic systems. Whether this hormonal action on brain neurotransmitters might interfere with a local effect of the hydrocarbon in specific brain centres remains to be elucidated.

Significant increases in the content of NA in the hypothalamus and telencephalon occur after acute naphthalene treatment to trout, while a similar effect was observed in telencephalon and preoptic region following implants. All these regions receive noradrenergic inputs from outer brain areas, mainly from the locus coeruleus complex of the mesencephalon (Meek, 1995). The effect of naphthalene on NA content follows a well different trend from those on DA in spite of the closed metabolic relationship between both monoamines. This suggests that different mechanisms are mediating the action of naphthalene on the metabolism of each catecholamine at the neuronal endings.

In summary, the present study shows that catecholaminergic and indoleaminergic systems in trout brain are very sensitive to acute and prolonged exposure to naphthalene and might be a cue for understanding the disruption of endocrine status following exposure to polycyclic aromatic hydrocarbons present in polluted environments. Recent research indicates that high doses of PAHs are likely to induce acute toxicity through a narcosis mechanism of action caused by hydrophobic chemical partitioning into cell membranes and nervous tissues that result in disruption of central nervous system function (Barron et al., 2001, 2004b). Such a nonspecific action on neuronal membranes may explain the widely spread changes in neurotransmitters affecting different brain regions after exposure to naphthalene. In addition, the mechanism of neural toxicity of PAHs may involve events which entail an oxidative stress and production of reactive oxygen species (Bagchi et al., 2002), and several studies have already demonstrated PAH-induced effects on intracellular calcium in mammalian cells (Mounho et al., 1997; Tannheimer et al., 1999). Changes in calcium homeostasis have been also suggested to be involved in PCBs-induced alterations in brain neurotransmission (Tilson and Kodavanti, 1998). Future studies should determine whether a similar mechanism could mediate the neuronal action of naphthalene and simultaneously estimate their implication as endocrine disruptor.

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