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Initial study on the possible mechanisms involved in the effects of high doses of perfluorooctane sulfonate (PFOS) on prolactin secretion

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ABSTRACT

Perfluorooctane sulfonate (PFOS) is a fluorinated organic compound. This chemical is neurotoxic and can alter the pituitary secretion. This is an initial study aimed at knowing the toxic effects of high doses of PFOS on prolactin secretion and the possible mechanisms involved in these alterations. For that, adult male rats were orally treated with 3.0 and 6.0 mg of PFOS/kg body weight (b.w.)/day for 28 days. At the end of the treatment, the serum levels of prolactin and estradiol as well as the concentration of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and gamma-aminobutyric acid (GABA) were quantified in the anterior and in the mediobasal hypothalamus. PFOS, at the administered doses, reduced prolactin and estradiol secretion, increased the concentration of dopamine and GABA in the anterior hypothalamus, and decreased the ratios DOPAC/dopamine and HVA/dopamine in this same hypothalamic area. The outcomes reported in this study suggest that (1) high doses of PFOS inhibit prolactin secretion in adult male rats; (2) only the periventricular-hypophysial dopaminergic (PHDA) neurons seem to be involved in this inhibitory effect but not the tuberoinfundibular dopaminergic (TIDA) and the tuberohypophysial dopaminergic (THDA) systems; (3) GABAergic cells from the paraventricular and supraoptic nuclei could be partially responsible for the PFOS action on prolactin secretion; and finally (4) estradiol might take part in the inhibition exerted by elevated concentration of PFOS on prolactin release.

1. Introduction

Perfluorooctane sulfonate (PFOS) is a fluorinated organic compound that has been extensively used in industry and consumer products (United States Environmental Protection Agency (US-EPA), 2000), being declared a Persistent Organic Pollutant (Stockholm Convention, 2009). This xenobiotic can reach high concentrations in serum and tissues of wildlife and humans because of its persistence and bioaccumulation (Fromme et al., 2009; Jeon et al., 2010; Wielsøe et al., 2014; Zeng et al., 2015). Therefore it is important to find techniques in order to get its degradation (Pramanik et al., 2015; Wang et al., 2015).

Food intake is the most significant route in the PFOS exposure in humans, being the estimated dietary exposure to PFOS 60 ng/kg body weight (b.w.)/day for average consumers, and 200 ng/kg (b.w)/day for high consumers of fish (EFSA, 2008). Drinking water is estimated to contribute less than 0.5% of the indicative exposure and the total contribution from non-food articles is estimated to be less than 2% compared to the average total PFOS exposure (EFSA, 2008). Taking into account a subchronic study in Cynomolgus monkeys (Seacat et al., 2003), the Tolerable Dose Intake (TDI) for PFOS is 150 ng/kg b.w. (EFSA, 2008). However, other animals such as rats are less sensitive than monkey to PFOS exposure. In fact, the no-observed–adverse-effect level (NOAEL) of PFOS in Sprague–Dawley rats orally treated with this xenobiotic, is 1.25 mg/kg b.w./day in 28-day repeated toxicity studies (Kim et al., 2011). Moreover, the lowest-observed-adverse-effect-level (LOAEL) of PFOS is 1.5 mg/kg b.w./day in adult male rats treated with the chemical in the diet for 4 weeks (Seacat et al., 2003).

Prolactin secretion is modified by many toxics such as pesticides, heavy metals and other endocrine disrupters like bisphenol (Caride et al., 2009, 2010; Jeng et al., 2010; Viñas and Watson, 2013; Jamal et al., 2015) but PFOS effects on the secretion of this pituitary hormone have been poorly studied. It is known that the exposure to this perfluorinated compound induces several neuroendocrine alterations on the reproductive system (Joensen et al., 2013; López-Doval et al., 2014, 2015; Zhao et al., 2014; van den Dungen et al., 2015; Zhang et al., 2015) and on the hypothalamic-pituitary-

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adrenal axis (Knox et al., 2011; Pereiro et al., 2014) as well as in the thyroid gland (Berg et al., 2015; Yu et al., 2015). Moreover, PFOS can cross the blood–brain barrier (Austin et al., 2003) and be accumulated in the hypothalamus, (Austin et al., 2003) presenting neurotoxicity (Long et al., 2013; Pereiro et al., 2014; Dong et al., 2015). This chemical even seems to induce some alterations in neurochemical signaling (Eggers Pedersen et al., 2015) and could have behavioral effects, which were observed in East Greenland polar bears and in rats, respectively (Wang et al., 2015).

Prolactin is synthesized by the lactothrops cells, located in the anterior pituitary gland. This hormone has manifold functions: immunological (Freeman et al., 2000), reproductive (Fitzgerald and Dinan, 2008; Egli et al., 2010), growth (Mosa et al., 2015), metabolic (Ben-Jonathan and Hugo, 2015) and behavioral (Babic et al., 2015). Moreover, it can be implicated in breast cancer (Surazynski et al., 2013) and in the regulation of cell proliferation and apoptosis (Ferraris et al., 2014; Hsieh et al., 2014) as well as the prostate gland activity (Rojas-Durán et al., 2015). Synthesis and secretion of this lactogenic hormone are subjected to multiple regulators. Among them, dopamine is the major prolactin inhibitory factor (PIF) through three hypothalamic neuronal systems: 1) the tuberoinfundibular dopaminergic (TIDA) neurons, which are located in the dorsomedial arcuate nucleus of the mediobasal hypothalamus, project to the median eminence, releasing dopamine into perivascular spaces of the portal vessels which reach the anterior pituitary; 2) the tuberohypophysial dopaminergic (THDA) neurons, which originate in the rostral arcuate nucleus of the mediobasal hypothalamus, send axons that course through the pituitary stalk and terminate in the neural and intermediate lobes of the pituitary gland and; 3) the periventricular-hypophysial dopaminergic (PHDA) neurons which have perikarya in the periventricular nucleus of the anterior hypothalamus and terminals in the intermediate lobe of the pituitary gland (Freeman et al., 2000). Among these three hypothalamic dopaminergic systems, the TIDA neurons play the predominant role in the regulation of the prolactin secretion (Andrews and Grattan, 2004).

Gamma-aminobutyric acid (GABA) is partially reponsible for the nondopaminergic PIF activity within the hypothalamus (Schally et al., 1977). There are two GABAergic systems affecting prolactin secretion: a tuberoinfundibular GABAergic system intrinsic to the mediobasal hypothalamus (Apud et al., 1989) and another one in the paraventricular (Meister et al., 1988) and supraoptic (Theodosis et al., 1986) nuclei, both located in the anterior hypothalamus. The acivity of hypothlamic GABAergic neurons and the responsiveness of lactotrophs cells to this neuromodulator are affected by estradiol (Diaz et al., 1989) and testosterone (Grattan and Selmanoff, 1994).

In lactotroph cells, estrogens stimulate the gene expression and the relase of prolactin, enhance prolactin storage capacity, and increase cell proliferation (Ben-Jonathan et al., 2009; Liu et al., 2015). Estradiol affects the secretion of prolactin at two different levels; directly in the pituitary lactotroph cells, by modifying its sensitivity to physiological stimulators and inhibitors of prolactin secretion, and whithin the hypothalamus by changing the activity of the neuroendocrine neurons known to control prolactin secretion (Freeman et al., 2000).

The present study was undertaken (1) to evaluate the possible effects of PFOS on prolactin secretion; (2) to know if these effects are mediated by the dopaminergic systems involved in the regulation of the prolactin release (TIDA, THDA and/or PHDA neurons); (3) to study whether GABA could be partially responsible for the PFOS action on prolactin secretion (through the tuberoinfundibular GABAergic neurons and/or through the GABAergic cells in the paraventricular and supraoptic nuclei), and finally; (4) to evaluate the possible mediation of estradiol in PFOS effects on prolactin release.

2. Material and methods

2.1. Animals and experimental design

Adult male Sprague–Dawley rats from the animal facilities of the University of Santiago (Santiago de Compostela, Spain) were used in this study, which were 60 day-old and their weight was 305 ± 16.4 g at the beginning of the experiment. All animals were remained under constant environmental conditions (temperature of 22 ± 2 °C and artificial lighting with a controlled photoperiod (14:10 light–dark, being the light phase from 07:00 to 21:00 h)). Animals received compound feed and water ad libitum. This study has been conducted according to the European and Spanish legislation (Guideline of the Council of the European Communities 2010/63/UE of 22/09/2010 and Real Ordinance 53/2013 of 01/02/2013), and it has been approved by the Ethical Committee of the University of Vigo.

PFOS was used as the potassium salt (Sigma-Aldrich) and it was orally administered by gavage and dissolved in 2.5% Tween 20 (Prolabo). Rats (n = 21) were randomly assigned to three groups (of 7 animals per group), one of which served as a control group (only treated with vehicle, 2.5% Tween 20) while the others two groups were treated with two different doses of PFOS: 3.0 and 6.0 mg of PFOS/kg b.w./day for 28 days. This experiment has been thought as a preliminary study. This is the reason why the chosen doses of PFOS in the present study are high compared to the LOAEL (Seacat et al., 2003) and the NOAEL (Kim et al., 2011) in adult rats, and much higher than its TDI (EFSA, 2008). Besides, for this same reason, male rats have been only used instead of female and male.

At the end of the treatment, animals were killed by decapitation. Care was taken to avoid any major stress before sacrifice and the decapitation procedure was completed within 5–7 s. Immediately after the sacrifice, the anterior and the mediobasal hypothalamus were quickly removed, weighed and kept at −80 °C until being used for analyzing the studied parameters. Trunk blood was also collected, centrifuged at 700 g for 15 min at 4 °C, and serum samples were kept frozen at −20 °C until prolactin and estradiol measurements.

2.2. Determination of serum prolactin and estradiol concentration

Serum prolactin and estradiol concentration were measured through ELISA methodology, by using the specific commercial kits from Cusabio Biotech (Wuhan, China). The assay sensibility was 0.05 ng/ml for prolactin and 23.1 pg/ml for estradiol. All samples were analyzed within the same assay to avoid inter-assay variations.

2.3. Preparation of the hypothalamic regions

After thawing, both the anterior and the mediobasal hypothalamus were immediately homogenized in cold 2N acetic acid (4 °C) in an ice-water bath, avoiding the heating of the sample and centrifuged (at 9000 g for 15 min, at 4 °C). The pellet was kept frozen at −20 °C until the determination of protein concentration according to Bradford method (Bradford, 1976), while one aliquot of (100 μl) of the supernatant was removed and kept frozen at −80 °C until dopamine and its metabolites determinations (3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)). The remaining fraction of the supernatant was heated for 7 min in a 100 °C water bath to avoid any analytical interference in the posterior determination of GABA and centrifuged at 21,500 g for 30 min at 4 °C. This second supernatant was removed and kept frozen at −80 °C until GABA determination.

2.4. Measurement of the concentration of dopamine and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)

Dopamine, DOPAC and HVA concentration was determined in the anterior and in the mediobasal hypothalamus by High Performance Liquid Chromatography (HPLC), using electrochemical detection (Coulochem, II, ESA; USA). A C-18 reverse phase column eluted with a mobile phase (pH 4; 0.1 M sodium acetate, 0.1 M citric acid, 0.7 mM sodium octylsulphate, and 0.57 mM EDTA containing 10% methanol v/v) was employed. Flow rate was 1 ml/min, at a pressure of 2200 psi. Fixed potentials against H2/H+ reference electrode were: conditioning electrode −0.4 V; preoxidation electrode +0.10 V; working electrode −0.35 V. The concentration of dopamine and its metabolites were calculated from the chromatographic peak areas by using external standards (Sigma-Aldrich). The linearity of the detector response for dopamine, DPOAC and HVA was tested within the concentration ranges found in supernatants of the studied hypothalamic regions.

2.5. Quantification of gamma-aminobutyric acid (GABA) concentration

GABA concentration was determined in the anterior and in the mediobasal hypothalamus by HPLC with fluorescence detection after precolumn derivatization reaction with O-phthalaldehyde (OPA) (Lafuente et al., 2007). A C-18 reverse-column filled with nucleosil (with a pore diameter of 5 μm, an internal diameter of 4.6 nm and a length of 150 mm (Phoenomenex)) was used for separation. GABA was eluted by two different mobile phases in gradient (phase A: pH 6.75; 0.1 M sodium acetate, containing 30% methanol v/v and phase B: 70% methanol/water (v/v)). Homoserine was used as internal standard. The selected wavelengths in the detector were 340 nm and 455 nm. The concentration was measured from the internal areas of the peaks, using calibration curves and internal standards and expressed as μg of protein.

2.6. Statistical analysis

The results of the measured parameters in this study were tested for variance homogeneity through the *Snedecor* test and the variance was homogenous. Statistical analysis of results was performed by a one-way analysis of variance (ANOVA) followed by post-hoc *Tukey* test to show which points differed significantly within each experimental group. Statistical treatment of the obtained results was made using SPSS software, version 22.0 for windows (SPSS Inc., Chicago, IL). The level for statistical significance was $P \leq 0.05$ for each analysis. All values represent the mean ± SEM.

3. Results

Although, stress or behavioral modifications have not been evaluated in this study, with a naked eye, these alterations were not observed in PFOS-exposed rats. Furthermore, the relative weight of the hypothalamus did not change after the treatment with this chemical. In addition, the animals treated with PFOS did not present any clinical sign such as variations of body weight, changes on food and water consumption or diarrhea throughout the experiment.

3.1. Effects on prolactin serum concentration

The prolactin serum concentration was modified after the PFOS treatment, descending with both administered doses (3.0 and 6.0 mg/kg/day) (Fig. 1; $P \leq 0.01$ with 3.0 mg of PFOS/kg/day and, $P \leq 0.05$ with 6.0 mg of PFOS/kg/day vs. control group).

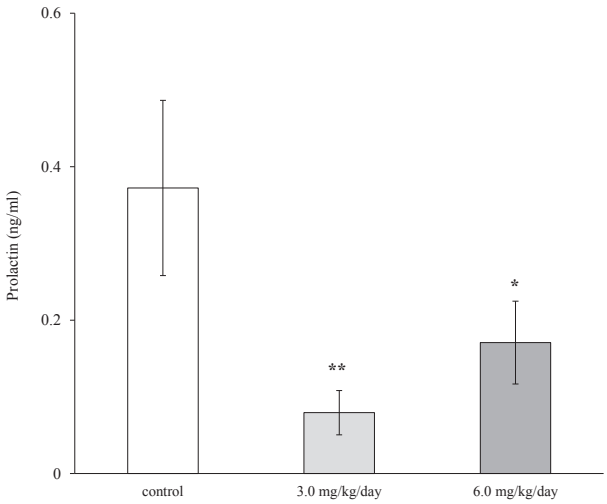

Fig. 1. Serum prolactin levels in adult male rats exposed to PFOS at the doses of 3.0 and 6.0 mg/kg b.w./day for 28 days. $^{**}P \leq 0.01$ and $^{*}P \leq 0.05$ vs. control group.

3.2. Effects on the concentration of dopamine and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)

In the anterior hypothalamus, PFOS at high doses seems to modify the concentration of dopamine and the ratios DOPAC/dopamine and HVA/dopamine in adult male rats. More concretely, dopamine content was increased in this region after the administration of this toxic (Fig. 2a; $P \leq 0.001$ for 3.0 and 6.0 mg of PFOS/kg/day vs. control group). The ratio DOPAC/dopamine was diminished for all the administered doses of PFOS (Fig. 2b; $P \leq 0.001$ vs. control group), and regarding the HVA/dopamine ratio in this same brain area, it was also decreased (Fig. 2c; $P \leq 0.001$ with 3.0 and 6.0 mg of PFOS/kg/day vs. control group). In the mediobasal hypothalamus, no statistically significant modifications were observed in the dopamine concentration (Fig. 2d) and in the ratios of DOPAC/dopamine (Fig. 2e) and HVA/dopamine (Fig. 2f) after PFOS exposure.

3.3. Changes on GABA concentration

High doses of PFOS induced a dose-dependent raise of the GABA concentration in the anterior hypothalamus in adult male rats (Fig. 3a; $P \leq 0.05$ with 3.0 mg of PFOS/kg/day and, $P \leq 0.01$ with 6.0 mg of PFOS/kg/day vs. control group). However, the content of this amino acid was not significantly modified in the mediobasal hypothalamus after PFOS treatment (Fig. 3b).

3.4. Variations on the estradiol serum level

The oral administration of high doses of PFOS for 28 days resulted in a decrease of the serum levels of estradiol with both administered doses, particularly with the 3.0 mg/kg/day dose (Fig. 4; $P \leq 0.01$ with 3.0 mg of PFOS/kg/day and, $P \leq 0.05$ with 6.0 mg of PFOS/kg/day vs. control group).

4. Discussion

The outcomes reported in this study indicate that the oral administration of PFOS at high doses (3.0 and 6.0 mg/kg/day) for 28 days seems to inhibit the synthesis and/or the secretion of prolactin in adult male rats. However, plasma prolactin concentration was

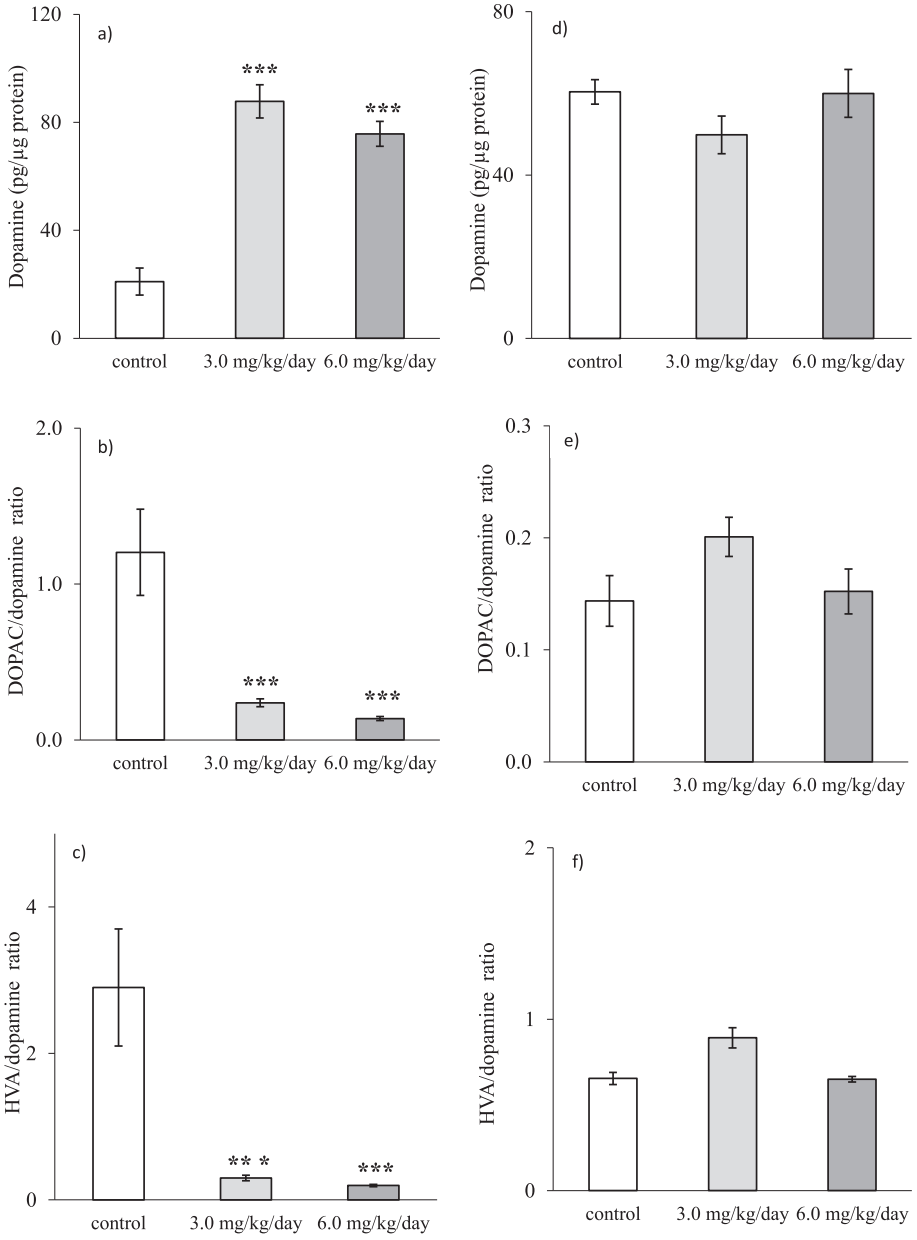

Fig. 2. Concentration of dopamine (a), 3,4-dihydroxyphenylacetic acid (DOPAC)/dopamine ratio (b) and homovanillic acid (HVA)/dopamine ratio (c) in the anterior hypothalamus and concentration of dopamine (d), DOPAC/dopamine ratio (e) and HVA/dopamine ratio (f) in the mediobasal hypothalamus in adult male rats exposed to PFOS at the doses of 3.0 and 6.0 mg/kg b.w./day for 28 days. ***$P \leq 0.001$ vs. control group.

not modified by PFOS exposure at the doses of 1.0, 2.0, 3.0, 5.0 and 10 mg/kg b.w./day in female rats when the chemical was administered during gestation (Thibodeaux et al., 2003). These apparent differences could be due to the specific prolactin activity through gestation and to the special hormone status, which occurs during this physiological period (López-Fontana et al., 2012). Furthermore, in rats, a sex-dependent sensitivity against PFOS exposure cannot be ruled out (Chen et al., 2013). On the other hand, PFOS induces development toxicity, so it might be interesting to analyze the concentration of prolactin-like hormones in the placenta.

Hypoprolactinemia induced by high doses of PFOS could have a very important pathophysiological significance because of the multiple functions exerted by prolactin (Fitzgerald and Dinan, 2008). Low serum prolactin levels are associated mainly with several reproductive diseases (Fitzgerald and Dinan, 2008; Egli et al., 2010) such as a low seminal quality, poor sperm motility and different testicular disorders like oligozoospermia, asthenospermia, hypofunction of seminal vesicles and hypoandrogenism (Gonzales et al., 1989). Moreover, hypoprolactinemia reduces basal testosterone secretion and its response to hCG stimulation (Oseko et al., 1991). In this sense, it has been reported in previous works by our group that large doses of PFOS inhibit testosterone secretion in adult male rats (López-Doval et al., 2014) and this inhibition might be caused by the hypoprolactinemia induced by these same doses of PFOS. Hypoprolactinemia is also recognized as a possible risk factor of arteriogenic erectile dysfunction (Galdiero et al., 2012). What is more, reduced serum prolactin levels could lead to disorders in the prostate gland (Crépin et al., 2007; Sánchez et al., 2008).

Immunotoxic effects of PFOS have been previously reported (Freeman et al., 2000; Osuna et al., 2014; Yan et al., 2014). These alterations on the immune function could be due to the

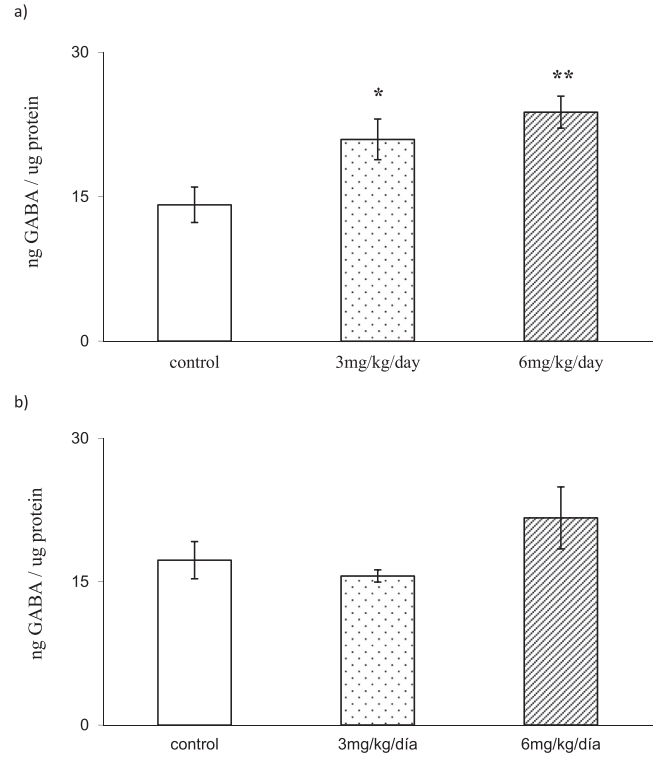

Fig. 3. Concentration of gamma-aminobutyric acid (GABA) in the anterior (a) and in the mediobasal (b) hypothalamus in adult male rats exposed to PFOS at the doses of 3.0 and 6.0 mg/kg b.w./day for 28 days. *P ≤ 0.05 and **P ≤ 0.01 vs. control group.

hypoprolactinemia induced by PFOS at high doses (Chikanza, 1999) because hyperprolactinemia and hypoprolactinemia are both immunosuppressive factors since physiological levels of circulating prolactin are necessary to maintain the normal immunocompetence (Matera, 1997). Finally, it should be pointed out that prolactin is involved in the regulation of the pancreatic islets function (Weinhaus et al., 2007). Therefore, hypoprolactinemia induced by PFOS might disrupt the activity of these cells. All these adverse health effects resulting from the hypoprolactinemia induced by PFOS at large concentrations inform us about the toxicity of this perfluorinated chemical at these doses, suggesting that evaluating the PFOS toxicity on prolactin secretion with doses close to the NOAEL would be very interesting.

According to the reported results, possible mechanisms of PFOS toxicity on prolactin secretion are represented like dashed lines (Fig. 5). Concerning the effects of PFOS in the dopamine metabolism in the anterior hypothalamus, high doses of this toxic seem to reduce the intraneuronal (expressed as the ratio DOPAC/dopamine) and interneuronal (expressed as the ratio homovanilic acid/dopamine) metabolism of this catecholamine, resulting in an increase of dopamine concentration.

In female rats, a previous study (Austin et al., 2003) reported that doses of 1.0 mg and 10 mg of PFOS/kg/day for 14 days did not modify the dopamine content in the paraventricular and in the medial preoptic nuclei (both nuclei located in the anterior hypothalamus) as a possible result of the lesser sensitivity to this chemical in female rats than in male ones (Chen et al., 2013). Moreover, a very lofty dose of PFOS (250 mg/kg) did not modify the dopamine concentration in the cerebellum or in the whole brain in male rats (Sato et al., 2009) but a later study showed a decrease of dopamine and DOPAC content in the caudate and putamen in mice treated with 10.75 mg of PFOS/kg/day for three months (Long et al., 2013).

The augmented concentration of dopamine reported in the anterior hypothalamus in PFOS-treated rats could lead to several alterations in the thermoregulation (Hasegawa et al., 2000), in defense (Sweidan et al., 1991) and aggressive (Ricci et al., 2009) behavior as well as in the reproductive axis activity (Henderson et al., 2008). In fact, PFOS inhibits the reproductive axis function (López-Doval et al., 2014, 2015) and this effect could be mediated by the increased concentration of dopamine, observed in the anterior hypothalamus after PFOS administration. Likewise, the diminution of the intraneuronal dopamine metabolism reported by the PFOS-treated animals might be due to changes in the activity of the dopamine transporter because this transporter is regulated by estrogens and PFOS can alter estrogen secretion (Fang et al., 2012; Du et al., 2013; Lafuente et al., 2013).

Dopamine concentration was not modified by PFOS in the mediobasal hypothalamus where the arcuate nucleus is. Both TIDA and THDA neurons have their origin in this nucleus, therefore high concentrations of PFOS do not seem to inhibit prolactin secretion by

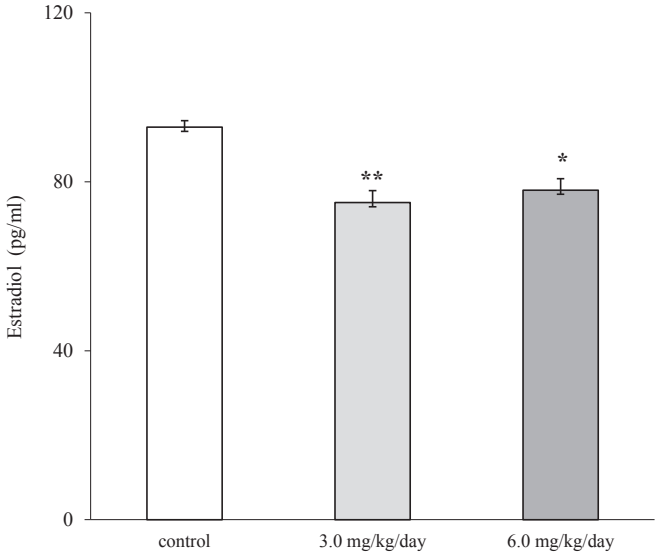

Fig. 4. Serum estradiol concentration in adult male rats exposed to PFOS at the doses of 3.0 and 6.0 mg/kg b.w./day for 28 days. **P ≤ 0.01 and *P ≤ 0.05 vs. control group.

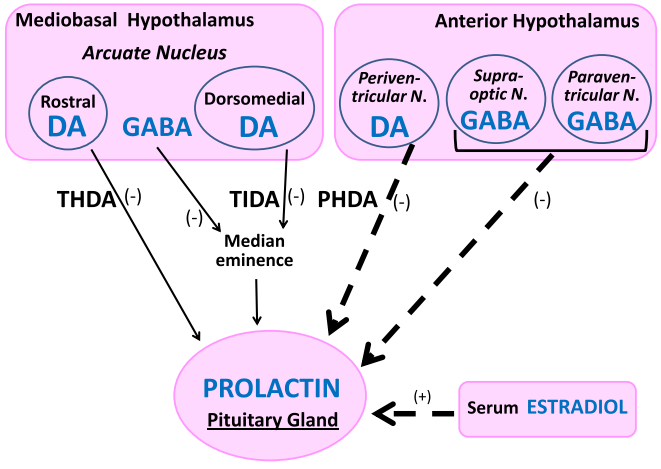

Fig. 5. The regulation of prolactin secretion by dopamine, GABA and estradiol is shown in this scheme. According to the obtained outcomes, possible mechanisms of PFOS toxicity on the secretion of this lactogenic hormone are represented like dashed lines.

altering these dopaminergic systems (Freeman et al., 2000). Nevertheless, the raise of the dopamine concentration found in the anterior hypothalamus after PFOS administration suggests that the PHDA system (dopaminergic neurons originated from the periventricular nucleus located in the anterior hypothalamus) might be involved in the inhibition of prolactin release induced by perfluorinated compounds (Freeman et al., 2000).

With respect to the possible role of GABA in the inhibitory PFOS effect on prolactin secretion, large concentrations of this chemical did not modify the concentration of this amino acid in the mediobasal hypothalamus, but the content of this neurotransmitter was augmented in the anterior hypothalamus, where paraventricular and supraoptic nuclei are. Therefore, the GABAergic neurons originated in these nuclei and involved in the regulation of prolactin release would be implicated in the inhibition of prolactin secretion exerted by PFOS (Theodosis et al., 1986), while the GABAergic system intrinsic to the mediobasal hypothalamus would not mediate this inhibition (Apud et al., 1989). In addition, other neuromodulators of prolactin secretion not evaluated in this study could also mediate PFOS toxicity on the secretion of this pituitary hormone.

The increase of the GABA concentration induced by PFOS in the anterior hypothalamus can have several pathological consequences. This fact would increase and/or explain, at least in part, some toxicity effects of this perfluorinated compound. Among these diseases, we can note reproductive pathologies (López-Doval et al., 2014, 2015; Watanabe et al., 2014), a disruption of the development and regulation of the stress response (Gunn et al., 2015), alterations in the corticotropin-releasing hormone (Tran et al., 1999), as well as desynchronization of the circadian rhythms (Kalsbeek et al., 2000), insomnia (Plante et al., 2012), and changes in the thermoregulation (Jha and Mallick, 2009).

Adult male rats exposed to elevated doses of PFOS for 28 days showed decreased serum estradiol levels. Taking into account that estradiol stimulates prolactin release within the hypothalamus and at the pituitary gland level (Freeman et al., 2000), we can think that PFOS would inhibit prolactin secretion by suppressing estradiol release, which could affect different neuromodulators in the anterior hypothalamus and the sensitivity of lactotroph cells to these modulators. However, norepinephrine and neuropeptide Y do not seem to be involved in the inhibition of prolactin secretion by PFOS because the changes observed in these neuromodulators after PFOS exposure (Pereiro et al., 2014; López-Doval et al., 2015) do not correlate with the low serum prolactin levels observed in PFOS-treated animals.

The results reported in this study suggest that (1) high doses of PFOS inhibit prolactin secretion in adult male rats; (2) only the PHDA neurons seem to be involved in this inhibitory effect but not the TIDA and the THDA systems; (3) GABAergic cells from the paraventricular and supraoptic nuclei could be partially responsible for the PFOS action on prolactin secretion; (4) estradiol might take part in the inhibition exerted by elevated concentration of PFOS on prolactin release; and finally (5) it would be interesting to assess PFOS effects on prolactin secretion in female rats administering similar doses to the acceptable daily intake in humans.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.fct.2015.05.013.

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