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The expression of several reproductive hormone receptors can be modified by perfluorooctane sulfonate (PFOS) in adult male rats

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HIGHLIGHTS

- PFOS can alter gene and protein expression of GnRHr, LHr, FSHr and Ar in the HPT axis.
- PFOS inhibits both gene and protein expression of FSHr and Ar at testicular level.
- PFOS surprisingly stimulates the protein GnRHr and the gene LHr expression in testis.
- PFOS can disrupt the HPT axis by altering several hormone receptors in adult male rat.

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ABSTRACT

This study was undertaken to evaluate the possible role of several reproductive hormone receptors on the disruption of the hypothalamic-pituitary-testis (HPT) axis activity induced by perfluorooctane sulfonate (PFOS). The studied receptors are the gonadotropin-releasing hormone receptor (GnRHr), luteinizing hormone receptor (LHr), follicle-stimulating hormone receptor (FSHr), and the androgen receptor (Ar). Adult male rats were orally treated with 1.0; 3.0 and 6.0 mg of PFOS kg^{-1} d^{-1} for 28 days. In general terms, PFOS can modify the relative gene and protein expressions of these receptors in several tissues of the reproductive axis. At the testicular level, apart from the expected inhibition of both gene and protein expressions of FSHr and Ar, PFOS also stimulates the GnRHr protein and the LHr gene expression. The receptors of the main hormones involved in the HPT axis may have an important role in the disruption exerted by PFOS on this axis.

1. Introduction

Perfluorooctane sulfonate (PFOS) is an organic fluorinated compound ubiquitously found in the environment (Houde et al., 2006; Miller et al., 2015; Xiao et al., 2012) that is extremely persistent in the ecosystem (Organisation for Economic Co-operation and Development (OECD), 2002). It is the final product of degradation of more than 50 fluorinated organic compounds, being detected in a variety of living organisms (Chu et al., 2015; Dietz et al., 2015) and human beings (Toms et al., 2014; Zeng et al., 2015). In the general population, a large part of the exposure to the chemical arises through the diet, primarily via consumption of fish, seafood, meat, and dairy products (Domingo et al., 2012; EFSA, 2008, 2012; Eriksson et al., 2013), being bioaccumulative (Houde et al., 2006). In addition, it is currently considered an emerging contaminant in the food chain. For these reasons, the Commission Recommendation 2010/161/EU urged the Member States to monitor the occurrence of PFOS, perfluorooctanoic acid (PFOA) and their precursors in food (EFSA, 2012). The serum half-life of PFOS can be as long as 5.4 years in humans (Olsen et al., 2007) while 33 days in adult male rat after a single oral dose of 400 μg kg^{-1} (Benskin et al., 2009). Besides, this chemical has the ability to cross the blood brain barrier (Austin et al., 2003) and the placenta (Kim et al., 2011b), affecting the central nervous system (Harada et al., 2006) and pre- and postnatal developing (Lee et al., 2015; Wang et al., 2015).

The liver is known as the primary target organ for PFOS toxicity (Qazi et al., 2013). PFOS also induces immunotoxicity (Brieger et al., 2011), neurotoxicity (Salgado et al., 2015) as well as endocrine (Du et al., 2013; Pereiro et al., 2014; Salgado et al., 2015) and reproductive toxicity (López-Doval et al., 2014, 2015; Qiu et al., 2013).

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Table 1
Primers used for quantitative polymerase chain reaction.

Gene	Primers	Accession No
β-actin	Forward 5′ -CTCTCTTCCAGCCTTCCTTC- 3′ Reverse 5′ -GGTCTTTACGGATGTCAA- 3′	NM_031144.3
GnRHr	Forward 5′ -GCAGAACCC CAGAACTTCGA- 3′ Reverse 5′ -TGCCCA GCTTCCTCTTCAAT- 3′	NM_031038.3
FSHr	Forward 5′ -AAGTCGATCCAGCTT TGCAT- 3′ Reverse 5′ -GTCCCAGCCCTCTTACAGTG- 3′	NM_199237.1
LHr	Forward 5′- ACCTTCACCACCAGCATCTGT- 3′ Reverse 5′- AGCTCACGGTAGGTGCACACT- 3′	NM_012978.1
Ar	Forward 5′- AGGAACTTGATCGCATCATTGC- 3′ Reverse 5′- CTGCCATCATTTCAGGAA- 3′	NM_012502.1

Moreover, gene ontology analysis in humans indicated that PFOS would exert toxic effects on L-02 cells by affecting multiple biological processes, including protein biosynthesis and degradation, mRNA processing and splicing, transcription, signal transduction and transport (Huang et al., 2014).

Among the neuroendocrine effects of PFOS, it should be emphasized that this chemical can affect the hypothalamic–pituitary–testicular (HPT) and the hypothalamic–pituitary–adrenal axes activity (López-Doval et al., 2014, 2015; Pereiro et al., 2014; Ribes et al., 2010). It is also able to exert its toxicity at testicular level (Qiu et al., 2013) having been reported in rats (Jensen and Leffers, 2008; López-Doval et al., 2014, 2015) and on testis models (Zhang et al., 2013). In addition to this, apoptosis induced by PFOS in mice Leydig cells was shown to be related to mitochondrially mediated pathways and to involve oxidative stress (Zhang et al., 2015).

On the other hand, it is well known that reproduction is mainly mediated in male by hormones such as the hypothalamic gonadotropin-releasing hormone (GnRH), the adenohypophyseal gonadotropins, the luteinizing hormone (LH) and the follicle-stimulating hormone (FSH), and the androgens, that regulate the HPT function through a negative feedback loop which involves the hypothalamus, pituitary, and testis (Chimento et al., 2014; Vadakkadath Meethal and Atwood, 2005). The synthesis and secretion of these hormones are regulated simultaneously by this complex feedback loop where activins stimulate the hypothalamic GnRH secretion, which through its receptor (GnRHr) located in the pituitary gonadotropes, stimulates the LH and FSH release by these cells (Millar et al., 2004; Miller and Gibson, 1994) into the bloodstream. On the testicular level, LH stimulates through its receptors (LHr), located in the Leydig cells, testosterone secretion, which has a local effect on the interstitium and seminiferous tubules and results in sperm production and maturation. In the testicular Sertoli cells, spermatogenesis is stimulated by FSH through its receptors (FSHr) (Reichlin, 1998). Moreover, FSH is involved in the paracrine control and the structural and nutritional support of the Sertoli cells development, exerting its effect directly or promoting and sustaining the spermatogenesis processes (Akingbemi, 2005; Atanassova et al., 2005; Huhtaniemi and Themmen, 2005) while at the same time, testosterone exerts its function in testis through androgen receptors (Ar) (O'Shaughnessy et al., 2012). Although other hormones facilitate the process of spermatogenesis, only testosterone is essential to maintain spermatogenesis, being required for at least four critical processes during spermatogenesis: maintenance of the blood–testis barrier (BTB), meiosis, Sertoli–spermatid adhesion and sperm release (Smith and Walker, 2014).

Despite that some of the previous studies have shown no effects of PFOS on reproductive functions in rats (Butenhoff et al., 2012; Luebker et al., 2005), other *in vivo* studies in marine organisms (Han et al., 2015; Zhang et al., 2014), insects (Mommaerts et al., 2011), mammals (Persson and Magnusson, 2015) as well as in *in vitro* works (Xu et al., 2013) showed alterations in reproductive function induced by this contaminant. Besides, several previous studies from our laboratory have reported various changes in relative gene expression and secretion of GnRH, LH and FSH as well as in testosterone synthesis in adult male rats treated with PFOS for 28 days (López-Doval et al., 2014), in which PFOS seems to alter these reproductive parameters by modifying different hypothalamic neuromodulators such as norepinephrine, serotonin and neuropeptide Y and also through oxidative mechanisms at testicular level (López-Doval et al., 2014, 2015). Although this toxic could also affect the receptors of the main hormones of the reproductive system, these possible effects have not yet been evaluated. The receptors of GnRH, LH, FSH and testosterone are expressed in the tissues where these hormones exert their activities (Chimento et al., 2014). Moreover, LHr, FSHr and Ar are present in the hypothalamus (Vadakkadath Meethal and Atwood, 2005), while Ar is also expressed in the pituitary (O'Hara et al., 2015) because these hormones regulate their own secretion through a negative feedback at these levels (Chimento et al., 2014). Additionally, LHr is also expressed in a number of extra gonadal sites (Rao and Lei, 2007). Aberrations in reproductive functions in the female and male have been reported due to naturally occurring mutations in LHr and FSHr (Ascoli and Puett, 1978; Menon and Menon, 2012; Segaloff, 2009). It has also been shown that in mice lacking FSHr and Ar only 3% of their normal germ cell numbers are present at the age of 20 days (O'Shaughnessy et al., 2012). These animals fail to generate post-meiotic germ cells; therefore, they are infertile (O'Shaughnessy, 2014).

Based on the explained regulatory mechanisms of the HPT axis activity and the reproductive effects of PFOS mentioned above, this contaminant could induce its reproductive toxicity by modifying gene and/or protein expression of GnRHr, LHr, FSHr and/or Ar. Therefore, the present study was undertaken to evaluate the possible role of these hormone receptors in the PFOS effects on the HPT axis in adult rats by analyzing both gene and protein expression of: (1) GnRHr in the pituitary and in the testis; (2) LHr and FSHr in the testis and in the hypothalamus, and (3) Ar in the hypothalamus, pituitary and in the testis.

2. Material and methods

2.1. Chemical

Perfluorooctane sulfonic acid has been used as potassium salt. It was purchased from Sigma-Aldrich (Madrid, Spain) and it was dissolved in 2.5% Tween 20, which was obtained from VWR International (Radnor, Pennsylvania, USA).

2.2. Animals and experimental design

Adult male Sprague-Dawley rats were obtained from the animal facilities of the University of Santiago (Santiago de Compostela, Spain). They were 60 days-old and weighed 305 ± 16.4 g at the beginning of the experiment. All animals remained in constant environmental conditions (temperature of 22 ± 2 °C and an automatic day–night cycle, light: 07:00–21:00 h). The animals were fed with 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. Six animals per experimental group were used to quantify the different studied parameters.

A study of repeated doses has been carried out and PFOS was orally administered by gavage. Rats were randomly assigned to four