Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/watres

Occurrence of androgens in sewage treatment plants influents is associated with antagonist activities on other steroid receptors

Virginie Bellet [a,b,c,d], Guillermina Hernandez-Raquet [e,f], Sonia Dagnino [a,b,c,d,g], Ludivine Seree [h], Patrick Pardon [i], Chrystelle Bancon-Montiny [g], Hélène Fenet [g], Nicolas Creusot [j], Selim Aït-Aïssa [j], Vincent Cavailles [a,b,c,d], Hélène Budzinski [i], Jean-Philippe Antignac [h], Patrick Balaguer [a,b,c,d,*]

[a] IRCM, Institut de Recherche en Cancérologie de Montpellier, Montpellier, F-34298, France
[b] INSERM, U896, Montpellier, F-34298, France
[c] Université Montpellier1, Montpellier, F-34298, France
[d] CRLC Val d'Aurelle Paul Lamarque, Montpellier, F-34298, France
[e] INRA, Institut National de la Recherche Agronomique, UR050, Laboratoire de Biotechnologie de l'Environnement, Avenue des Etangs, Narbonne F-11100, France
[f] Laboratoire d'Ingénierie des Systèmes Biologiques et Procédés, UMR5504, UMR792, CNRS, INRA, INSA. 135, avenue de Rangueil, 31077 Toulouse Cedex 04, France
[g] UMR 5569 Hydrosciences, Université de Montpellier I, Avenue Charles Flahault, 34060 Montpellier, France
[h] ONIRIS, Laboratoire d'Etude des Résidus et Contaminants dans les Aliments (LABERCA), USC INRA 1329 – BP 50707 – 44307 Nantes Cedex 3 – France
[i] EPOC, Environnements et Paléoenvironnements Océaniques et Continentaux, UMR 5805 CNRS, Laboratoire de Physico- et Toxico-Chimie de l'environnement (LPTC), 351 cours de la Libération, 33405 Talence, France
[j] INERIS, Unité Écotoxicologie in vitro et in vivo, Parc ALATA, BP2, f-60550 Verneuil-en-Halatte, France

ARTICLE INFO

Article history:
Received 28 September 2011
Received in revised form
11 January 2012
Accepted 14 January 2012
Available online 25 January 2012

Keywords:
Estrogens
Androgens
Pregnenolone
Steroid receptors
Sewage treatment plants

ABSTRACT

The occurrence of endocrine disrupting chemicals such as estrogens in raw urban sewage is well documented. By contrast, the presence of other steroidal activities in wastewater has been poorly studied, although they can cause undesirable biological responses in the environment. In this work, extracts of raw wastewater were tested for agonist and antagonist activities on estrogen, androgen, progesterone, mineralocorticoid and glucocorticoid receptors. We detected strong estrogenic activities that correlated well with the concentration of natural estrogens (estrone, estriol and 17β-estradiol) measured by chemical analysis. We also measured strong androgenic activities which were not due to estrogen receptor ligands based on the use of recombinant estrogen receptor α affinity columns. Several molecules with androgenic activities were identified in wastewater samples, testosterone, dihydrotestosterone and epiandrosterone being the most abundant. However, they explain only a small part of the detected androgenic activity, as indicated by the comparison of the detected biological responses with the results of the targeted chemical analysis. Finally, we found that our samples also contained strong antagonist

Abbreviations: ER, estrogen receptor; AR, androgen receptor; PR, progesterone receptor; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; STPs, sewage treatment plants; EDC, endocrine disrupting compound.

* *Corresponding author.* IRCM, Institut de Recherche en Cancérologie de Montpellier, Montpellier, F-34298, France. Tel.: +33 467612409; fax: +33 467613787.
E-mail address: Patrick.Balaguer@montpellier.unicancer.fr (P. Balaguer).

0043-1354/$ – see front matter
doi:10.1016/j.watres.2012.01.013

activities on progesterone, glucocorticoid and mineralocorticoid receptors. Very interestingly, we identified pregnenolone (the precursor to all steroid hormones in humans) as a major endocrine disrupting chemical which accounts for most of the anti-mineralocorticoid activities present in raw wastewater. In conclusion, this study demonstrates the occurrence of androgen agonists as well as other steroid receptor antagonists such as pregnenolone in raw wastewater. Further research is needed to assess the fate of such compounds during sewage treatment and their potential effect on living organisms.

1. Introduction

Wastewater has often been identified as a source of endocrine disrupting compounds (EDCs) for the environment. EDCs include a great range of substances such as natural and synthetic hormones, pesticides, phytocompounds as well as industrial chemicals (Hotchkiss et al., 2008). Up until now, studies on the presence of EDCs in the receiving aquatic environment have predominantly focused on estrogenic compounds (Muller et al., 2008; Dagnino et al., 2010). Estrogenic contamination has been reported at concentrations of nanograms per liter in sewage influents and has been related to the amount of estrogens excreted in urine and feces either as biologically active, free forms or as inactive forms, conjugated with glucuronide and/or sulfate groups (Ternes et al.,1999, Baronti et al., 2000). As a consequence, most studies have focused on the effects of estrogenic environmental contaminants on aquatic species (Sumpter et al., 1996; Toppari et al., 1996), while the potential effects of non-estrogenic steroids on wildlife have been overlooked.

In addition to estrogens, other EDC classes, such as androgens, progestogens, glucocorticoids and mineralocorticoids, can be released into the aquatic environment from sewage treatment plants (STPs) and therefore they may also represent a risk for the aquatic fauna (Ellis et al., 2003; Mnif et al., 2010; Van der Linden et al., 2008). Some studies have described androgenic activities in environmental samples (Urbatzka et al., 2007; Liu et al., 2009; Yamamoto et al., 2006) that have been attributed to human androgens, like androstenedione, testosterone and androsterone. Furthermore other studies have described the presence of progesterone (PR) and glucocorticoid (GR) receptors agonistic activities (Van der Linden et al., 2008; Fan et al., 2011; Liu et al., 2011).

In this study, we determined steroid receptor profiling of raw sewage extracts using a panel of *in vitro* bioassays that allowed the detection of ER and AR agonist activities as well as antagonist activities for GR, PR and MR in all the samples tested.

2. Methods

2.1. Chemicals and materials

Cell culture material was from Life Technologies (Cergy-Pontoise, France) except the 96-well Cellstar plates, which were from Greiner Labortechnic (Poitiers, France). Luciferin (sodium salt) and geneticin were purchased from Promega (Charbonnières, France). R1881 was from NEN Life Science Products (Paris, France). The other natural or pharmaceutical ligands and puromycin were purchased from Sigma Aldrich (Saint-Quentin Fallavier, France). These ligands were dissolved in dimethyl sulfoxide (DMSO) at 10 mM and dilutions from this stock solution were prepared in culture medium.

All solvents and reagents were of analytical or HPLC grade quality and purchased from Solvent Documentation Synthesis (SDS, Peypin, France). All SPE cartridges (ChromP, SiOH) were also provided by SDS. *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) was purchased from Fluka (Buchs, Switzerland). Dithiothreitol (DTT) and trimethyliodosilane (TMIS) were from Sigma–Aldrich (St. Quentin Fallavier, France). Standard reference steroids (Supplementary Table 1) were purchased from Sigma (St. Louis, MO). Deuterated internal standards (etiocholanolone-*d*5, 5α-androstane-3α, 17β-diol-*d*3, 4-androstenedione-*d*3, 17α-testosterone-*d*3 and 17α-methyltestosterone-*d*3), used for quantification according to the isotopic dilution method, were provided by Steraloids (Wilton, NY). The deuterated estrogens βE2-*d*4, T-*d*3 and ethynilestradiol (EE2-*d*4) (isotopic purity >99%), which were used as internal standards (IS) for hormone analysis, were supplied by Cluzeau Info Labo (Ste. Foy –La-Grande, France).

2.2. Samples preparation

Six STPs located in the Languedoc Roussillon region (South of France) were selected for this study to represent urban STPs receiving sewage from populations of different sizes (population equivalent from 500 to 300,000 people; Table 1). All STPs received wastewater mainly of domestic origin.

Grab influent wastewater samples (10 L) were collected in the morning in winter (from 4 to 12 December, 2008) over 1-h period and combined to constitute a composite sample of 10 L. triplicate (chemical analysis) or duplicate (*in vitro* measurements) samples were conditioned in 1 L polypropylene bottles previously rinsed with acetone. Formaldehyde was added to samples prepared for chemical analysis (1%, v/v) to prevent bacterial degradation. All samples were stored at 4 °C and analyzed within 24 h or immediately frozen at −20 °C until further processing.

For steroid activity measurements, all samples were extracted according to the procedures described by Labadie and Budzinski (2005). Briefly, samples were acidified to pH 3–5 with hydrochloric acid (10%) and filtered on Whatman GF-F glass fiber filters (pore size, 0.7 μm; VWR, Strasbourg, France). Analytes were extracted by solid phase extraction (SPE) from 1 L of liquid samples using 200 mg Oasis HLB cartridges (Waters, St. Quentin en Yvelines, France).