Environmental Research 158 (2017) 660–668

Contents lists available at ScienceDirect

Environmental Research

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

Effects of environmental pollutants on signaling pathways in rat pituitary GH3 adenoma cells

Nicoletta Fortunati[a], Federica Guaraldi[b], Valentina Zunino[a], Federica Penner[c], Valentina D'Angelo[a], Francesco Zenga[c], Francesca Pecori Giraldi[d], Maria Graziella Catalano[e], Emanuela Arvat[a,e,*]

[a] Division of Oncological Endocrinology, Città della Salute e della Scienza University Hospital, I-10126 Turin, Italy
[b] Division of Endocrinology, Diabetes and Metabolism, Department of Medical Sciences, Città della Salute e della Scienza University Hospital, I-10126 Turin, Italy
[c] Division of Neurosurgery, Città della Salute e della Scienza University Hospital, I-10126 Turin, Italy
[d] Neuroendocrinology Research Laboratory, Istituto Auxologico Italiano IRCCS, Cusano Milanino, (MI), Italy and Department of Clinical Sciences and Community Health, University of Milan, I-20149 Milan, Italy
[e] Department of Medical Sciences, University of Turin, I-10126 Turin, Italy

ARTICLE INFO

Keywords:
Pollution
Benzene
GH
DEHP
PCB
Somatostatin receptor
Pituitary adenoma

ABSTRACT

An increased rate of acromegaly was reported in industrialized areas, suggesting an involvement of environmental pollutants in the pathogenesis and behavior of GH-secreting pituitary adenomas. Based on these premises, the aim of the study was to evaluate the effects of some widely diffused pollutants (i.e. benzene, BZ; bis(2-ethylhexyl) phthalate, DEHP and polychlorinated biphenyls, PCB) on growth hormone secretion, the somatostatin and estrogenic pathways, viability and proliferation of rat GH-producing pituitary adenoma (GH3) cells. All the pollutants induced a statistically significant increase in GH secretion and interfered with cell signaling. They all modulated the expression of SSTR2 and ZAC1, involved in the somatostatin signaling, and the expression of the transcription factor FOXA1, involved in the estrogen receptor signaling. Moreover, all the pollutants increased the expression of the CYP1A1, suggesting AHR pathway activation. None of the pollutants impacted on cell proliferation or viability. Present data demonstrate that exposure to different pollutants, used at in vivo relevant concentrations, plays an important role in the behavior of GH3 pituitary adenoma cells, by increasing GH secretion and modulating several cellular signaling pathways. These observations support a possible influence of different pollutants in vivo on the GH-adenoma aggressiveness and biological behavior.

1. Introduction

Pituitary adenomas account for 10–25% of intracranial tumors (Dworakowska and Grossman, 2009). They derive from clonal expansion of highly differentiated anterior pituitary cells and are mainly sporadic (Dworakowska and Grossman, 2009; Melmed, 2003). Although the great majority of pituitary tumors are histologically benign, they may have a very aggressive behavior characterized by rapid cell proliferation, infiltration of surrounding nervous and vascular structures, and resistance to medical treatment. GH-secreting adenomas, clinically manifesting with signs and symptoms of acromegaly, are the third most common type of pituitary adenoma after prolactinomas and non-functioning adenomas. Though rare, their prevalence and incidence is reportedly increasing (Beckers, 2010).

Hormone secretion and growth of GH-adenoma cells are under the control of many signaling pathways, including somatostatin (SS) pathway that mainly exerts an inhibitory function. SS and its analogs (SSA) – considered the gold-standard medical treatment for acromegaly – bind to five different seven-domain G-protein-coupled receptors, SSTR1-5, through which SS regulates a complex downstream pathway, that includes the zinc-finger protein (ZAC1), the aryl hydrocarbon receptor (AHR), the aryl hydrocarbon receptor-interacting protein (AIP), and several AIP-interacting proteins (Trivellin and Korbonits, 2011; Gadelha et al., 2013). Furthermore, it has been demonstrated that

Abbreviations: AF2, activation function 2; AHR, aryl hydrocarbon receptor; AIP, aryl hydrocarbon receptor-interacting protein, A; BZ, benzene; CYP1A1, cytochrome-P 1A1; DEHP, bis (2-ethylhexyl) phthalate; ERα, estrogen receptor α; FIPA, familial isolated pituitary adenoma; FOXA1, Forkhead box protein A1; GH, growth hormone; PAC1-R, adenylate cyclase activating polypeptide type 1 receptor; PCB, polychlorinated biphenyls; PPARγ, peroxisome proliferator-activated receptor γ; PR, progesterone receptor; PRL, prolactin; SD, standard deviation; SRC1, steroid receptor co-activator 1; SS, somatostatin; SSA, somatostatin analogs; SSTR, somatostatin receptor; ZAC1, zinc-finger protein

* Corresponding author at: Department of Medical Sciences, University of Turin, I-10126 Turin, Italy.
E-mail address: emanuela.arvat@unito.it (E. Arvat).

http://dx.doi.org/10.1016/j.envres.2017.07.015
Received 12 January 2017; Received in revised form 26 May 2017; Accepted 6 July 2017
Available online 18 July 2017
0013-9351/

estrogens directly stimulate GH synthesis and secretion through the estrogen receptor and the classic nuclear hormone receptor pathway (Avtanski et al., 2014).

Although several genetic, such as germ-line mutations of AIP, (Chahal et al., 2010; Guaraldi and Salvatori, 2011; Cazabat et al., 2012) and epigenetic factors have been suggested (Melmed, 2006), the exact ethiopathogenesis underlying the development of pituitary adenomas, and the mechanisms responsible for their different behavior are not completely understood. Pollution has significant impact on human health and it is implicated in different types of cancers (Engström et al., 2015; Hashim and Boffetta, 2014). Among several pollutants, benzene (BZ) is widespread and relevant worldwide (WHO Expert consultation, 2015). It derives from industrial effluents, vehicle emissions and cigarette smoking. Its carcinogenic and non-cancerous systemic toxic effects have been largely demonstrated, and they appear secondary to the cytotoxic effect of free radicals and quinone metabolites produced by its metabolism, as well as to genotoxic effects and altered global DNA methylation (Bahadar et al., 2014; Snyder et al., 1993). Moreover, a large number of chemicals have been identified as endocrine disruptors, compounds that alter the normal functioning of the endocrine system of both humans and wildlife. Bis-(2-ethylhexyl)-phthalate (DEHP) is the most commonly used plasticizer in plastic industries and it has been detected in different environmental compartments (Magdouli et al., 2013). Polychlorinated biphenyls (PCB) are banned from production and use in most countries but they are persistent organic pollutants of concern for environment and health. Adverse effects of PCB and their metabolites include neurotoxicity, immunosuppression, effects on thyroid hormones and retinoic acid transport to target tissues, reproductive effects, porphyria and carcinogenicity (AMAP Assestment 2002, 2004; Vorkamp, 2016).

The first description of the detrimental effects of environmental pollutants on the pituitary dates back to late 1950s, when Iannacone and Cicchella (1958) described the histological changes associated with benzene intoxication in rats. To our knowledge, no other studies were performed in this field up to 2008, when a modest increase in the incidence of pituitary tumors was reported in the population from Seveso (Italy), exposed thirty years earlier to intermediate-high concentrations of 2,3,7,8-tetrachlorodibenzo-p-dioxin, a strong toxic and carcinogenic agent, because of an industrial accident. The involvement of AHR signaling pathway was suggested, although not experimentally demonstrated (Pesatori et al., 2008). Soon later, Cannavò et al. (2010) demonstrated a significantly greater rate of acromegaly in a highly industrialized area close to Messina (Italy) with respect to farther areas and the general population, not attributable to genetic/family predisposition. The authors therefore hypothesized the involvement of environmental pollutants in the pathogenesis of somatotropinomas. Recently, Tapella et al. (2016) demonstrated in vitro that the pollutants BZ and DEHP are able to interfere with normal rat pituitary cell proliferation and to promote gene expression changes at AIP and AHR levels, providing a link between epidemiological and genomic findings in pituitary tumors. However, to date, there are no other studies about the influence of these environmental chemical pollutants on the behavior of pituitary tumors in terms of aggressiveness and potentially drug therapy resistance. Based on these premises, aim of the study was to evaluate the in vitro effects of BZ, DEHP and PCB on GH production, gene and protein expression of the main constituents of the somatostatin and estrogen signaling pathways, and on cell growth and viability, using a GH-producing pituitary adenoma cell line (GH3).

2. Materials and methods

2.1. Chemicals

Benzene (BZ), bis(2-ethylhexyl) phthalate (DEHP), and polychlorinated biphenyls (PCB) were purchased from Sigma-Aldrich Co. (St. Louis, MO). BZ was dissolved in DMEM-F12 medium added with dextran-charcoal stripped sera (12.8 μM), PCB was purchased in solution (10 μg/ml in isooctane); DEHP was dissolved in ethanol (2.5 μM). Further dilutions were made in culture medium. PCB concentration is given in grams since PCB is a mixture of compounds # 28, 52, 101, 138, 153, 180 and 209, and it was not possible to calculate the molarity of the solution. Experimental doses were within the ranges observed in blood of exposed people (Hines et al., 2009; Yan et al., 2009; Hays et al., 2012; Chovancová et al., 2012; Hsu et al., 2014).

2.2. Cell cultures

Rat pituitary adenoma GH3 cells, obtained from the American Type Culture Collection (ATCC), were maintained in DMEM-F12 culture medium (Lonza, Switzerland) supplemented with 2.5% fetal calf serum and 15% horse serum (Gibco, UK), at 37 °C in a humidified atmosphere of 95% O_2 and 5% CO_2. Antibiotics (100 UI/ml penicillin and 100 μg/ml streptomycin) were added to growth media. Cells were routinely screened for mycoplasma contamination, and kept in serum-free medium for the 24 h preceding the treatment with pollutants. Cells were cultured into polystyrene plasticware from Greiner Bio-One (Kremsmünster, Austria). As reported elsewhere, no significant release of bisphenol A from polystyrene at 37 °C occurred (Biswanger et al., 2006).

2.3. Gene expression profile

Real Time PCR was used to evaluate the expression of growth hormone (GH), prolactin (PRL), somatostatin receptor type 1–5 (SSTR1-5), ZAC1, AIP, AHR, cytochrome-P 1A1 (CYP1A1), estrogen receptor α (ERα), progesterone receptor (PR) and Forkhead box protein A1 (FOXA1) genes. To assess gene expression profile, GH3 cells were seeded in cell culture flasks (25 cm^2) at a density of 1×10^6 cell/flask, and then treated with 130 pM BZ, 250 pM DEHP, and 100 ng/l PCB for 24, 48 and 72 h.

Total RNA was extracted using TRIzol Reagent (Invitrogen Ltd, Paisley, UK), and 1 μg of the total RNA reverse-transcribed with iScript cDNA Synthesis Kit (BioRad Laboratories, Inc.), following manufacturer's protocol. Specific primers were designed using Beacon Designer 5.0 software; primers sequences are shown in **Supplementary Table 1**.

Real-time PCR was performed using a BioRad MiIQ Detection System (BioRad Laboratories, Inc.) with SYBR green fluorophore. Reactions were performed in 15 μl volume, which included 7.5 μl IQ SYBR Green Supermix (BioRad Laboratories, Inc.), 0.3 μl each primer at 10 μM concentration, 1.90 μl RNAse-free distilled water, and 20 ng/5 μl of the previously reverse-transcribed cDNA template. For each primer set, the reaction was optimized using seven serial 5X dilutions of template cDNA obtained from cells in basal conditions (100, 20, 4, 0.8 and 0.16 ng). A melting curve analysis was performed following each run to ensure a single amplified product for each reaction. All reactions were carried out at least three times for each sample. Every gene expression level was normalized on the expression of three house-keeping genes (β-Actin, L13A and β-2-microglobulin), and expressed as relative expression fold *vs* untreated controls. Before RNA extraction viable cells were counted by Trypan Blue exclusion assay in order to assess absence of cytotoxicity induced by pollutants exposure.

2.4. Western blot

GH3 cells were seeded in cell culture flasks (25 cm^2) at a density of 1×10^6, and treated with 130 pM BZ, 250 pM DEHP, and 100 ng/l PCB. At different times after treatment, cells were scraped from the flask in the presence of 1 ml lysis buffer (containing 50 mM Tris–HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 0.5% sodium deoxycholate, 1% Nonidet P-40, 0.1% SDS, 10 mg/ml PMSF, 30 l/ml aprotinin and 100 mM sodium orthovanadate). Cell lysates were incubated in ice for 30–60 min. At completion, tubes were centrifuged at 4 °C for