Molecular and Cellular Endocrinology 367 (2013) 98–108

Contents lists available at SciVerse ScienceDirect

Molecular and Cellular Endocrinology

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

Peri-conceptional changes in maternal exposure to sewage sludge chemicals disturbs fetal thyroid gland development in sheep

Sabine Hombach-Klonisch [a,b,*], Adrian Danescu [a], Farhana Begum [a], Maria R. Amezaga [c], Stewart M. Rhind [d], Richard M. Sharpe [e], Neil P. Evans [f], Michelle Bellingham [f], Corinne Cotinot [g], Beatrice Mandon-Pepin [g], Paul A. Fowler [c,1], Thomas Klonisch [a,h,i,1]

[a] Dept. of Human Anatomy & Cell Science, University of Manitoba, Winnipeg, Canada
[b] Dept. of Obstetrics, Gynecology & Reproductive Sciences, University of Manitoba, Winnipeg, Canada
[c] Centre for Reproductive Endocrinology and Medicine, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, UK
[d] The James Hutton Institute, Craigiebuckler, Aberdeen, AB15 8QH, UK
[e] MRC Human Reproductive Sciences Unit, The Queen's Medical Research Institute, University of Edinburgh, 47 Little France Crescent, Edinburgh, UK
[f] Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G61 1QH, UK
[g] INRA, UMR 1198, Biologie du Developpement et de la Reproduction, 78350 Jouy-en-Josas, France
[h] Dept. of Medical Microbiology & Infectious Diseases, University of Manitoba, Winnipeg, Canada
[i] Dept. of Surgery, University of Manitoba, Winnipeg, Canada

ARTICLE INFO

Article history:
Received 23 May 2012
Received in revised form 14 November 2012
Accepted 13 December 2012
Available online 4 January 2013

Keywords:
Endocrine disruptors
Thyroid gland
Sheep
Fetal
Sewage sludge
Development

ABSTRACT

Ewes were exposed to sewage sludge-fertilized pastures in a study designed investigate pre-conceptual and/or gestational exposure to environmental chemicals. The in utero impact on fetal thyroid morphology and function at day 110 (of 145) of pregnancy was then determined.

Pre-conceptual exposure increased the relative thyroid organ weights in male fetuses. The number of thyroid follicles in thyroids of fetuses after pre-conceptual or gestational exposure was reduced. This correlated with an increase in Ki67 positive cells. Pre-conceptual exposure to sewage sludge reduced small blood vessels in fetal thyroids. Thyroid tissues of exposed fetuses contained regions where mature angiofollicular units were reduced exhibiting decreased immunostaining for sodium-iodide symporter (NIS). Fetal plasma levels of fT3 and fT4 in exposed animals, however, were not different from controls suggesting compensatory changes in the thyroid gland to maintain homeostasis in exposed fetuses. The regional aberrations in thyroid morphology may impact on the post-natal life of the exposed offspring.

1. Introduction

Multiple organ systems including the adrenal, thyroid, mammary and pituitary glands, gonads and other parts of the reproductive tract can be adversely affected by developmental exposure to environmental chemicals (ECs), including heavy metals and endocrine-disrupting compounds (EDCs) (Woodruff and Walker, 2008). Over 100 naturally occurring and synthetically derived substances, including many EDCs (e.g., halogenated arylhydrocarbons, bisphenols, phthalates and pesticides), are either suspected or known to have thyroid-disrupting properties and their ability to interfere with normal thyroid development, thyroid hormone (TH) levels, and thyroid function has important implications for animal and human health (Boas et al., 2006, 2009; Crofton, 2008; Kohrle, 2008; Zoeller, 2007). The thyroid gland is a major regulator of cellular metabolic programming and an intact hypothalamic-pituitary-thyroid (HPT) axis is essential for normal pre- and post-natal neuronal and reproductive development and functions (Gauger et al., 2007). Prenatal exposure to polychlorinated biphenyls (PCBs) has been associated with poor mental performance and altered motor functions in children and monkeys (Jacobson and Jacobson, 2003; Levin et al., 1988; Tilson et al., 1979; Walkowiak et al., 2001; Wilhelm et al., 2008). Prenatal exposure

Abbreviations: ECs, environmental chemicals; EDCs, endocrine-disrupting compounds; NIS, sodium-iodide symporter; fT3, free triiodothyronine; fT4, free thyroxine; TH, thyroid hormone; TSH, thyroid stimulating hormone; TR, thyroid hormone receptor; TTR, transthyretin; HPT, hypothalamic-pituitary-thyroid axis; PCBs, polychlorinated biphenyls; PBDE, polybrominated diphenyl ether; DEHP, di(2-ethylhexyl) phthalate; CV, coefficient of variation; DAB, 3,3′-diaminobenzidine tetrahydrochloride; HRP, horseradish peroxidase; RT, room temperature; HE, hematoxylin-eosin; GnRH, gonadotropin releasing hormone; GD, gestational day; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

* Corresponding author. Address: Dept. of Human Anatomy and Cell Science, University of Manitoba, 130-745 Bannatyne Avenue, Winnipeg, Manitoba, Canada R3E 0J9. Tel.: +1 204 789 3982; fax: +1 204 789 3920.
E-mail address: hombach@cc.umanitoba.ca (S. Hombach-Klonisch).

[1] These authors contributed equally to this work.

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http://dx.doi.org/10.1016/j.mce.2012.12.022

to PBDEs was shown to be associated with long-lasting hyperactivity, hearing impairment, and impaired learning and memory (Goldey et al., 1995; Kuriyama et al., 2005). The mechanisms through which ECs interact with the HPT axis are complex, occur at various levels, and the effects may be dependent on the developmental state of exposed cells and tissues in this regulatory circuit. ECs have been reported to alter thyroid homeostasis by blocking iodide uptake and enzymatic inhibition of thyroid peroxidase and deiodinase activity in follicular thyrocytes (Dohan et al., 2007; Gauger et al., 2007; Jugan et al., 2010; Mastorakos et al., 2007; Schmutzler et al., 2007). Some ECs have also been reported to cause the selective and competitive displacement of THs from their receptors and the three main TH binding proteins in serum (Brouwer et al., 1998; Hallgren and Darnerud, 2002; Lema et al., 2008; McKinney and Waller, 1994; Schriks et al., 2007). Detailed reviews on the interaction of EDCs with thyroid physiology have been published (Jugan et al., 2010; Schmutzler et al., 2007) (Boas et al., 2012; Kohrle, 2008; Zoeller, 2007).

Work with specific classes of ECs has documented possible thyroid dependent mechanisms through which specific developmental effects might be mediated. For example, PCBs have a structure similar to TH and can bind to TH receptors (Fritsche et al., 2005; McKinney and Waller, 1994). Hydroxylated PCBs were shown to decrease T4 hormone levels by competing with the binding to the TH-binding protein transthyretin (TTR) (Brouwer et al., 1998). Similar in structure to PCBs, the newly emerging polybrominated diphenyl ether (PBDE) flame retardants and para-hydroxylated PBDE metabolites, and isopropylidenediphenol or bisphenol A can also bind to and antagonize TH receptors (Kitamura et al., 2005; Kojima et al., 2009) (Moriyama et al., 2002; Zoeller et al., 2005), as does di(2-ethylhexyl) phthalate (DEHP) (Ishihara et al., 2003).

In addition to direct effects such as those illustrated above, EDCs can also have secondary effects on health as many ECs are able to induce the expression of the hepatic xenobiotic metabolizing sulfotransferases (SULTs) and uridine diphosphate glucuronyltransferases (UDGPTs) (Brouwer et al., 1998; Nishimura et al., 2005), which through the production of reverse-T3 (rT3) from sulfated T4 can influence TH signaling (Mol and Visser, 1985). Such an EC-induced effect could be developmentally important as plasma T3 concentrations in the ovine fetus remain low for most of gestation because T4 is largely metabolized to biologically inactive rT3 and sulphated TH derivatives and placental enzymes inactivate T3 (Polk, 1995). It is only in the latter part of pregnancy when preferential deiodination of T4 to T3 occurs and this results in higher plasma T3 levels in the ovine fetus near term (Forhead et al., 2006; Polk, 1995).

While studies of the effects of individual chemicals can be important mechanistically, environmental exposure is normally to a complex mixture of chemicals. We have been working with an ovine model in which pregnant animals have been exposed to environmental concentrations of a mixture of EDCs through grazing pastures fertilized with human sewage sludge. Sheep provide an ideal model to study the effects of EC exposure on HPT development and function as, like humans, sheep are long-lived and have a relatively long gestation period of 145 days, during which timing and sequence of organogenesis is similar to that seen in humans. Despite the relatively low concentrations of individual chemicals that are found in the environment and tissues collected from animals maintained in this experimental paradigm (Rhind et al., 2005a,b), including concentrations in the tissues of animals used in the current study (Rhind et al., 2009, 2010), we have reported exposure effects on multiple organ systems including the fetal hypothalamus and pituitary (Bellingham et al., 2009, 2010), fetal gonads (Fowler et al., 2008; Paul et al., 2005), adult gonads (Bellingham et al., 2011; Fowler et al., 2012), offspring behavior (Erhard and Rhind, 2004) and adult bone structure (Lind et al., 2009, 2010).

In the present study, we have investigated the effects of preconceptual, gestational, and continuous maternal exposure to sewage sludge on fetal thyroid gland development and the levels of circulating thyroid hormones in the exposed ovine fetus.

2. Materials and methods

2.1. Animals and treatments

The study was approved by the James Hutton Institute's Local Ethical Committee and fully licensed by the United Kingdom's Animals (Scientific Procedures Act 1986) under Project License PPL 60/4028. Animals and exposure regimens to sewage sludge were described earlier (Rhind et al., 2010). Briefly, sheep derived from a single flock of Texel ewes were maintained on pastures treated twice annually with thermally dried digested sewage sludge (2.25 metric tonnes of dry matter per hectare; Treated: T) or with inorganic fertilizer containing equivalent amounts of nitrogen (225 kg per hectare per year; Control: C). Sewage sludge contained numerous EDCs, among them PCBs, phthalates, PAHs, PBDEs, and the analysis was reported previously for a similar batch of sludge (Rhind et al., 2009) and for the soil levels for treated and untreated plots (Rhind et al., 2010). Groups of sheep were exposed to the respective treatments before or after mating or continuously (Fig. 1): exposure of sheep throughout their lives before and after mating (TT) resulting in pre-conceptual and gestational exposure in the fetuses; exposure only until mating, but not thereafter (TC) resulting in exclusively pre-conceptual exposure; exclusively gestational exposure was achieved by exposure between mating and euthanasia (CT). Control animals (CC) were exposed only to pasture treated with inorganic fertilizer, throughout their lives. Pregnant ewes were euthanized at day 110 of gestation, i.e. in the last trimester of pregnancy. Thyroid glands of maternal ewes and fetal lambs were collected within 15 min (min) of euthanasia and their wet weight determined prior to fixation of one lobe in 10% buffered formalin and snap-freezing of the other lobe in liquid nitrogen. The sizes and weights of the fetuses were determined at the time of euthanasia and the thyroid weights expressed relative to the body mass of the fetuses.

2.2. Detection of thyroid hormones and thyrotropin (TSH)

Maternal and fetal blood samples were obtained at euthanasia and plasma was separated and stored at −20 °C. Plasma concentrations of free triiodothyronine (fT3) and free tetraiodothyronine (thyroxine, fT4) were measured with the automated ADVIA Centaur® XP competitive immunoassay system (Siemens Healthcare Diagnostics, Camberley, UK), which uses direct chemilumines-

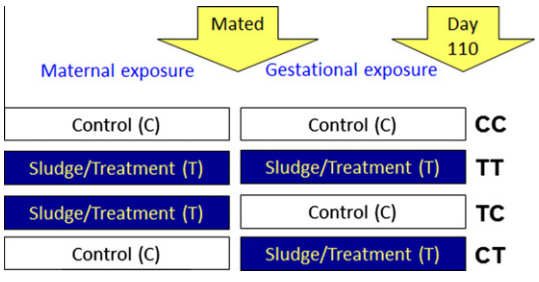

Fig. 1. Schematic illustration of the four treatment groups. Groups of sheep were exposed to sewage sludge fertilized pastures at different stages: throughout life before and after mating (TT); only until mating, but not thereafter (TC); only between mating and euthanasia (CT); controls (CC) were always exposed to pasture treated with inorganic fertilizer. Animals were euthanized at GD 110 (of 145 days to term) of pregnancy.