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Review

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Thomas R. Zoeller

Biology Department and Program in Molecular and Cellular Biology, University of Massachusetts, Amherst, U.S.A.

ABSTRACT

Thyroid hormones (THs) are required for normal brain and somatic development and for the proper regulation of physiology in both children and adults. Thyroid function is controlled by the dynamic interrelationships between the hypothalamus, the pituitary and the thyroid. These dynamic relationships maintain circulating levels of THs within a narrow range under normal conditions. Normally, there is likely to be a tight relationship between changes in circulating levels of THs and changes in TH action in various target tissues. This relationship is maintained by tissue-level mechanisms that include TH metabolism and transport. Environmental chemicals that interfere with TH signaling mechanisms (Endocrine Disrupting Chemicals, EDCs) may produce adverse effects both in the individual and in a population. Because of the complex nature of the regulation of thyroid function and TH action, the consequences of EDC exposure is also likely to be complex and our ability to understand these effects as well as to screen for potential EDCs must consider this complexity. Specifically, if there are chemicals in the environment that directly interfere with TH action through their receptors but do not affect circulating TH levels, they would not be identified as thyroid toxicants by currently applied screening methods or by epidemiological studies. The goal of this review is therefore to identify the issues that must be clearly resolved before effective risk assessment can be performed.

Key words: Thyroid hormones, Endocrine disruptors, Environmental chemicals, Thyroid toxicants

THE HYPOTHALAMIC PITUITARY THYROID AXIS (HPT) AXIS

To develop efficient risk assessment strategies for thyroid toxicants, it is important to understand both the normal functioning of the hypothalamic-pituitary-thyroid axis (HPT) and what we know about individual thyroid toxicants. To this end, background information about the HPT axis and its relevance to thyroid toxicology is reviewed here.

Circulating levels of Thyroid Hormones (THs) are maintained within a relatively narrow range[1] in large part by a negative feedback relationship between circulating levels of THs and those of Thyroid Stimulating Hormone (TSH). THs appear to exert this effect by acting on one subtype of the TH receptor (TR). TRs are encoded by two separate genes, α and β, the primary transcripts of which are alternatively spliced

Address for correspondence:
Dr. R. Thomas Zoeller, Biology Department, University of Massachusetts Amherst, 611 N Pleasant St., Morrill Science Center, Amherst, MA 01003, Tel.: +1 (413) 545-2088, Fax: +1 (413) 545-3243, E-mail: tzoeller@bio.umass.edu

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to generate three major products capable of mediating the response to TH; these transcripts – TRα1, TRβ1, and TRβ2 – exhibit a very selective spatial and temporal pattern of expression in the developing and adult brain.[2-4] Interestingly, TRβ2 is expressed in the pituitary gland[5] and in the hypothalamic Paraventricular Nucleus (PVN),[6] and appears to be the predominant mediator of the negative feedback action of TH on TSH.[7] Thus, if the TRβ2 isoform is solely or predominantly responsible for the negative feedback actions of TH on the hypothalamus and pituitary, then environmental chemicals that interact with TRs will affect this negative feedback system if, and only if, they interact with the TRβ2 isoform.

Finally, although circulating levels of thyroid hormones are generally maintained within a narrow range, the range is far narrower for an individual than for the population,[1,8] and individual genetics is a major contributor defining the set-point around which the HPT axis is regulated.[9] The variability in measures of thyroid hormones should be considered both in calculating the power of studies designed to identify associations between environmental exposures and TH signaling[8] and in interpreting the findings.

The principal pathway of TH clearance from serum is by conjugation to glucuronic acid or sulfate.[10] The Constitutive Androstane Receptor (CAR) and the Pregnane X Receptor (PXR) play central roles in the xenobiotic-induced clearance of T_3 and T_4 and the subsequent ability of environmental chemicals to reduce serum concentrations of these hormones.[11] Both CAR and PXR are nuclear (orphan) receptors that respond to classes of xenobiotic chemicals and induce the expression of phase I-III enzymes involved in the detoxification and elimination of steroids, bile acids and xenobiotics.[12-14] Phase I enzymes (the cytochrome P450) largely hydroxylate hydrophobic chemicals (including xenobiotic chemicals and steroid hormones), which can then be further modified by phase II enzymes such as Glucuronyl Transferases (UDPGTs), Glutathione-S-Transferases (GSTs), and Sulfo Transferases (SULTs). These chemical metabolites are then transported across plasma membranes for elimination by phase III transporters such as the Multidrug Resistance-associated Protein 2 (MRP2) and the Multidrug Resistance Protein 1 (MRP1).

MECHANISMS OF INTERACTIONS OF HPT WITH XENOBIOTICS

Serum half-life of thyroxin (T_4) is also controlled in part by serum binding proteins. Two important elements of serum T_4 in humans are its half-life of 7-10 days and its serum concentration of 100 nM.[15] No other hormone has a half-life this long and few hormones are found in serum at these concentrations. These elements are largely the result of tight noncovalent binding to three principal serum T_4-binding proteins, Thyroxin-Binding Globulin (TBG), Transthyretin (TTR) and albumin. The bound T_4 is in rapid equilibrium with unbound or "free" T_4 that is available for cellular uptake. Because T_4 is more avidly bound to these proteins, it has a much longer half-life than T_3. Adult male rats do not appear to produce TBG,[16] but pregnant females and pups produce high levels of TBG.[17-19] There are chemicals that are well known to displace T_4 from serum binding proteins (e.g., Polychlorinated Biphenyls (PCBs)[20,21] and Polybrominated Diphenyl Ethers (PBDEs).[22-24]

Chemicals that can displace TH from these binding proteins may cause a very rapid decline in serum hormone levels. For example, salicylate can cause a rapid (within 2 minutes) and dramatic reduction in serum T_4 that can be attributed to biliary clearance.[25] This is also true for environmental chemicals such as PCBs.[26] Thus, it may well be the combination of the ability of xenobiotics to displace TH from serum binding proteins and their ability to induce phase I-III enzymes. However, this scenario has not been fully explored.

Recent observations from the Privalsky lab provide valuable information about the regulation of TH action mediated by TRs. This group is identifying somatic mutations of TRs that are associated with human cancers and studying the consequences of these mutations on TR function.[27] Their studies indicate that structural changes associated with mutations in the TR can alter transcriptional regulation and DNA recognition.[27] In addition, mutations in the TR can affect the transcriptional potency of T_3 without affecting the affinity of the TR for T_3.[28] These observations are important because they establish a precedent and a strategy for testing the hypothesis that xenobiotic TR ligands might fit imprecisely into the ligand binding

pocket of the TR – or into an allosteric site – to alter the structure of the TR, causing it to function in ways that are not a simple activation/inhibition but rather a qualitative change, the consequence of which will be difficult to predict.

Role of TH in Development and in Adult Physiology

Thyroid hormone is essential for normal brain development. This statement is supported by decades of study both in experimental animals and in humans,[29] though a detailed analysis of this literature is beyond the scope of the current review. Nevertheless, there are several issues that are important to highlight within the framework of thyroid toxicology and these will be developed here.

Idealized Model of the HPT Axis in Development

A key issue to consider is that the dynamic interactions among hormones and tissues that make up the HPT axis may be altered by exposure to various xenobiotics in ways that are not fully understood. For example, according to an idealized model of the HPT axis, if a xenobiotic chemical causes a decrease in circulating levels of T_4, then serum TSH should increase. However, there are a number of chemicals that cause a decrease in serum total and free T_4 without causing a concomitant increase in serum TSH. One of the best-known examples is that of PCBs. Exposure to the technical mixture Aroclor 1254 causes a significant decrease in serum T_4, but does not cause an increase in serum TSH, while the thyroid gland does not show signs of stimulation such as thyrocyte proliferation.[30,31]

The mechanism by which a toxicant can cause a decrease in the circulating level of T_4 without affecting serum TSH is not clear. Moreover, it may differ between toxicants that induce this effect. This is a poorly studied area of research but is critical because a large number of chemicals produce such effects. Moreover, failure of the HPT axis to respond in an idealized way makes identification of biomarkers with adverse effects more complicated. For example, the U.S. Environmental Protection Agency (EPA) proposes that the effect of chemical exposure on thyroid histopathology is an important endpoint for a testing program designed to identify thyroid toxicants (http://www.epa.gov/endo/pubs/assayvalidation/status.htm). However, this is true only for those thyroid toxicants that affect serum TSH and it seems both unwise and premature to assume that if TSH does not increase, serum T_4 does not decrease.

Compensation

Another critical issue is that of compensation. This concept posits that as thyroid hormone (both T_4 and T_3) levels decline, a number of systemic and tissue-level responses are activated which abrogate or ameliorate the consequences of low TH. This concept is extremely important for risk assessment, but despite its implication, it appears to lack empirical support. We also know that T_4 metabolism and tissue uptake may represent major elements of compensation, especially in the developing brain. We recently addressed this issue directly but failed to identify evidence for functional compensation to low thyroid hormone in the developing rat brain.[32] These findings are consistent with a number of studies indicating that very small changes in serum T_4 can exert adverse effects on brain development.[32] The above observations indicate that we may not fully understand the degree to which "compensation" occurs or the conditions under which it occurs.

Complexity of TRs

There are many factors that we need to understand about the receptors mediating TH action in the developing brain. Clearly, there are studies that support the hypothesis that different TR isoforms mediate different actions of TH.[33-37] Moreover, the different TR isoforms have different responses to T_3[27,28] as well as to different toxicants.[38] This is of great interest because the consequences of thyroid toxicants that interact with TRs in an isoform-specific manner may well produce a mosaic of effects that do not recapitulate the effects of global hypothyroidism (or hyperthyroidism).[39]

CHEMICALS THAT INTERFERE WITH THE HPT AXIS

There is a large number of chemicals that have been shown to affect the HPT axis.[40,41] However, all of these chemicals have been identified as thyroid toxicants because of their ability to reduce circulating levels

of thyroid hormones. Although it is clear that this is an important/key mechanism of thyroid toxicity, it is not the only mechanism and an increasing number of chemicals have been shown to interfere with the TR directly or with enzymes or transporters that play important roles in mediating TH action. These issues are highlighted in the classes of chemicals discussed below.

Polychlorinated Biphenyls (PCBs)

PCBs are a family of industrial compounds consisting of two linked phenyl rings and varying degrees of chlorination, resulting in 209 different "congeners".[42,43] Before their production was banned in the 1970s, over a billion kilograms of PCBs were produced[44] and they are now persistent and ubiquitous environmental contaminants that are routinely found in samples of human and animal tissues.[42,45] PCBs are developmental neurotoxins. Schantz and Rice[46] reviewed this literature for humans prior to 2003 and concluded that, on balance, there was strong evidence indicating that PCB exposure is associated with negative effects on cognitive development. Interestingly, many different neuropsychological strategies have been used to identify effects of PCB exposure on cognitive function, providing additional strength for this conclusion. Several studies published since 2003 provide additional support for the conclusion that PCB are neurotoxic. For example, Stewart et al[47] reported that cord blood PCBs were significant predictors of deficits in McCarthy performance at 38 months of age in children enrolled in the Oswego Newborn and Infant Development Project. These relationships were however not observed at 54 months, indicating that functional compensation may occur. In a later study, Stewart et al[48] reported a significant negative association between prenatal PCB exposure (cord blood PCBs) and both full scale and verbal IQ in children 9 years old. In addition, this group found that the size of the corpus callosum on MRI images was a significant predictor of the strength of the association between PCB exposure and response inhibition[49] in children aged 4 years and that this effect was retained in these children at age 9 years.[50]

Hertz-Picciotto et al[51] identified a different kind of potential effect of PCB exposure – the negative association between measures of prenatal PCB exposure and thymus size in the infant. This observation is important both because there may be a link between the neurodevelopmental effects of PCB exposure and effects on immune development, but also because of considerable potential consequences of immune dysfunction. Finally, this group also identified a unique negative association between a specific hydroxylated PCB metabolite (4-OH-PCB 107) and Mental Developmental Index (MDI) in children at 16 months of age in eastern Slovakia.[52]

An active area of investigation has been on the mechanism(s) by which PCBs may produce neurodevelopmental effects. One mechanism proposed early was that PCBs could interfere with the thyroid system.[53] Studies of the relationship between PCB body burden and thyroid hormone levels in human populations have produced variable results. This issue has been extensively reviewed previously[54,55] and will not be repeated here. However, there are several issues that are important to highlight and update. An early observation indicated that people highly contaminated with PCBs that occurred in rice oil ("Yusho" disease) exhibited *elevated* T_4 and T_3 with normal levels of TSH when measured over a decade after the accident.[56] Thus, an industrial accident provides evidence in humans that high levels of PCB exposure are associated with measures of altered thyroid function. Nonetheless, the findings are not consistent with work in animals, which nearly uniformly confirm that PCB exposure causes a reduction in circulating levels of thyroid hormone. An interesting and potentially significant paradox was reported by Goldey et al,[57] who found that exposure to the technical mixture Aroclor 1254 caused a reduction in serum total and free T_4 but did not affect serum TSH. This issue was further developed by Klaassen,[30] who showed that not only is TSH not raised by A1254 but that the thyroid gland did not exhibit histological features of stimulation by TSH.

Salay and Garabrant[58] recently reviewed the complexity of this issue and concluded that, among studies they deem strongest, there tends to be an inverse relationship between serum T_3 and T_4, but not for free T_4 or TSH. There is a notable additional observation recently reported. Specifically, birth delivery mode (i.e., vaginal versus cesarean) modified the association between prenatal exposure to PCBs and neonatal

thyroid hormone measures.[59] This is an important observation indicating that a number of biological sources of variation may exist that serve to obscure vital relationships between toxicant exposure and thyroid hormone levels.

Because PCB effects on circulating levels of TH may not accurately reflect an effect on TH action itself, we initially pursued the hypothesis that PCBs interfere with TH action in the developing rodent brain producing a state of relative hypothyroidism. Surprisingly, we found that A1254 reduced circulating levels of T_4 to below the detection limit for the radioimmunoassay, yet the TH -responsive genes RC3/Neurogranin and Myelin Basic Protein (MBP) were up-regulated as if T_4 levels were increased.[60] To pursue this hypothesis, we developed a mixture of 6 PCB congeners.[119] In pregnant rats, this mixture reproduced the effect of A1254 in that it significantly decreased serum T_4 levels but increased the expression of a TH-responsive gene (malic enzyme). Moreover, we showed that this mixture could enhance transcription through a specific TH response element (a DR4), which minimally required the combination of PCBs 105, 118, and 126. Finally, using pharmacological approaches, we showed that the ability of PCBs to increase TR activity required CYP1A1, indicating that parent PCBs must be hydroxylated to form TR agonists. Interestingly, the PCB metabolite predicted to be formed by CYP1A1 activity is 4-OH-PCB107, which was uniquely associated with Mental Development Index (MDI) by Park et al.[120]

Polybrominated Diphenyl Ethers (PBDEs)

Polybrominated diphenyl ethers are primarily employed as flame retardants in consumer products to delay ignition and burning of materials. Because they are added to the materials rather than chemically bound, they can leach from the materials into the environment over time and use.[62] Because of their lipophilicity, PBDEs bioaccumulate and levels of PBDEs in human tissues have exhibited significant increases over the past decade,[63] including serum,[64] milk[65-69] and cord blood.[59,62] Interestingly, PBDE body burden of North Americans is the highest in the world[70] with toddlers being exposed to nearly 10 times as much as adults.[69] Thus, it is clear that PBDEs have made their way into the human population, that PBDE levels are rising, this indicating continual exposures, and that PBDEs have gained access to human development during fetal[71] and neonatal life.

Because of the ability of PBDEs to bioaccumulate and because blood levels are increasing, especially in children, it is essential to evaluate their endocrine toxicity. Moreover, their structure – with two halogenated phenyl rings attached by an ether link – is considerably more similar to thyroid hormone than are other known thyroid disruptors like PCBs. Hence, a number of studies have recently evaluated the ability of PBDEs to disrupt thyroid function both in animals and in humans. In rodents, PBDE exposure has been repeatedly found to decrease serum total and free T_4 and increase serum TSH.[72-77] These observations are dependent to some extent upon the specific PBDE mixture employed in the study, with the lower brominated compounds appearing more potent in reducing serum thyroid hormone levels. Stoker et al[75] and Szabo et al[72] investigated some of the potential mechanisms by which PBDE exposure may lead to a reduction in circulating levels of thyroid hormone. These studies show that a combination of changes in serum T_4 binding, T_4 conjugation and elimination, and changes in T_4 metabolism by deiodination may all be involved in the pathway by which PBDE exposure may reduce circulating levels of thyroid hormone.

Fewer studies have evaluated the relationship between PBDE exposure and thyroid status in humans. Early work did not reveal a relationship between PBDE body burden and thyroid hormone levels in human serum.[71,78] These two small studies focused on mother-infant pairs in central Indiana (USA)[71] and occupational exposure in an electrical recycling plant in Sweden.[78] More recently, Herbstman et al[59] reported on a larger study of mother-infant pairs. This study was enhanced by their background work indicating that delivery mode affects thyroid hormone levels in the infant.[79] Thus, using this information, they then found that PBDE levels in cord blood at birth were negatively associated with both total and free T_4 in those babies born by vaginal delivery. In contrast, the stress associated with conditions leading to a cesarean delivery apparently were such that they masked this relationship. This observation is critical first because it indicates that PBDE body burden may produce a stressor on the thyroid system during development and

second because it indicates that our ability to identify these relationships is dependent upon eliminating variables that can mask this relationship.

Interestingly, several animal studies revealed that PBDE exposure could lower circulating levels of vitamin A.[77,80] This is noteworthy because retinoic acid signaling is a potentially important co-regulator of gene expression with thyroid hormone signaling.[81] It is therefore essential to ascertain whether PBDE exposure can potentially reduce the circulating levels of both thyroid hormone and vitamin A. Moreover, the condition of marginal vitamin A deficiency renders rodents significantly more sensitive to the TH-lowering effect of PBDEs.[82] These novel studies indicate that PBDE exposure may exert independent effects on circulating levels of thyroid hormone and vitamin A, but that the cross-talk between these two signaling systems may be a target of PBDE action.

Limited data also indicate that some PBDEs, or their hydroxylated metabolites, can interact directly with thyroid hormone receptors. Marsh et al[83] reported that 4'-hydroxy-1,3,3',5-tetrabromodiphenyl ether could bind to the beta isoform of the TR (TRβ1) with an affinity about 150-fold lower than that of T_3. In addition, this hydroxylated PBDE exhibited a 40-fold lower affinity for TRα1 than for TRβ1. Kojiba et al[84] have evaluated the ability of a different set of PBDEs and their metabolites to affect the thyroid hormone receptor. In this case, they evaluated the ability of these compounds to affect TR function in transcription assays using CHO-K1 cells and a palindromic TH response element. They found that 4-OH-BDE90 exerted a significant antagonistic effect on TRα1 and TRβ1 at 10^{-5}M. There were no apparent differences between the effects on TRα1 or TRβ1.

Taken together, these studies indicate that PBDEs are thyroid toxicants that clearly reduce measures of thyroid function in the animal system and for which there is credible evidence for an effect in humans. Moreover, specific PBDE congeners and/or metabolites may affect thyroid hormone binding to its receptor or receptor function directly.

Perchlorate

Perchlorate is a powerful oxidant used as a rocket propellant, in ordinance, fireworks, airbag deployment systems, and others.[85] Because of the environmental stability and prevalence of use of perchlorate, it has become a widespread contaminant in drinking and irrigation waters and in food,[86] such that perchlorate contamination is nearly ubiquitous in the US population.[87] Experimental studies in humans indicate that the serum half-life of perchlorate is about 8 hours and that an exposure level of about 5.2 μg/kg/day is sufficient to begin to reduce iodide uptake into the thyroid gland.[88] Because the adult thyroid gland stores a great deal of hormone in the form of iodinated thyroglobulin, it was surprising that Blount et al[89] found that urinary perchlorate levels were associated with serum TSH in the general population of women (not in men). It is perhaps not surprising that this association was greater in women with urinary iodine below 100 μg/L, and stronger still among these women who smoke,[90] since cigarettes contain thiocyanates that also inhibit iodine uptake. Because infants are particularly vulnerable to thyroid hormone insufficiency[29] and because perchlorate levels are particularly high in breast milk,[91] it is of concern that perchlorate may be affecting TH signaling in early infant development in some proportion of the US population.[92]

Predicting the consequences of environmental perchlorate exposure on thyroid function and thyroid hormone action reveals several serious weaknesses in our understanding of the thyroid system as we employ this information for risk assessment. Perhaps most importantly, it remains enigmatic that the high-dose short-term human exposure studies of Greer et al[88] and Lawrence et al[93,94] failed to predict the association of low-dose long-term perchlorate exposure reported by Blount et al[89] and Steinmaus et al.[90] The mechanism of perchlorate action on the thyroid system is relatively simple and is believed to be mediated solely by inhibiting iodide uptake into the thyroid gland.[95,96] As a result, perchlorate is expected to interfere with thyroid function by reducing iodide uptake and, ultimately, thyroid hormone synthesis. Thus, perhaps the single most important lesson to be learned from the study of perchlorate is why early studies did not allow us to identify potential adverse effects of low-dose chronic exposures.

Bisphenol-A

Bisphenol-A (BPA, 4,40-isopropylidenediphenol)

is one of the highest volume chemicals produced worldwide. Over 6 billion pounds are produced each year and over 100 tons are released into the atmosphere by yearly production.[104] This chemical is the primary building block of polycarbonate plastic, but is also found in a large number of consumer products, epoxy resins that coat food cans, and in dental sealants.[105,106] Howe et al[106] estimated human consumption of BPA from epoxy-lined food cans alone to be about 6.6 mg/person/day. Several recent reviews have comprehensively discussed the known sources and human exposures to BPA[107-110] and will not be further explored here. However, it is important to note that BPA is found in serum of pregnant women, in the amniotic fluid of their fetus, in placenta, and in cord serum taken at birth.[111,112] Finally, it is of considerable interest to highlight a recent study that found a longer than expected retention time for BPA in humans with substantial non-food sources of exposure.[108] It is thus clear that the human population is ubiquitously exposed to BPA through sources that are incompletely understood.

BPA is also halogenated (brominated or chlorinated) to produce flame retardants. Tetrabromobisphenol-A (TBBPA) is the most commonly used, with over 60,000 tons produced annually.[113,114] Thomsen et al[115] have recently reported that brominated flame retardants, including TBBPA, increased in human serum from 1977 to 1999 with concentrations in adults ranging from 0.4 to 3.3 ng/g serum lipids, while infants (0-4 years) exhibited serum concentrations that ranged from 1.6 to 3.5 times higher.

Of potential significance is the fact that BPA was shown to bind to the TR acting as an antagonist on gene expression.[116] Best characterized as a weak estrogen binding to the estrogen receptor with a Ki of approximately 10^{-5}M,[117,118] BPA binds to and antagonizes T_3 activation of the TR with a Ki of approximately 10^{-4}M, but as little as 10^{-6}M BPA significantly inhibits TR-mediated gene activation. Moreover, Moriyama et al[116] found that BPA reduced T_3-mediated gene expression in culture by enhancing the interaction of the TR with the corepressor N-CoR. Interestingly, Kitamura reported that tetrachloro- and tetrabromobisphenol-A can bind to the TR with a higher affinity than does parent BPA, and that they act as an agonist on the TR when the rat pituitary cell line GH3 growth is used as an index of thyroid hormone action.[119] These early studies indicated that BPA could exert a direct action on thyroid hormone signaling through the TR, which complicates our ability to predict the outcome of BPA exposure on thyroid hormone-regulated developmental or physiological endpoints.

These issues have become further complicated by recent in vitro studies showing that BPA is an antithyroid agent in the absence of a canonical Thyroid Response Element (TRE). In general, BPA is identified as an antagonist and the halogenated forms as an agonist, but this is not uniformly observed. Although the active dose of these compounds typically is reported to be in the micromolar range, some effects were reported in the sub-nanomolar range.

Considering these findings *in vitro*, it is not surprising that actions of BPA on thyroid hormone signaling have also been reported in *in vivo* assays. Iwamuro[129] first reported that BPA could block thyroid hormone-induced tail resorption during *Xenopus* metamorphosis. This report was followed by a study using primary mouse Oligodendrocyte Precursor Cells (OPCs) finding that 10^{-5}M BPA could inhibit T_3-induced specification of oligodendrocytes.[130] Our lab then found that developmental exposure to BPA in rats produces an endocrine profile similar to that observed in thyroid resistance syndrome.[131] Specifically, T_4 levels were elevated during development in the pups of BPA-treated animals, but TSH levels were not different from controls. This profile is consistent with BPA inhibition of TRβ-mediated negative feedback. By contrast, the TH-response gene RC3 was elevated in the dentate gyrus of these BPA-treated animals.[131] Because the TRα isoform is expressed in the dentate gyrus, we concluded that BPA may be a selective TRβ antagonist *in vivo*. These observations were partly confirmed by Xu et al,[132] who reported that BPA exposure increased serum free T_4 in dams and in pups prior to P21. However, they did not observe RC3 expression alteration in the hippocampus. Given that SRC-1 appears to be up-regulated by thyroid hormone in these regions of the hippocampus,[133] the findings of Xu et al may indicate either that the BPA-induced increase in free T_4 is responsible for increasing SRC-1 expression or that BPA is having this effect directly. Because of the consistent finding that BPA is a TR

agonist, it seems likely that the former interpretation has the greatest support.

If BPA is a selective TRβ antagonist *in vivo*, it is possible that it could produce effects similar to thyroid resistance syndrome in which the TRβ receptor is defective leading to elevated serum T_4 and either normal or slightly elevated TSH.[134] In this regard, it is potentially important to note that there may be an association between thyroid resistance and attention-deficit hyperactivity disorder (ADHD) in humans[101,135,136] and in rats.[137] Therefore, it is possibly significant that BPA-exposed rats exhibit ADHD-like symptoms.[138] In addition, BPA exposure alters neocortical histogenesis in the mouse.[139,140] Although no specific link was made to TH action in this study, the findings are consistent with the hypothesis that BPA alters early development of the cortex by interfering with TH signaling.

These experimental studies provide strong evidence that BPA can interfere with TH signaling both *in vitro* and *in vivo*. However, because of the complex nature of the interaction of BPA (and halogenated derivatives) with the TH receptor, it is likely to be difficult to identify thyroid disrupting effects of BPA in humans. Despite this, Sugiura-Ogasawara et al[141] found that BPA exposure was associated with recurrent miscarriage, and early fetal loss associated with both elevated[142] and reduced[143] serum T_4. Thus, considering the widespread exposure of the human population to BPA, it will be of considerable interest to explore the potential relationship between BPA exposure and thyroid disruption.

SUMMARY AND CONCLUSIONS

Animal studies are revealing both the complexity of the thyroid system and the complexity of the ways in which EDCs may interfere with TH signaling. A significant conclusion that needs to be drawn from these studies is that the current clinical strategy of evaluating thyroid disease (i.e. measure blood levels of hormones, antibodies and proteins) is not sufficient to identify EDC actions on thyroid hormone signaling that may well be associated with disease in the human population. Moreover – and less defensible – screening and testing strategies to identify thyroid toxicants focus on an extremely limited number of endpoints.[144] Clearly recent studies of EDC actions on the thyroid system need to be taken into account in both clinical and regulatory studies.

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