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Review article

Thyroid hormone- and estrogen receptor interactions with natural ligands and endocrine disruptors in the cerebellum

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ABSTRACT

Although the effects of phytoestrogens on brain function is widely unknown, they are often regarded as "natural" and thus as harmless. However, the effects of phytoestrogens or environmental pollutants on brain function is underestimated. Estrogen (17beta-estradiol, E2) and thyroid hormones (THs) play pivotal roles in brain development. In the mature brain, these hormones regulate metabolism on cellular and organismal levels. Thus, E2 and THs do not only regulate the energy metabolism of the entire organism, but simultaneously also regulate important homeostatic parameters of neurons and glia in the CNS. It is, therefore, obvious that the mechanisms through which these hormones exert their effects are pleiotropic and include both intra- and intercellular actions. These hormonal mechanisms are versatile, and the experimental investigation of simultaneous hormone-induced mechanisms is technically challenging. In addition, the normal physiological settings of metabolic parameters depend on a plethora of interactions of the steroid hormones. In this review, we discuss conceptual and experimental aspects of the gonadal and thyroid hormones as they relate to *in vitro* models of the cerebellum.

1. Introduction

Numerous studies provided evidence for the role of 17β-estradiol (estrogen, E2) (Ikeda, 2008; Fan et al., 2010) and thyroid hormones (THs), i.e., triiodothyronine, thyroxine (T3 and T4) (Koibuchi, 2008; Horn and Heuer, 2010) in the regulation of normal cerebellar development. Estrogen, as a traditionally known female reproductive hormone and THs, best known as regulators in energy homeostasis, are unique in that they play key roles in the regulation of several other physiological processes as well. Such E2-TH regulated processes are neuronal/glial maturation (also involved in other somatic cells), cell migration (Kirby et al., 2004; Belcher et al., 2009), and the regulation of the intracellular metabolism, latter which significantly affects most intracellular events on its own. The listed hormonal regulatory effects are mediated by at least three known major mechanisms: 1. Specific (cognate) receptors (E2-, TH receptors, ERs and TRs) that function as transcription factors when activated by bound hormone ligands (generally considered as genomic effects) (Ikeda, 2008; Fan et al., 2010; Jakab et al., 2001; Belcher and Zsarnovszky, 2001); 2. Putative plasma membrane-bound/incorporated ligand-receptor complexes that

Abbreviations: AgRP, agouti-related protein; AMPK, adenosin monophosphate activated protein kinase; AN, arcuate nucleus; As, arsenic; AraC, cytosine β-D-arabinofuranoside; BDNF, brain derived neurotrophic factor; BPA, bisphenol A; Ca, calcium; cDNA, complementary deoxyribonucleic acid; CNS, central nervous system; D1-3, deiodinase type I-III; db, genetically diabetic mouse; DNA, deoxyribonucleic acid; E2, 17β-estradiol; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol tetraacetic acid; ER, estrogen receptor; GDX, gonadectomy; GFAP, glial fibrillary acidic protein; Glia+, Glia containing; Glia−, Glia reduced; GLUT, glucose transporter; GnRH, gonadotropin releasing hormone; HRE, hormone response element; IGF, insulin-like growth factor; IgG, immunoglobulin G; LHN, lateral hypothalamic nucleus; MAPK, mitogen-activated protein kinase; MBC, 4-methylbenzylidene camphor; ME, median eminence; mRNA, messenger ribonucleic acid; NEB, negative energy balance; NPY, neuropeptide Y; ntC, non-treated controls; OT, oxytocin; P0-14, postnatal day 0-14; PMSF, phenylmethanesulfonylfluoride or phenylmethylsulfonyl fluoride; POMC, pro-opiomelanocortin; PPE, preproenkephalin; PVN, paraventricular nucleus; qPCR, quantitative Polymerase chain reaction; rT3, reverse triiodothyronine; SCN, suprachiasmatic nucleus; SON, suptraoptic nucleus; STAT, signal transducer and activator of transcription; T2, diiodothyronine; T3, triiodothyronine; T4, thyroxine; TG, thyroid gland; TH, thyroid hormone; TR, thyroid hormone receptor; TRH, thyreotrop releasing hormone; TSH, thyroid-stimulating hormone; UCP2, uncoupling protein 2; VMH, ventromedial hypothalamus; VMN, ventromedial nucleus

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activate rapid, non-genomic intracellular signaling cascades (e.g., Pekary et al., 2006; Belcher, 2008; Leonard, 2008); and 3. Crosstalk on multiple levels of genomic and/or non-genomic E2- and TH-activated intracellular signaling pathways (Vasudevan et al., 2001a; Zhao et al., 2005), where hormone effects are evident but the exact role of the ligand alone or ligand-receptor complex is not yet clarified. Thus, the numerous trophic effects of E2 and THs that are mediated by ERα,β and TRα,β, are the result of the two hormone's interactive effects on the expression level of each-other's receptors, thereby modulating intracellular mechanisms that depend on the receptor's signal-mediating functions. ERs (Shughrue et al., 1997) and TRs (Murray et al., 1988; Hodin et al., 1989) are widespread in the brain, however, their expression level depends on the brain region, age (Bernal, 2007; Al-Bader et al., 2008) and functional-hormonal status of the organism. Thus, it is not known, how ER-TR receptor expression levels correlate with real-time hormonal conditions and to what extent ER-TR gene transcriptional activity correlates to ER-TR protein synthesis in the developing cerebellum.

Environmental estrogens are a large and structurally diverse group of compounds that can mimic, and in some cases antagonize the effects of endogenous estrogens and THs, and they are therefore often referred to as endocrine disruptors (EDs). As a result of their estrogen-like activities and the potential for some to block the normal actions of endogenous E2, there is currently much debate within the scientific community, and also considerable interest within the general public, regarding the relative benefits or threats associated with exposure to environmental estrogens and endocrine disruptors in general. Although most of the EDs mentioned herein are referred to as estrogenic compounds, it has recently been generally accepted that many of them can also disrupt thyroid actions as well.

Compounds characterized as having estrogenic or thyroid properties are typically divided into three general categories, the xenoestrogens, the phytoestrogens and the mycoestrogens. Xenoestrogens are a diverse group of synthetic compounds that include pesticides, the widespread industrial pollutants poly-chlorinated biphenyls; bisphenol-A, the synthetic estrogen, diethylstilbesterol; and many others. As a result of their negative actions on reproductive tissues, xenoestrogens are a potential threat to wildlife and human populations and are therefore the subject of much active research.

The phytoestrogens and mycoestrogens are a group of naturally occurring compounds with estrogenic (and thyroid) activity that are present in plants or that arise from bacterial or fungal metabolism of plant precursor compounds. To varying degrees, phytoestrogens (and mycoestrogens) can also act as agonists or antagonists of the normal actions of E2, and in adults they may have protective effects against certain forms of cancer, cardiovascular disease, and osteoporosis and may also prevent undesirable menopausal symptoms (Bingham et al., 1998). As a result of these potentially beneficial effects, phytoestrogens, especially soy isoflavones have increasingly gained widespread acceptance as safe and beneficial dietary components and as a "natural" alternative to estrogen-based hormone replacement therapies. This increased use of phytoestrogens has occurred even though their mechanisms of action and their effects (either positive or negative) on the developing and mature brain are not well understood. It is also of interest to note that the ready acceptance of the safety and the benefits associated with exposures to increased concentrations of the "natural" estrogenic compounds by the general public and the medical community is in sharp contrast to the common (and potentially accurate) perception that the actions of xenoestrogens are a threat to the health and well-being of human and wildlife populations.

Some phytoestrogens have obvious structural similarity with E2 and are typically considered to act as E2 mimetics; however, many compounds characterized as having estrogen-like properties have few obvious structural similarities to E2 (this is especially true with regard to xenoestrogens and also arsenic, for example). It is also likely that some estrogenic compounds act through mechanisms unrelated to those through which E2 mediates its effects. For example, numerous studies indicate that changes in the expression or activity of E2-metabolizing enzymes or changes in the levels of E2-binding serum proteins can influence the rate of E2 metabolism resulting in alterations in the availability of free E2 at target tissues (Rosselli et al., 2000). This structural and mechanistic diversity of different estrogenic compounds suggests that E2 normally influences multiple mechanisms (i.e., genomic and rapid nongenomic signaling mechanisms) whose sum-effects result in a cell-type specific E2 responsive phenotype.

EDs, such as phytoestrogens or environmental pollutants (also some mycotoxins, such as zearalenone) are selective ER and/or TR modulators and can act as agonists or antagonists of the hormones in question. During development, EDs can influence normal hormonal homeostasis and lead to immediate and/or life-long consequences (e.g., Zsarnovszky et al., 2007; Miodovnik et al., 2014). Here, we discuss effects of some frequently studied EDs, specifically bisphenol A (BPA), zearalenone (Zea), arsenic (As) and methyl-benzilidene-camphor (MBC) on TRα,β and ERβ in cells seeded into cell cultures and obtained from the cerebella of newborn rat pups. It should be noted at this point that this frequently deployed *in vitro* experimental system carries some advantages that make the primary cerebellar cell culture an excellent choice for the investigation of neurocellular effects: the absence of aromatase activity and serum-free medium allows full control over experimental estrogenic (or other steroidogenic) effects; the expression of both ERs and TRs allows for the investigation of substrate effects on these receptors, although effects on ERα cannot be reliably considered due to its involvement in brain reparation processes; inhibition of the growth of glia in this experimental system allows for the investigation of glial effects. Thus, results from this experimental model may not only apply for the cerebellum, but can also be suggestive to other basic celular processes in the CNS.

1.1. Bisphenol A (BPA)

BPA is produced in large amounts for use as a monomer in the production of polycarbonate plastics and epoxide resins that are used as coatings for food cans and plastic packaging, dental sealants, and water pipes. As a result, there is extensive human exposure to BPA that has been estimated to range from 2 to 20 μg/kg/day (vom Saal et al., 1998). During pregnancy, BPA is detectable in maternal (0.3–18.9 ng/ml) and fetal (0.2–9.2 ng/ml) serum, demonstrating ready passage of BPA through the placenta (Schonfelder et al., 2002). Compared with other tissues, BPA is concentrated approximately 5-fold in amniotic fluid during early pregnancy, further indicating significant fetal exposure during important periods of human development (Ikezuki et al., 2002). The levels of BPA to which adult and fetal humans are exposed negatively impacts reproductive function, neurodevelopment, and hippocampal synapse formation in rodents (Palanza et al., 2002; Takai et al., 2000, 2001; Honma et al., 2002; MacLusky et al., 2005).

With regard to ED effects in the cerebellum, it was previously shown that BPA can rapidly activate ERK1/2 in primary cerebellar granule cell cultures (Wong et al., 2003) and also, after injection of BPA into the cerebella of newborn rat pups (Zsarnovszky et al., 2005). These effects were dose-dependent, with a U-shaped dose-response curve, which could indicate compound actions of BPA. In support of these findings, Mathisen et al. (2013) described that perinatal BPA exposure increased Pax6 (transcription factor playing a role in granule cell development and migration) in newborn mice cerebella and in cerebellar cell cultures. In the hippocampus, BPA modulated dendritic morphogenesis via effects on ER. Likewise, BPA also promoted dendritic growth in maturing cerebellar Purkinje cells (Shikimi et al., 2004). This is consonant with previous results from our laboratory (Wong et al., 2003; Zsarnovszky et al., 2005).

In addition to interactions between BPA and ERs, BPA can alter thyroid-specific gene expression (Gentilcore et al., 2013) and functions (Iwamuro et al., 2006; Delfosse et al., 2014). Our studies indicated that