Neuroscience 346 (2017) 203–215

EARLY POSTNATAL GENISTEIN ADMINISTRATION PERMANENTLY AFFECTS NITRERGIC AND VASOPRESSINERGIC SYSTEMS IN A SEX-SPECIFIC WAY

G. PONTI, [a,b]* **A. RODRIGUEZ-GOMEZ,** [b,c] **A. FARINETTI,** [b,c] **M. MARRAUDINO,** [b,c] **F. FILICE,** [c†] **B. FOGLIO,** [b,c] **G. SCIACCA,** [b,c] **G. C. PANZICA** [b,c] **AND S. GOTTI** [b,c]

[a] *Department of Veterinary Sciences, Largo Braccini 2, 10095 Grugliasco (TO), University of Torino, Torino, Italy*

[b] *Neuroscience Institute Cavalieri Ottolenghi (NICO), Regione Gonzole, 10 – 10043 Orbassano (TO), Torino, Italy*

[c] *Laboratory of Neuroendocrinology, Department of Neuroscience, Via Cherasco 15, 10126-University of Torino, Torino, Italy*

Abstract—Genistein (GEN) is a natural xenoestrogen (isoflavonoid) that may interfere with the development of estrogen-sensitive neural circuits. Due to the large and increasing use of soy-based formulas for babies (characterized by a high content of GEN), there are some concerns that this could result in an impairment of some estrogen-sensitive neural circuits and behaviors. In a previous study, we demonstrated that its oral administration to female mice during late pregnancy and early lactation induced a significant decrease of nitric oxide synthase-positive cells in the amygdala of their male offspring. In the present study, we have used a different experimental protocol mimicking, in mice, the direct precocious exposure to GEN. Mice pups of both sexes were fed either with oil, estradiol or GEN from birth to postnatal day 8. Nitric oxide synthase and vasopressin neural systems were analyzed in adult mice. Interestingly, we observed that GEN effect was time specific (when compared to our previous study), sex specific, and not always comparable to the effects of estradiol. This last observation suggests that GEN may act through different intracellular pathways. Present results indicate that the effect of natural xenoestrogens on the development of the brain may be highly variable: a plethora of neuronal circuits may be affected depending on sex, time of exposure, intracellular pathway involved, and target cells. This raises concern on the possible long-term effects of the use of soy-based formulas for babies, which may be currently underestimated.

Key words: phytoestrogens, endocrine disruptors, sexual dimorphism, hypothalamus, limbic system.

INTRODUCTION

Many brain regions, including amygdala, hypothalamus and the bed nucleus of the stria terminalis are sexually dimorphic (for a recent review see McCarthy, 2015). Sex hormones, 17-β-estradiol (E_2) and testosterone, have important roles in their normal development (for recent reviews see Ball et al., 2014; Panzica and Melcangi, 2016), whereas several endocrine disruptor compounds (EDCs), at least those classified as xenoestrogens or xenoandrogens, may affect their normal development (Panzica et al., 2011). The presence of EDCs in the environment increased in the last 50 years and only recently their effects were under scientific evaluation and legislative regulation (Bourguignon et al., 2016; Kortenkamp et al., 2016; Slama et al., 2016). Many studies indicate that EDC stimulation, during peri- and post-natal critical periods, may interfere with the formation of neuronal networks in a sex specific manner either binding or biasing the turnover of estrogen receptors (for a reviews see Panzica et al., 2007; Gore and Dickerson, 2012). Furthermore, blood–brain barrier may be more permeable to EDCs than to estrogens in the perinatal period (Doerge et al., 2001). During this age, long-lasting effects may be caused by a much lower dose than the one considered not toxic by laws that, in general take into account only acute toxic effects on adults (Richter et al., 2007).

The pre- or/and peri-natal exposure to EDCs may lead to permanent neuroanatomical changes in adults (Panzica et al., 2011) in particular in neuronal Nitric Oxide Synthase (nNOS) expression in bed nucleus of the stria terminalis and medial preoptic nucleus (MPOA) (Gotti et al., 2010; Martini et al., 2010) and in amygdala (Rodriguez-Gomez et al., 2014) that may affect aggressive (Wisniewski et al., 2005), anxiety-related

*Correspondence to: G. Ponti, Department of Veterinary Sciences, Largo Braccini 2, 10095 Grugliasco (TO), University of Turin, Turin, Italy. Fax: +39-011-6709138.
E-mail address: giovanna.ponti@unito.it (G. Ponti).
† Department of Medicine, Anatomy Unit, University of Fribourg, 1700 Fribourg, Switzerland.

Abbreviations: ARC, arcuate nucleus; AVP, arginin-vasopressin; BLA, basolateral amygdala; BPA, Bisphenol A; BSTmv, ventromedial part of the bed nucleus of the stria terminalis; E_2, 17-β-estradiol; EDC, endocrine disruptors compound; GEN, genistein; La, lateral amygdala; LS, lateral septum; MPOA, medial preoptic nucleus; NO, nitric oxide; nNOS, neuronal isoform of Nitric oxide synthase; PaAP, anterior parvicellular part of the paraventricular nucleus; PaDC, paraventricular dorsal cap; PaLM, lateral magnocellular part of the paraventricular nucleus; PaMM, paraventricular medial magnocellular; PaMP, paraventricular medial parvicellular; PaV, ventral part of the paraventricular nucleus; PBS, phosphate saline buffer; ROI, region of interest; SCN, suprachiasmatic nucleus; VMHvl, ventromedial hypothalamus.

http://dx.doi.org/10.1016/j.neuroscience.2017.01.024
0306-4522/

(Rodriguez-Gomez et al., 2014) and sexual behaviors (Hull and Dominguez, 2006).

Among EDCs, phytoestrogens are a class of compounds widely distributed in the plant kingdom potentially able to influence all processes regulated by estrogens (Wang et al., 2002). Genistein (GEN) is a phytoestrogen of the group of isoflavones, highly present in soy (Mazur, 1998). GEN is considered a potential EDC due to the fact that is able to interact with the neural pathways related to estrogens in a complex and multidirectional manner. In particular, the exposure to GEN during developmental critical periods could have several long-term consequences on the nervous system of male and female higher vertebrates, as well as on related behaviors (Jefferson et al., 2012; Rodriguez-Gomez et al., 2014). In recent years there has been an ever-increasing consumption of soy because of its easy cultivation, and its high protein levels. Moreover it is a substitute for dairy products for the feeding during childhood; this has caused a significant increase in exposure to phytoestrogens.

EDCs may interfere with the expression of many neuropeptides and enzymes (Panzica et al., 2011). Therefore, we focused here our morphological analysis on the nitrergic and vasopressinergic circuits. Both systems are regulated by gonadal hormones' signaling in adulthood [arginin-vasopressin, AVP, (Nomura et al., 2002; Grassi et al., 2010; Piet et al., 2015); nitric oxide, NO, (Scordalakes et al., 2002; Grassi et al., 2013b,c, 2017)]. In addition they are sexually dimorphic in several regions (AVP, De Vries and Panzica, 2006; NO, Panzica et al., 2006), and are sensitive to hormonal fluctuation during the estrous cycle (NO, Gotti et al., 2009; Sica et al., 2009; Martini et al., 2011; AVP, Skowsky et al., 1979; Levin and Sawchenko, 1993).

NO is a gaseous neurotransmitter produced by three isoforms of Nitric Oxide Synthase: endothelial, inducible and neuronal NOS (Alderton et al., 2001), the latter being expressed in several regions of rodent brain (Rodrigo et al., 1994; Gotti et al., 2005). NO is involved in many physiological activities such as neuroprotection, neural degeneration, long-term potentiation and secretion of many neurotransmitters and neuropeptides (for reviews see: Nelson et al., 1997; Prast and Philippu, 2001; Hull and Dominguez, 2006). NO also modulates several behaviors including reproduction and sexual behavior (for reviews see Nelson et al., 1997; Panzica et al., 2006) and mediates AVP release (Zhang et al., 2009; Vega et al., 2010).

AVP is a nonapeptide produced in two major neural subpopulations: the magnocellular neurons of the supraoptic and paraventricular nucleus projecting to the neurohypophysis (where the peptide is released within the blood as antidiuretic hormone, regulating osmolarity and blood pressure) (Bourque, 1999), and the parvocellular neurons mainly located within the medial amygdala, bed nucleus of the stria terminalis, medial subdivision of the paraventricular nucleus (PVN), and the suprachiasmatic nucleus (SCN) (Rood and De Vries, 2011). These cells project to a number of brain sites where AVP acts as a neuropeptide modulating several behaviors, i.e., social memory, parental behavior, sex behavior, aggression, anxiety, circadian rhythms, body temperature regulation, and others (for a review see Caldwell et al., 2008). In rodents, many of those behaviors are affected by E_2 modulation of the AVP system (Scordalakes and Rissman, 2004; Grassi et al., 2010).

The critical age in which neural circuits may be permanently affected by EDC exposure such as bisphenol A (BPA) (Martini et al., 2010) or GEN (Wisniewski et al., 2005; Panzica et al., 2011), depends on the species and on the considered brain area. Many of these circuits develop prenatally (McCarthy, 2008). For instance prenatal GEN exposure resulted in a demasculizing effect on AVP (or arginin vasotocin) system in MPOA and BST in mammals (Scallet et al., 2003) and birds (Viglietti-Panzica et al., 2007). Other neural systems are sensitive to steroid hormones during early postnatal development. In this period sexual hormones shape BST circuits and disrupt typical aspects of the female reproductive system in rats (Fukushima et al., 2013), as well as the SCN nucleus and circadian rhythms (Royston et al., 2016).

In our previous study we have shown that indirect exposure to GEN (administered with a pipette to the mother), during late gestation (through the placenta) and the first post-natal week (through the mother milk), resulted in changes in anxiety and aggressive behaviors of male offspring mice, paralleled with changes in the nNOS system in the amygdala (Rodriguez-Gomez et al., 2014). However, the developmental effects attributable to GEN exposure in our study are more likely to result from fetal exposures rather than from the postnatal one. In fact, GEN can easily cross the placental barrier (Doerge et al., 2001), while its accumulation in the milk is limited due to the mammary gland barrier (Doerge et al., 2006). Therefore, the purpose of this experiment was to focalize on the effects of a post-natal exposure (i.e. the first postnatal week) on both sexes, mimicking the exposure to soy-milk during infancy (Rozman et al., 2006). Moreover, we broadened our analysis also to the AVP system, since, in other models, it was affected by GEN treatment through E_2-similar mechanisms (Forsling et al., 2003; Scallet et al., 2003; Grassi et al., 2013a). Some of the nNOS and AVP sexually dimorphic circuits are controlled by estrogens for both their development and their activation in adulthood. Therefore, they are major putative targets for the disruptive action of GEN if this molecule is acting by interfering with the cellular mechanisms of estrogenic action.

EXPERIMENTAL PROCEDURES

Animals

Two-month-old virgin CD-1 mice (10 females and 5 males) were purchased from HARLAN Italy and maintained as an outbreed colony at the University of Torino. Mice were housed and treated according to European guidelines (European Union Council Directive of 24th November 1986 n° 86/609/EEC). All the procedures were approved by the Italian Ministry of Health and by the Ethical Committee of the University of Turin.