

Potential roles of the aryl hydrocarbon receptor in female reproductive senescence

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Received: 22 October 2004; accepted: 13 November 2004

SUMMARY

The transition to reproductive senescence involves changes in neuroendocrine and ovarian functions, and is accelerated by activation of the aryl hydrocarbon pathway by environmental toxicants such as 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD). In this article, studies which provide evidence as to the possible mechanisms by which the aryl hydrocarbon receptor (AhR) acts in this capacity (i.e. disruption of ovarian, hypothalamic or suprachiasmatic nucleus function, or any combination of these) are reviewed, along with the normal physiological changes that occur during the transition to reproductive senescence in female humans and rodents. Based on findings that the AhR is evolutionarily conserved and necessary for normal fertility, we suggest that the AhR has not only a pathological but also a physiological role in the process of aging. Studies of realistic lifelong AhR activation by dioxins on the hypothalamic-pituitary-ovarian axis and its impact on the transition to

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reproductive senescence in the aging female are a previously neglected area of research that warrants further consideration. *Reproductive Biology* 2004 4(3): 243-258.

Key words: aryl hydrocarbon receptor (AhR), 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD), reproductive senescence, menopause, ovary, hypothalamus, neuroendocrine

INTRODUCTION

In many mammalian species the transition to reproductive senescence occurs relatively early in life compared to other physiological systems [69, 72]. Activation of the aryl hydrocarbon pathway by environmental toxicants such as 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD) acutely hastens the age-related changes in reproductive function [19]. The purpose of this review is to re-examine what is known about the neuroendocrine and ovarian changes that occur during reproductive senescence in female humans and rodents, and review recent studies that provide evidence for potential mechanisms by which aryl hydrocarbon receptor (AhR) activation may accelerate these changes, as well as point out neglected areas of research on this function of the aryl hydrocarbon pathway. We propose that the AhR has both a pathological and physiological role in the process of reproductive senescence.

Normal aging of the female reproductive system

Reproductive senescence is a general term referring to the loss of reproductive function with age. In the female, this involves a loss of normal ovarian and hypothalamic or pituitary function and is most evident in women as a cessation of cyclic menstruation (menopause) that occurs around age 50 [2, 54, 63, 69]. Normal reproductive senescence and menopause are not disease processes and are useful in the sense that cessation of ovarian cyclicity prevents unnecessary expenditure of energy upon reproduction once the time of fertility is past. The actual end of ovarian activity is preceded by a period of irregular reproductive cycles of suboptimal fertility in both women and

laboratory rodents [40, 42, 53, 65, 69]. This transitional period is of variable duration but generally lasts approximately 10 years in the woman beginning at age 35-40 [2, 5, 69]. It has been hypothesized that menopause occurs once all ovarian follicles are expended, while the prolonged transition to reproductive senescence, during which reproductive cycles are irregular and result in impaired fertility, involves alterations in hypothalamic, pituitary and ovarian mechanisms (tab. 1; [6, 69]).

The correlation between the loss of oocytes and declining fertility with age is well characterized and it is generally accepted that depletion of follicular reserves is the harbinger of the final loss of cyclicity at the menopause in women [38, 69]. Loss of oocytes due to autoimmune disease is thought to be a central mechanism of premature ovarian failure leading to accelerated menopause [41]. Similarly, cigarette smoking has been correlated with diminished follicular reserves and early menopause [59].

The role of changes in follicular function during the transition to reproductive senescence is less clear. This is due in part to difficulties in dissecting ovarian vs. hypothalamo-hypophyseal mechanisms of aging and aggravated by controversies concerning rodent models of reproductive aging. Changes in the hypothalamus and pituitary gland with age in the rat appear to bring about a final cessation of ovarian cyclicity before follicular depletion occurs [72]. This is in contrast to women, where exhaustion of ovarian follicular reserves appears to drive the menopause [69]. Despite these apparent differences in final reproductive senescence, the transition period and the mechanisms leading to prolonged cycles with aberrant endocrinology appear similar in primates and rodents [6, 72].

While depletion of ovarian follicular reserves certainly accompanies reproductive aging in the female, changes in neuroendocrine function also contribute to the loss of normal cyclicity with age. This is particularly true for the prolonged transition to reproductive senescence. Neuroendocrine changes common to middle aged female rats and humans include elevated follicle stimulating hormone (FSH), altered luteinizing hormone (LH) release (generally increased duration and decreased frequency of pulses) and increased variability in cycle length [72]. Gonadotropin releasing hormone (GnRH) neuronal activation during the normal cycle [31, 70] and following

Table 1. Ovarian and neuroendocrine changes during the transition to reproductive senescence and possible mechanisms of action of aryl hydrocarbon pathway

Known changes during the transition to reproductive senescence	Evidence from rodent studies suggest possible mechanisms by which activation of the AhR accelerates reproductive senescence
Ovary	Germ cell death is induced and follicular reserves are diminished [28, 29].
Hypothalamus and pituitary	FSH and LH release are disrupted independent of inhibin or estradiol secretion, and can be largely attenuated by treatment with exogenous GnRH [16, 40].
Suprachiasmatic nucleus	Desensitization of hypothalamo-hypophyseal axis to positive feedback of estradiol [15, 40]. Delays ovulation with 24 hour periodicity [33]. Effects of TCDD on FSH and LH are blocked by pentobarbital [38].

stimulation with estradiol [54] is markedly reduced in middle aged rats. This occurs before changes in the pituitary gland's response to GnRH are detected [71]. This suggests that hypothalamic dysfunction is potentially responsible for the early alterations in gonadotropin secretion that occur with age. Furthermore, the sensitivity of the hypothalamus to estradiol is decreased in this model and in middle aged women as well [53, 72].

The transition to reproductive senescence in the female is also accompanied by a loss of circadian rhythmicity in gene expression in the primary neuroendocrine pacemaker, the suprachiasmatic nucleus (SCN; [72, 73]). Neurotransmitter and neurotransmitter receptor expression, such as proopiomelanocortin and alpha-1-adrenergic receptors, along with monoamine processing, all are blunted in middle aged rats [72, 73]. Furthermore, the 24 hour rhythm in vasointestinal peptide (VIP) mRNA expression disappears by the time animals are middle aged [72]. This is critical in that VIP has the role of providing time-of-day information to the GnRH neurons, and removal of this input leads to a delay in the timing of the LH surge, such as seen in middle-aged rats [72].

The aryl hydrocarbon receptor pathway

Dioxins are environmental contaminants of great concern derived from combustion and synthetic industrial processes [47]. One of the most potent environmental toxicants and the most toxic dioxin is 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD). In addition to dioxins, a number of other environmental and smoking-related toxicants, such as dibenzofurans and biphenyls, act via the AhR pathway [47]. Activation of the aryl hydrocarbon receptor (AhR) by dioxins and subsequent transduction of the dioxin signal through the aryl hydrocarbon receptor nuclear translocator (ARNT) protein and dioxin-response element (DRE) to regulate gene transcription has been well described in many tissues [47, 52]. In the absence of ligand, AhR is present in the cytosol in a complex with heat shock protein 90 (Hsp90), hepatitis B virus X-associated protein (XAP2) and p23 protein [52]. Upon ligand binding, the AhR complex translocates into the nucleus where AhR dissociates from the Hsp90 complex and binds to ARNT [52]. The AhR/

ARNT heterodimer recognizes DRE in the promotor region of target genes and results in the enhanced expression of these genes [52]. AhR and ARNT are proximately 20% identical in amino acid sequence and show a strong resemblance in structure to each other [20]. They are members of the PAS superfamily of proteins (so named for the original members of the family: Period, ARNT, and Single-minded) which are known to play roles in the detection of and adaptation to environmental changes [20].

Mechanisms of action of AhR ligands in the female

Short term AhR activation in young laboratory animals causes male and female endocrine disruption, altered sexual behavior, decreased spermatogenesis, diminished fertility, endometriosis-like symptoms, teratogenesis and abortion [8, 19, 46, 60]. Recent studies also suggest that short term prepubertal AhR activation with TCDD can shorten the reproductive lifespan by accelerating reproductive senescence. Gray and Ostby [19] similarly found that a single gestational exposure to TCDD hastens the loss of fertility with age and increases the age-related onset of acyclicity in the female rat offspring. This experiment, however, did not include environmentally-relevant doses of the dioxin and failed to address potential mechanisms of action. Experiments that focus upon both neuroendocrine and ovarian mechanisms of reproductive senescence and their interaction with the AhR pathway have been neglected in the past.

At the level of the ovary, activation of the AhR system by polycyclic aromatic hydrocarbons has been shown to induce germ cell apoptosis and diminish ovarian follicular reserves [33]. Transgenic or chemical blockade of the AhR or the apoptotic pathways activated by the AhR in the embryonic ovary appears to protect against fetal follicular death and results in expanded follicular reserves and a prolonged reproductive lifespan [7, 43, 50, 61]. TCDD, however, acts on the AhR in a manner distinct from other polycyclic aromatic hydrocarbons in that it does not promote fetal follicular apoptosis [34]. Instead, TCDD appears to attenuate follicular maturation by reducing the number of antral and preantral follicles without inducing apoptotic cell death in rat offspring exposed *in utero* and during lactation [23]. Other studies

have found no effect of lower doses of TCDD in utero on follicular populations in the rat [15]. Acute ovotoxic effects are seen with other ligands of the aryl hydrocarbon receptor, particularly smoking-related toxicants [32, 35, 36, 62]. TCDD blocks ovulation in gonadotropin-primed immature rats and this effect is also seen in hypophysectomized rats [45, 46], suggesting direct ovarian actions of the dioxin. Furthermore, direct application of TCDD to the ovary blocks ovulation as well [45]. Thus, activation of the ovarian AhR does alter ovarian function directly in short term studies, potentially through ovotoxic and steroidogenic effects. As described above, oocyte depletion [69] and hypothalamic dysfunction [40, 42, 53, 65] are central to most theories of reproductive senescence; however, the role of the AhR pathway in the loss of reproductive function with age is unclear (fig. 1).

Several other relevant studies concerning the mechanisms of AhR action on the developing reproductive axis provide clues to the sites of AhR action during aging. Our studies and others, to date, support actions of AhR at both the ovary and hypothalamus or pituitary of the immature rat during the acute blockade of ovulation [46]. In this model, disruption of FSH and LH release following exposure to dioxins appears to involve acute modulation of the daily neural signal driving ovulation. This is followed by desensitization of the hypothalamo-hypophyseal axis to the positive feedback of estrogen just prior to the time of ovulation [16, 46]. This dysregulation of gonadotropin release appears to be independent of endogenous inhibin and estradiol secretion from the ovary [16, 46] and is sensitive to pentobarbital (which blocks the neural signal for GnRH release) and exogenous estradiol [44]. The anovulatory effects of TCDD accompanying these alterations in gonadotropin secretion are largely but not entirely reversed by treatment with exogenous GnRH [17]. Intrauterine and lactational exposure of female rats to TCDD alters estrogen concentrations and gonadotropin synthesis [11, 12, 26, 56]. Furthermore, Hays et al. [22] localized TCDD action to the hypothalamic GABAergic system during development of the female reproductive system postnatally, supporting a hypothalamic site of TCDD action during dysregulation of FSH and LH secretion. Such dysregulation of gonadotropin release is a key feature of the transition from cyclicity to reproductive senescence [72].

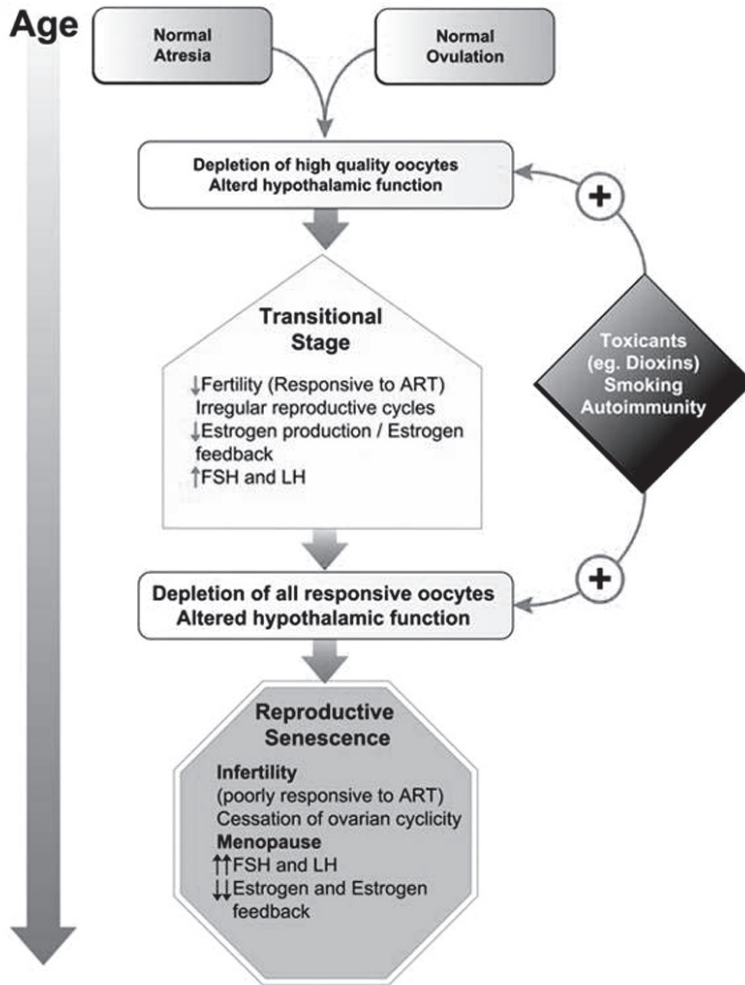


Fig. 1. Hypothetical mechanisms of reproductive aging in the female. ART: assisted reproductive technology; FSH: follicle stimulating hormone, LH: luteinizing hormone.

While the impact of TCDD on SCN function is unknown, the dioxin delays ovulation with a 24 hour periodicity in the immature rat model [39]. Pentobarbital can prevent the premature release of LH and FSH seen in this model only when delivered during the critical period of the neural signal for gonadotropin release in this species [14, 44]. Further evidence is given by

examining other members of the PAS superfamily, the clock proteins, MOP3 and CLOCK, which are essential components of the mammalian circadian pacemaker [9]. The molecular homology among AhR, ARNT, and the clock proteins, along with *in vitro* evidence of MOP3 and AhR dimerization [24], suggests that pathways mediated by MOP3 and CLOCK may influence or respond to dioxin signaling pathway. The potential interaction between these proteins is a connection between activation of AhR and disruption of circadian processes such as gonadotropin release [9].

Also of recent interest is crosstalk between AhR and the estrogen receptor (ER). Several studies have shown that TCDD inhibits estrogen-induced responses in rodent mammary gland and uterus [37, 48]. The antagonistic effects of AhR ligands on ER α are carried out by several different mechanisms. AhR activation increases the metabolism of estradiol by the induction of the cytochrome P450 monooxygenases CYP 1A1 and CYP 1B1 [21, 64]. Also, TCDD enhances degradation of ER α protein by activation of proteasome machinery [55]. Furthermore, TCDD suppresses the transcription of many estradiol-induced genes by blocking or disrupting binding of ER α to DNA [13, 67, 75] and by competing for shared cofactors [28]. Potentially, AhR activation could mediate the hypothalamic desensitization to estradiol of that occurs during the transition phase of reproductive senescence.

Pathological effects of AhR activation on reproductive aging

Approximately one third of unexplained human infertility has been attributed to environmental factors including environmental endocrine disruptors such as dioxins [29, 66]. Current demographic and economic trends, including an aging of the population seeking pregnancy, rising body fat content within the population of developed countries and progressive industrialization, all increase the exposure to lipophilic persistent organic pollutants such as TCDD and the risk of reproductive sequelae [47, 60]. The cumulative exposure to dioxin-like compounds reaches biologically active levels for some endpoints in countries such as the United States [57] that are striving to maintain environmental quality. Smith et al. [57] estimated the exposure of women in the United States to dioxin-like compounds as 3 pg/kg/d which

results in a cumulative exposure of approximately 50 ng/kg across the reproductive lifespan. Environmental contamination with AhR ligands is also an immediate threat to fertility and general health in many developing countries [4, 25, 49]. Occupational exposure to dioxins has been suggested in the pathogenesis of human female reproductive disorders such as endometriosis, infertility and premature reproductive ovarian failure [46, 60]. Gesau and colleagues [18] have reported symptoms consistent with marked premature menopause in women following accidental exposure to dioxins, and smoking (which is a source of many notable AhR agonists) has long been associated with accelerated reproductive senescence [62]. Research into the role of AhR agonists in human disease has been given renewed interest because of the possibility of developing therapeutic agents targeted at the AhR signaling pathway and due to the discovery of natural and synthetic chemopreventive agents that appear to confer health benefits via modulation of the AhR signal [3, 10, 30, 58]. However, a thorough understanding of the impact of toxicants such as dioxins on the female reproductive system across the reproductive lifespan is needed before logical preventive measures can be justified.

In the United States, about 13% of the population experiences early menopause (ages 40-47; [74]). While reproductive senescence is a normal process, premature reproductive senescence is pathological and undesirable for several reasons [62]. The estrogen provided by ovarian activity maintains proper bone homeostasis and premature loss of this estrogen due to accelerated reproductive senescence predisposes women to osteoporosis and bone fractures [5, 27]. This can be alleviated by hormonal replacement therapy, but this therapy in itself may increase other health risks (coronary heart disease, stroke, pulmonary embolism, and invasive breast cancer; [51]). Perhaps as importantly, premature menopause is preceded by premature decreased fertility associated with the transitional period [62, 69]. As women increasingly delay attempts at pregnancy into their 30s and beyond, any acceleration of the onset of the transitional period has dire consequences for the possibility of successful pregnancy. On the whole, accelerated reproductive senescence in women is associated with decreased lifespan and quality of life [62] and exposure to AhR agonists such as dioxins that have

the potential to accelerate reproductive aging is an important health risk. We suggest that activation of the AhR pathway reiterates the hypothalamic and ovarian mechanisms of aging, potentially contributing to the loss of normal cyclicity with age.

Physiological role of AhR activation in aging

While the acute effects of activation of the AhR pathway by environmental toxicants have been a focus of past study, the role of this pathway in normal physiology and aging is only beginning to be appreciated. Although significant exposure to dioxins and similar compounds is a recent phenomenon, the AhR pathway is evolutionarily conserved [68], suggesting an importance outside of adaptation to pollutants. Furthermore, recent studies examining the phenotype of transgenic mice lacking a functional AhR support a role for the aryl hydrocarbon receptor pathway in normal physiological processes including immune function and reproduction [1, 7]. Transgenic ablation of AhR activity in the mouse results in aberrant follicular development [7] during early postnatal life and compromised fertility in the adult involving difficulties in maintaining pregnancy [1]. The importance of the AhR pathway in the biology of normal aging should be addressed. Because the female reproductive system undergoes senescence at a relatively young age and prior to coincident decline of other organ systems, it is viewed as an ideal model for the biology of senescence [72].

To summarize, activation of the aryl hydrocarbon pathway accelerates reproductive senescence, possibly by disrupting ovarian, hypothalamic or SCN function, or any combination of these (tab.1). Furthermore, we suggest that the AhR has not only a pathological but also a physiological role in the process of aging that has previously been overlooked. The impact of a realistic lifelong AhR activation by dioxins on the hypothalamic-pituitary-ovarian axis during the transition to reproductive senescence in the aging female is an area of research that warrants further consideration. This would fill the deficits in our understanding and serve as a basis for efforts to determine the interaction between environmental toxicants and age-related reproductive decline.

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