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The effect of perinatal exposure to ethinyl oestradiol or a mixture of endocrine disrupting pesticides on kisspeptin neurons in the rat hypothalamus

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ABSTRACT

Early life exposure to endocrine disruptors is considered to disturb normal development of hormone sensitive parameters and contribute to advanced puberty and reduced fecundity in humans. Kisspeptin is a positive regulator of the hypothalamic–pituitary–gonadal axis, and plays a key role in the initiation of puberty. In the adult, *Kiss1* gene expression occurs in two hypothalamic nuclei, namely the anteroventral periventricular nucleus (AVPV) and the arcuate nucleus (ARC), which are differentially regulated by peripheral sex steroid hormones. In this study we determined the effects on puberty onset and Kiss1 mRNA levels in each of the two nuclei after long-term perinatal exposure of rats to ethinyl oestradiol (EE_2) or to five different pesticides, individually and in a mixture. Rat dams were per orally administered with three doses of EE_2 (5, 15 or 50 μg/kg/day) or with the pesticides epoxiconazole, mancozeb, prochloraz, tebuconazole, and procymidone, alone or in a mixture of the five pesticides at three different doses. Kiss1 mRNA expression was determined in the AVPV and in the ARC of the adult male and female pups in the EE_2 experiment, and in the adult female pups in the pesticide experiment.

We find that perinatal EE_2 exposure did not affect Kiss1 mRNA expression in this study designed to model human exposure to estrogenic compounds, and we find only minor effects on puberty onset. Further, the Kiss1 system does not exhibit persistent changes and puberty onset is not affected after perinatal exposure to a pesticide mixture in this experimental setting. However, we find that the pesticide mancozeb tends to increase Kiss1 expression in the ARC, presumably through neurotoxic mechanisms rather than *via* classical endocrine disruption, calling for increased awareness that Kiss1 expression can be affected by environmental pollutants through multiple mechanisms.

1. Introduction

Perinatal exposure to substances that interfere with endogenous hormones, thereby disrupting their normal function, is suspected to contribute to advanced pubertal maturation and reduced fecundity in women (Buck Louis et al., 2008; Crain et al., 2008; Euling et al., 2008; Mouritsen et al., 2010; Wohlfahrt-Veje et al., 2012). Such substances, termed endocrine disruptors (EDs), have various mechanisms of action, such as changing the rate of steroidogenesis and aromatase activity, agonizing/antagonizing steroid receptors, and changing steroid receptor level or function (Frye et al., 2012; Roa et al., 2008). Girls of mothers exposed to pesticides in early pregnancy experience advanced breast development (Wohlfahrt-Veje et al., 2012), and there is an increased prevalence of cryptorchidism in boys of mothers exposed to pesticides (Weidner et al., 1998). Many EDs cross the placenta and are found in breast milk (Frye et al., 2012). Fetuses and children are especially vulnerable to EDs, since steroid hormone levels during this period are critical for proper development of both the reproductive organs and the nervous system, including the hypothalamus (Frye et al., 2012; Patisaul and Adewale, 2009a).

Kisspeptin, encoded by *Kiss1*, is a hypothalamic peptide expressed in two hypothalamic nuclei; the anteroventral periventricular nucleus (AVPV) and the arcuate nucleus (ARC), and kisspeptin potently elicits GnRH release, thereby stimulating the hypothalamic–pituitary–gonadal axis (Pinilla et al., 2012). Kisspeptin is essential for puberty onset, as described by the hypogonadotropic hypogonadistic phenotype of humans with loss-of-function mutations in the kisspeptin receptor Kiss1R (de Roux et al., 2003; Seminara et al., 2003). Kiss1 neurons express

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estrogen receptor (ER)-α, progesterone receptor and androgen receptor (AR) in a high degree, while fewer Kiss1 neurons express ER-β (Lehman et al., 2010). Kiss1 neurons are thus considered to be important targets for steroid feedback in the adult, and *Kiss1* expression is differentially regulated by steroid hormones in the two areas, with sex steroids stimulating kisspeptin expression in the AVPV and inhibiting expression in the ARC (Clarkson et al., 2012; Smith et al., 2005a, b). During pubertal development, sex steroids are essential for the increase in kisspeptin (Clarkson et al., 2009, 2012; Smith et al., 2005a, b), and ablation of ER-α in kisspeptin neurons advances puberty, decreases kisspeptin expression, and disrupts estrous cyclicity in mice (Mayer et al., 2010). Furthermore, the Kiss1 system is highly sexual dimorphic and sexual dimorphic hypothalamic regions are shaped during developmental windows where differences in sex steroid levels are high, particularly the perinatal period (Bakker and Brock, 2010; Clarkson et al., 2009; Kauffman et al., 2007). Collectively, kisspeptin is a putative target for perinatal endocrine disruption.

Several studies have shown that perinatal or prepubertal exposure to estrogenic EDs can advance puberty onset in female rats (Bateman and Patisaul, 2008; Dickerson et al., 2011; Marty et al., 1999; Rasier et al., 2007), whereas other EDs have the capability to delay puberty onset (Davis et al., 2011; Laws et al., 2000; Marty et al., 1999; Stanko et al., 2010). The delay in puberty onset was induced by atrazine, which disturbs GnRH release (Cooper et al., 2007; Davis et al., 2011; Laws et al., 2000; Stanko et al., 2010), and ketoconazole, which decreases oestradiol levels through aromatase inhibition (Marty et al., 1999). Reduced kisspeptin expression after perinatal exposure to potent ER agonists has also been reported (Bateman and Patisaul, 2008; Bellingham et al., 2009; Dickerson et al., 2011; Losa-Ward et al., 2012; Navarro et al., 2009; Patisaul et al., 2009b), whereas the environmental EDs bisphenol-A and genistein only affect kisspeptin expression at high doses (four neonatal sc. injections of 50 mg/kg and 10 mg/kg, respectively) and not at low doses (four sc. neonatal injections of 50 μg/kg BPA) (Bateman and Patisaul, 2008; Losa-Ward et al., 2012; Patisaul et al., 2009b). Also, a reduced kisspeptin level has been reported in fetuses of ewes exposed to a mixture of environmental EDs at an undefined exposure level, but timing of puberty was not assessed (Bellingham et al., 2009).

These studies have a number of limitations in comparison to human exposure, as high doses of a single compound are injected in early postnatal life in most studies. By contrast, humans are exposed to several EDs at low levels, primarily *via* skin and digestion, and pesticide residues in food are an important exposure route. Pesticides have therefore been tested for their potential endocrine disrupting effects, and European recommendations to limit human exposure of single compounds have been made (Regulation(EC)No. 396/2005). However, it is increasingly recognized that the effects of EDs can be additive, such that exposure to

Table 1
Compounds, dosing, effects and mechanisms of action.

Study 1 compound	Doses (μg/kg/day)	Dosing period	Effects and mechanisms of action	References
Ethinylestradiol	5; 15; 50	GD 7–21 + PD 1–22	Endogenous estrogen receptor agonist	Ryan et al. (2010)
			Accelerated mammary gland development	Mandrup et al. (2012)
			Increased anogenital distance and advanced puberty in female offspring	Ryan et al. (2010)
Study 2 compounds	**Doses (mg/kg/day)**	**Dosing period**	**Effects and mechanisms of action**	**References**
Epoxiconazole	3.75; 15	GD 7–21 + PD 1–16	AR antagonism *in vitro*	Hass et al. (2012) and Kjaerstad et al. (2010a)
			Reduction of estradiol and testosterone and increase in progesterone synthesis *in vitro*	Hass et al. (2012) and Kjaerstad et al. (2010a)
			Increased anogenital distance in female rats	Taxvig et al. (2007)
			Anti-estrogenic and aromatase inhibiting	Kjaerstad et al. (2010b)
Mancozeb	6.25; 25	GD 7–21 + PD 1–16	Manganese-dependent mitochondrial dysfunction in neurons	Harrison Brody et al. (2013)
			Thyroid hormone disruption	Axelstad et al. (2011) and Hurley (1998)
			Increased risk of Parkinson's disease in exposed humans	Kamel et al. (2007)
Prochloraz	8.75; 35	GD 7–21 + PD 1–16	AR antagonism *in vitro*	Hass et al. (2012) and Kjaerstad et al. (2010a)
			Central and peripheral AR antagomism *in vivo*	Vinggaard et al. (2002)
			Reduction of estradiol and testosterone and increase in progesterone synthesis *in vitro*	Hass et al. (2012) and Kjaerstad et al. (2010a)
			Anti-estrogenic and aromatase inhibiting	Kjaerstad et al. (2010b)
Tebuconazole	12.5; 50	GD 7–21 + PD 1–16	AR antagonism *in vitro*	Hass et al. (2012), Orton et al. (2011) and Kjaerstad et al. (2010a)
			Reduction of estradiol and testosterone and increase in progesterone synthesis *in vitro*	Hass et al. (2012) and Kjaerstad et al. (2010a)
			Increased anogenital distance in female rats	Taxvig et al. (2007)
			Anti-estrogenic and aromatase inhibiting	Kjaerstad et al. (2010a)
Procymidone	12.5; 50	GD 7–21 + PD 1–16	AR antagonism *in vitro*	Hass et al. (2012) and Ostby et al. (1999)
			Decreased anogenital distance in male rats	Ostby et al. (1999)
Pesticide mixture	14.58; 29.17; 43.75	GD 7–21 + PD 1–16	Additive effects on gestational length, pup survival and nipple retention	Hass et al. (2012)
			Additive effects on sperm count, decreased weight of reproductive organs and altered spatial learning in males	Jacobsen et al. (2012)

In vivo mechanisms of actions is reported predominantly for females.
GD, gestational day; PD, pup day; AR, androgen receptor.