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Review

Endocrine disruption by environmental gestagens in amphibians – A short review supported by new *in vitro* data using gonads of *Xenopus laevis*

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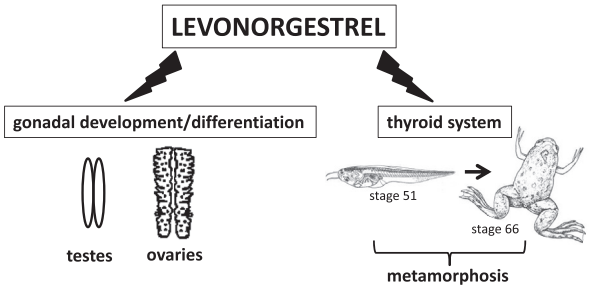
HIGHLIGHTS

- Gestagens have impacts on both, reproductive and thyroid system, in amphibians.
- Female gonads are more susceptible to gestagens than male ones.
- Levonorgestrel delayed and disrupted metamorphosis in *Xenopus laevis*.

GRAPHICAL ABSTRACT



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ABSTRACT

Endocrine disruption caused by various anthropogenic compounds is of persisting concern, especially for aquatic wildlife, because surface waters are the main sink of these so-called endocrine disruptors (ED). In the past, research focused on (anti)estrogenic, (anti)androgenic, and (anti)thyroidal substances, affecting primarily reproduction and development in vertebrates; however, other endocrine systems might be also targeted by ED. Environmental gestagens, including natural progestogens (e.g. progesterone (P4)) and synthetic progestins used for contraception, are supposed to affect vertebrate reproduction via progesterone receptors. In the present paper, we review the current knowledge about gestagenic effects in amphibians, focussing on reproduction and the thyroid system. In addition, we support the literature data with results of recent *in vitro* experiments, demonstrating direct impacts of the gestagens levonorgestrel (LNG) and P4 on sexually differentiated gonads of larval *Xenopus laevis*. The results showed a higher susceptibility of female over male gonads to gestagenic ED. Only in female gonads LNG, but not P4, had direct inhibitory effects on gene expression of steroidogenic acute regulatory protein and P_{450} side chain cleavage enzyme, whereas aromatase expression decreased in reaction to both gestagens. Surprisingly, beyond the expected ED effects of gestagens on reproductive physiology in amphibians, LNG drastically disrupted the thyroid system, which resembles direct effects on thyroid glands and pituitary along the pituitary-thyroid axis disturbing metamorphic development. In amphibians, environmental

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gestagens not only affect the reproductive system but at least LNG can impact also development by disruption of the thyroid system.

Contents

1. Introduction

Surface waters are the main sink for environmental pollution by various chemical compounds of anthropogenic origin and therefore there is persisting concern that a number of compounds, including some pharmaceuticals, cause adverse effects on aquatic wildlife by endocrine disruption rather than by direct toxic impacts (Damstra et al., 2002; Kloas et al., 2009; Bergmann et al., 2013; Kumar et al., 2015). Initially, researchers focused on (anti)estrogenic, (anti)androgenic, and (anti)thyroidal substances, the so-called endocrine disruptors (ED), affecting mainly reproduction and development in vertebrates while interacting with their endocrine system (Le Blanc et al., 2011).

Lately environmental gestagens, including the natural progestogens (e.g. progesterone (P4)) and synthetic progestins, have been identified as potential EDs, too (Jenkins et al., 2003; Carson et al., 2008; Besse and Garric, 2009; Liu et al., 2011; Mansell et al., 2011; Fent, 2015; Avar et al., 2016). Gestagens are steroid hormones used for cancer treatment and female contraception. Apart from pharmaceutical products for human use, a variety of industrial chemicals and pesticides displays (anti)gestagenic activities as well (Li et al., 2008, 2010).

Gestagens bind to progesterone receptors (PR) and display important functions in vertebrate reproduction particularly, with regard to gamete maturation, behaviour, and negative feed-back regulation of reproductive cycles (Norris and Carr, 2013; Fent, 2015; Frankel et al., 2016). Progesterone (P4), a derivative of cholesterol and pregnenolone, is the first steroidal hormone giving rise to further gestagens, androgens, estrogens, and corticosteroids. Gestagens are pivotal for final gamete maturation in fish (Tokumoto et al., 2005) and amphibians (Arias Torresa et al., 2016; Ogawa et al., 2011), where they affect the germinal vesicle breakdown (GVBD) (Pickford and Morris, 1999) and sperm motility (Murack et al., 2011). In addition to interactions with nuclear PR, gestagens are able to bind to membrane PR, causing rapid gestagen-mediated biological responses (Thomas, 2000; Thomas and Doughty, 2004). It is known that some progestins can display also androgenic activities by binding to androgen receptors (AR) (Kumar et al., 2015).

However, despite the great importance of gestagens for reproduction in all vertebrates, the close interaction of gestagens, androgens, and estrogens in the regulation of reproductive processes and the crosstalk between these signalling pathways makes clear identification of (anti)gestagenic modes of action inherently difficult. Beyond their endocrine disruptive effects, additional but poorly investigated targets (e.g. photo-transduction cascade and circadian rhythm network) can be also affected (Zhao and Fent, 2016).

Amphibians are suitable and sensitive models to assess endocrine disruption (Kloas, 2002; Kloas and Lutz, 2006). In amphibians, gestagenic effects (Kloas et al., 2009) were found not only on reproductive physiology (Kvarnryd et al., 2011; Lorenz et al., 2011b) and mating behaviour (Hoffman and Kloas, 2012a) but also on the thyroid system (Kloas et al., 2009; Lorenz et al., 2011a, 2016).

Here, we review the current knowledge about impacts of environmental gestagens on amphibians, focussing on the endocrine systems, gonadal development and reproduction as well as the thyroid system. In addition to this brief overview, we provide first empirical *in vitro* data on direct effects of the progestins levonorgestrel (LNG) and progesterone (P4) on immature but sexually differentiated gonads of larval *Xenopus laevis*.

2. Gonadal development

In contrast to fish (Zeilinger et al., 2009; Paulos et al., 2010; Hua et al., 2015; Frankel et al., 2016; Zhao and Fent, 2016), the number of studies investigating impacts of gestagens as ED on reproductive processes in amphibians is rather limited.

2.1. Review of gestagenic impacts on gonads

One potential endpoint for gestagenic effects, the germinal vesicle breakdown (GVBD), was already used as early as 1999 to characterize impacts of ED (Pickford and Morris, 1999). Depending on the examination of intact follicles or denuded oocytes, co-incubation protocols with gonadotropin and/or gestagen and the potential ED of interest are available. Such assays can be performed either directly *in vitro* or after in vivo exposure of the test animals, and the results are evaluated by visual inspection. The natural gestagen P4 stimulated GVBD already at a concentration of 10 nM and the estrogenic pesticide methoxychlor (MCL) was a potent inhibitor of P4 causing inhibition of oocyte maturation with a median concentration of 72 nM, and thus demonstrated clear antigestagenic activity. However, additional experiments, using in parallel the antiestrogen ICI 182,780, revealed that the antigestagenic effect of MCL is not due to suggested estrogenic activity. The authors further showed that MCL did not displace [^{3}H]-P4 from