Opinions and Hypotheses

Reconsidering the roles of endogenous estrogens and xenoestrogens: the membrane estradiol receptor G protein-coupled receptor 30 (GPR30) mediates the effects of various estrogens

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Abstract. Estrone (E1) and estriol (E3) are considered “weak” estrogens, which exert suppressive effects through estrogen receptors α and β. However, recent studies have demonstrated that E1 and E3, as well as estradiol (E2), suppress gonadotropin-releasing hormone-induced luteinizing hormone secretion from bovine gonadotrophs via G-protein-coupled receptor 30, which is expressed in various reproductive organs. Currently, there is a lack of fundamental knowledge regarding E1 and E3, including their blood levels. In addition, xenoestrogens may remain in the body over long time periods because of enterohepatic circulation. Therefore, it is time to reconsider the roles of endogenous estrogens and xenoestrogens for reproduction.

Key words: Gonadotroph, G protein-coupled estrogen receptor-1, Pituitary, Xenoestrogen, zearalenone

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Estrogen receptors

Estradiol (E2), which is secreted from the ovaries, is a powerful feedback regulator, controlling the secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus and the secretion of luteinizing hormone (LH) form the anterior pituitary. Blood E2 concentrations fluctuate in heifers and cows between 0.004 nM (1 pg/ml) and 0.030 nM (8 pg/ml) during the luteal phase of the estrous cycle [1, 2]. Small concentrations exert a negative feedback effect on GnRH secretion from the hypothalamus [3–5], and suppress the amounts of LH mRNA in the anterior pituitary [6].

E2 binds to nuclear-localized estrogen receptors α or β (ERα or ERβ), and changes the mRNA expression of the genes that produce GnRH and LH in the hypothalamus or the pituitary, respectively [7–9]. However, E2 also suppresses LH production in a rapid, nongenomic manner within the pituitary via binding to the G protein-coupled receptor 30 (GPR30; or G protein-coupled estrogen receptor 1) [10–14]. GPR30 can bind to E2 to initiate several rapid, nongenomic signaling events in the cytoplasm [15].

Significant effects of estrone and estriol

Estrone (E1) and estriol (E3) have been explained as “weak” estrogens compared to E2, with respect to genomic actions [16]. However, according to a recent study, both E1 and E3 have been shown to be strong estrogens in the nongenomic signaling pathways, and elicit functional responses in rat lactotroph-like cells [16]. Additionally, E1 exerts a direct, nongenomic action on rat aortic metabolism [17]. However, there are no reports on the nongenomic effects of E1 and E3 on LH secretion by gonadotrophs in animals, including women and rodents.

Therefore, we evaluated whether GPR30 mediated E1 and E3 to suppress GnRH-induced LH secretion from bovine pituitary [18]. As shown in Fig. 1, pre-treatment with picomolar levels, but not femtomolar or nanomolar levels, of E2, E1, or E3 suppressed the GnRH-induced LH secretion. The GPR30 antagonist (G36), but not ERα antagonist (MPP), abrogate such suppressing effects of picomolar levels of E2, E1, and E3 on LH secretion [20]. Therefore, E1 and E3, as well as E2, suppress GnRH-induced LH secretion from anterior pituitary cells in a nongenomic manner, via GPR30 binding (Fig. 2). Previous studies reported that in ruminants, the intramuscular injection of E3 induces an LH surge earlier than an E2 injection [19, 20]. The nongenomic effect of E1 on LH secretion may have an important role in controlling the number of ovulations in heifers treated for superovulation [21]. Therefore, we need to reconsider the functions of E1 and E3, and further studies are required to evaluate the functional significance of the findings, given that E2, E1, and E3 exert similar, nongenomic suppressive effects via GPR30 binding in gonadotrophs.

As shown in Fig. 1, both E1 and E3 can markedly affect the GnRH-induced LH secretion rapidly. Iqbal *et al.* [11] had suggested that E2 might have a biphasic effect on LH secretion by ovine gonadotropes *in vivo*, with

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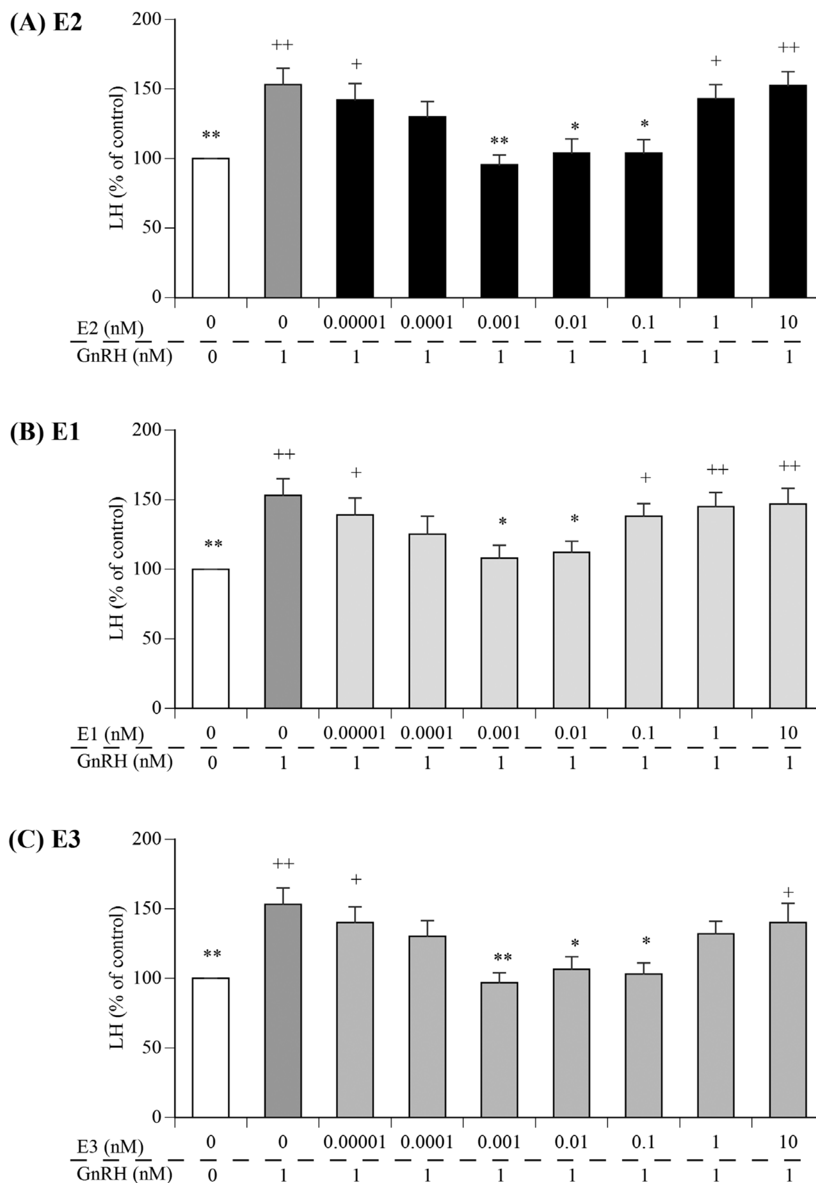

Fig. 1. The effects of pretreatment with femtomolar, picomolar, or nanomolar levels of E2, E1, or E3 on GnRH-induced LH secretion from cultured bovine anterior pituitary cells (from Otsuka and Kadokawa [18]). + indicates P < 0.05, and ++ indicates P < 0.01 compared to the control (white bar); * indicates P < 0.05, and ** indicates P < 0.01 compared to GnRH alone (dark grey bar).

a rapid suppression of LH release (negative feedback) initially, followed by a positive feedback event many hours later. Iqbal *et al.* [11] had also reported that E2 might activate the MAPK pathway for exerting its time-delayed positive feedback effect. Therefore, the nanomolar concentration of E1, E2, and E3 may have activated the MAPK pathway earlier than the picomolar concentrations, suggesting that both ligand dosage and duration of action are important for their suppressive effects on LH secretion by gonadotrophs.

Cytoplasmic pathway of GPR30

Unlike the cytoplasmic pathways for ERα and ERβ, which control gene expression in various cells, little is known about the cytoplasmic signaling pathway of activated GPR30. Few studies have recently revealed that protein kinase A (PKA) and phosphorylated extracellular signal-regulated kinase (pERK) are involved in the cytoplasmic signaling pathways of GPR30 in mouse trigeminal ganglia [22] and rat liver [23]. Within 15 min of E2 treatment, LH secretion from ovine AP decreases, and pERK levels increase [11, 24]. A GPR30 antagonist, G36, inhibits ERK phosphorylation by estrogen in SKBr3 cells [25]. Furthermore, we have previously reported that PKA and pERK are the intracellular mediators downstream of GPR30, which induce the nongenomic suppression of GnRH-induced LH secretion from bovine AP cells by E2 or GPR30-specific agonists [26].

Crosstalk between ERs and GPR30 in gonadotrophs

Whether GPR30 acts as an autonomous ER *in vivo* or interacts with nuclear estrogen receptor signaling pathways in response to estrogens is controversial. As reviewed by Romano and Gorelick [27], several lines of evidence support the role of GPR30 as an autonomous estrogen receptor *in vivo*. However, evidence supporting the interaction of GPR30 with ER signaling is also available. These reports suggest that GPER may either activate ER or form a complex with ER. Romano and Gorelick [27] concluded that the degree to which GPR30 influences nuclear estrogen receptor signaling likely depends on the cell type, developmental stage, and pathology. In our study, GPR30 antagonists, but not ERα antagonists, abrogated the suppressive effect of picomolar concentrations of E2, E1, and E3 on LH secretion from bovine gonadotrophs [18]. Our data suggested that GPR30 is an autonomous estrogen receptor that suppressed the GnRH-induced LH secretion from bovine gonadotrophs. However, any crosstalk between ER and GPR30 may have important roles in the nongenomic effects of E1, E2, and E3, which induce prolactin secretion from GH3/B6/F10 rat pituitary tumor cells [16]. Therefore, further studies are required to clarify the mechanism underlying the nongenomic effects of endogenous estrogens in ruminants and other species.

Lack of fundamental knowledge regarding E1 and E3

Little is known about blood E1 concen-

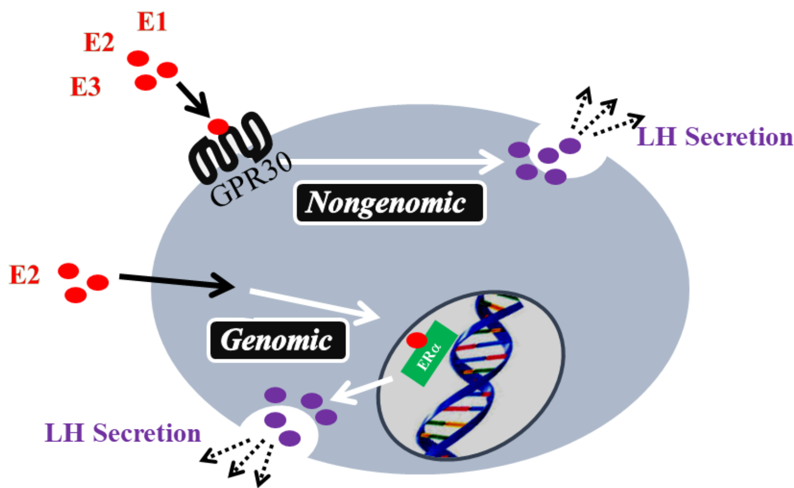

Fig. 2. Genomic and nongenomic pathways controlling LH secretion from bovine gonadotrophs.

trations in domestic animals. The blood E1 concentration in cows is probably in the picomolar range during the estrous cycle, and increases to nanomolar levels during pregnancy [28]. However, we found no reports about blood E3 concentration in cows. In animals, E3 is synthesized from E1 and E2 [29, 30]. Thus, it is reasonable to assume that blood E3 concentrations in cows might follow the same trend as blood E1 concentrations. Although further studies are required to clarify the blood concentrations of E1 and E3 in domestic animals, the picomolar concentrations of E1 and E3, as well as E2, may exert similar negative feedback effects from the ovaries to gonadotrophs to control GnRH-induced LH suppression during the estrous cycle.

Xenoestrogens

We must be cautious that the GPR30 expressed on cells in various reproductive organs may bind xenoestrogens as well as endogenous estrogens. Xenoestrogens include plant estrogens, mycoestrogens, and environmental estrogens.

Plants have chemical mechanisms to defend against attacks by animals, including insects and vertebrate herbivores. Phytochemical options exist by which plants can modulate the fertility of herbivores [31]. Phytoestrogens are most well-known group of phytochemical mimics of vertebrate reproductive hormones [32]. However, little is known about whether phytoestrogens are GPR30 ligands. A previous study demonstrated that the phytoestrogen genistein is a GPR30 ligand in human periodontal ligament cells [33]. Although phytoestrogens have been studied in menopausal women, many of their effects remain unstudied in both human and domestic animals, as reviewed by other authors [34, 35].

α-Zearalanol (or Zeranol; α-ZAL) is a non-steroidal estrogenic compound, derived from zearalenone (ZEN), the mycoestrogen produced by *Fusarium* [36]. It has been established that α-ZAL implantation delays puberty, increases the incidence of non-ovulatory estrus, and retards reproductive tract development in heifers [37]. Previous studies also indicate that in ruminants, α-ZAL suppresses LH secretion in a rapid, nongenomic manner *in vivo* [38–41].

We previously reported that ZEN and five known ZEN metabolites rapidly suppressed GnRH-induced LH secretion, by decreasing cytoplasmic cyclic adenosine monophosphate without decreasing expression of the LHα and LHβ subunits [14, 42]. It is important to note that α-ZAL has been detected in cereal grains and animal feeds [43], cattle urine, and ruminant bodies worldwide [44].

In our study, we estimated that ZEN had the greatest nongenomic inhibiting effect on LH secretion in terms of magnitude and effective concentration range, followed by α-ZAL, zearalanone (ZAN), α-zearalenol (α-ZOL), β-zearalenol (β-ZOL), and β-zearalanol (β-ZAL) [42]. It is important to note that the relative strengths of the nongenomic inhibiting effects caused by each ZEN analog differ from the reported relative strengths of their genomic effects measured by the MCF7 human breast cell proliferation assay [45], as summarized in Fig. 3.

Biehl *et al.* reported that ZEN administered orally was rapidly and efficiently absorbed and metabolized by pigs, reaching maximum plasma concentrations within 2 or 3 h [46]. These authors also reported that 45% of the administered ZEN was excreted in bile, of which 85% was reabsorbed into the systemic circulation [45]. It is unknown whether ZEN analogs are subject to this type of "enterohepatic circulation" in ruminants. However, E2 undergoes enterohepatic circulation [47]; the bile of ruminants contains ZEN, α-ZOL, and β-ZOL [48]. The half-life for the elimination of ZEN from the blood of ruminants is quite long (28.6 h) [49]. Therefore, ZEN remains in ruminant bodies fed with ZEN-contaminated feed for long periods; it is possible that ZEN concentrations attain values at which LH secretion is inhibited *in vivo*.

Thomas and Dong [50] reported that bisphenol A (BPA) and nonylphenol, both well-known environmental estrogens and endocrine disruptors, imperfectly mimic the effects of physiologic estrogens via binding to ERα, ERβ, and GPR30. These authors also reported that the binding affinity of BPA and ZEN to GPR30 is less than 3% relative to that of E2, and that BPA shows a higher affinity to GPR30 than to ERα and ERβ. If both ligand dosage and duration of action are important for the suppressive effect on LH secretion by gonadotrophs, the low affinity of xenoestrogens to GPR30 may modulate the duration of action to allow the biphasic effect on LH secretion by gonadotrophs. Thus, there is a need to test the hypothesis that the rapid negative feedback from low-affinity xenoestrogens may appear before the effects of E2, while the positive feedback may appear after the effects of E2.

Bisphenol S (BPS) is an alternative to BPA in plastic consumer products and thermal paper. However, Viñas and Watson [51] reported that BPS, once considered a safe substitute for BPA, disrupts membrane-initiated E2-induced cell signaling, leading to altered proliferation, cell death, and prolactin release in a rat cell line. Substituting BPA with BPS in consumer products is a fast process, especially in the U.S. [52]; BPS is now ubiquitous in the envi-

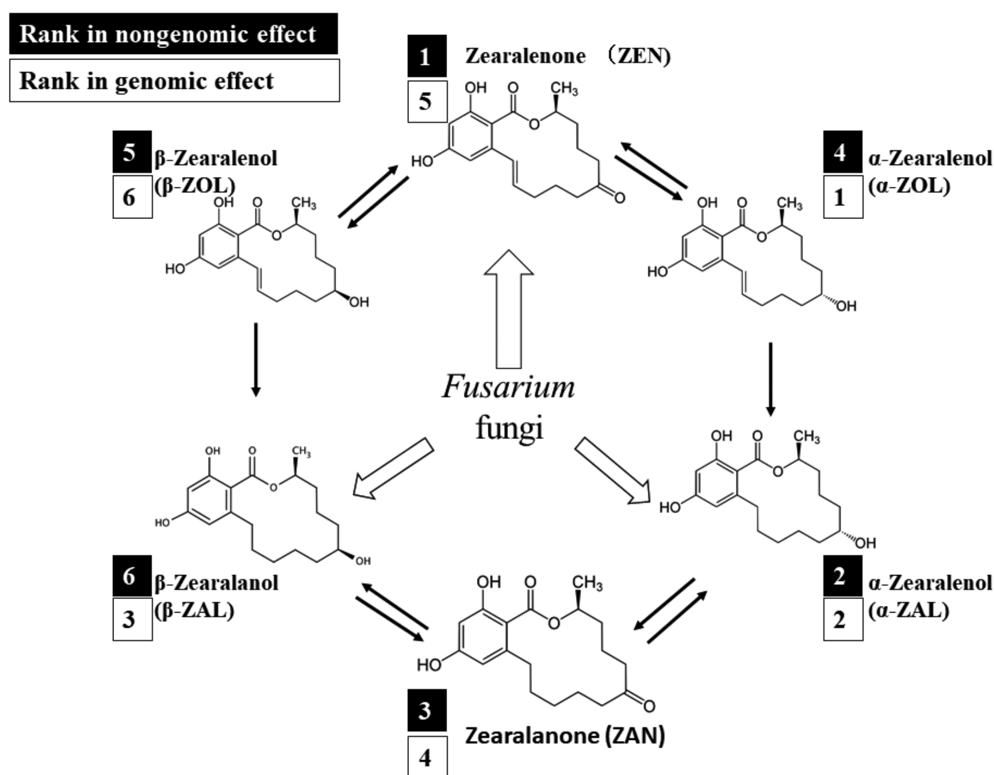

Fig. 3. Relative strengths of the nongenomic inhibiting effects of ZEN analogs on bovine gonadotroph LH secretion (white font in black boxes) [42] and genomic effects of the ZEN analogs measured by the MCF7 human breast cell proliferation assay (black font in white boxes) [45]. White and black arrows indicate mycoestrogens synthesized by the *Fusarium* fungi and their metabolic pathways according to Erasmuson *et al.* [44], respectively.

ronment (including water, sediment, sludge, indoor dust and air, consumer products, food, and human urine) worldwide [52]. Recent studies have demonstrated that BPS may have adverse effects on the reproductive, endocrine, and nervous systems of animals and humans, and may trigger oxidative stress [52]. Further studies are required to clarify the effects of environmental estrogens mediated by GPR30 in domestic animals.

GPR30 is expressed in various reproductive organs

Recently, GPR30 expression has been shown to be important in various reproductive functions such as testosterone production in human testes [53], lordosis behavior in rats [54], SBD-1 gene expression in ovine oviduct epithelial cells [55], mouse oocyte maturation [56], and mediation of the direct effects of E2 in immortalized GnRH neurons [57]. The *GPR30* gene is expressed in the granulosa and theca cells, although its role in the ovary is not yet clear [58]. GPR30 rapidly stimulates GnRH secretion from GnRH neurons [59]. Therefore, further studies are required to evaluate the hypothesis that various endogenous estrogens and xenoestrogens are risk factors for reproductive suppression in domestic animals.

Species difference in GPR30

As mentioned previously, further studies are required to clarify the physiological significance of E1 and E2 in other animals. Matthews *et al*. [60] reported that ERs from different species exhibit differential ligand preferences and relative binding affinities for estrogenic compounds due to the differences in their amino acid sequences. The *GPR30* gene is conserved in *Homo (H.) sapiens*, *Macaca (M.) mulatta*, *Bos (B.) taurus*, *Canis (C.) lupus*, *Mus (M.) musculus*, *Rattus (R.) norvegicus*, *Gallus (G.) gallus*, *Xenopus (X.) tropicalis*, and *Danio (D.) rerio* (https://www.ncbi.nlm.nih.gov/homologene/15855). The amino acid sequence of *B. taurus* GPR30 (XP_002698215.1) has 7 transmembrane helices, similar to the GPR30 of other animals (determined by the SOSUI algorithm; http://harrier.nagahama-i-bio.ac.jp/sosui/). However, the pairwise alignment scores of GPR30 of *H. sapiens* with that of *B. taurus* is lower (84.6% of identity) than that with those of other mammals [*M. mulatta* (98.1%), *C. lupus* (89.7%), *M. musculus* (86.9%), and *R. norvegicus* (86.1%)]. This could be because the amino acid sequences of the extracellular N-terminus region of GPR30 of different species differ substantially from each other. Therefore, it can be assumed that GPR30 plays a wide variety of roles in different organs of carnivores, omnivores, and herbivores that consume large quantities of phytoestrogens.

Conclusion

Endogenous estrogens and xenoestrogens may play important roles in the reproduction process via binding to GPR30 in various cells; however, little is known about the mechanism by which activated GPR30 mediates these effects. Therefore, we need to reconsider the

roles of endogenous estrogens and xenoestrogens in different cells in the reproduction process of various species.

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