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Non-classical mechanisms of steroid sensing in the ovary: Lessons from the bovine oxytocin model

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ABSTRACT

Steroidogenic tissues such as the ovary, testes or adrenal glands are paradoxical in that they often indicate actions of steroid hormones within a dynamic range of ligand concentration in a high nanomolar or even micromolar level, i.e. at the natural concentrations existing within those organs. Yet ligand-activated nuclear steroid receptors act classically by direct interaction with DNA in the picomolar or low nanomolar range. Moreover, global genomic studies suggest that less than 40% of steroid-regulated genes involve classical responsive elements in gene promoter regions. The bovine oxytocin gene is a key element in the maternal recognition of pregnancy in ruminants and is regulated via an SF1 site in its proximal promoter. This gene is also regulated by steroids acting in a non-classical manner, involving nuclear receptors which do not interact directly with DNA. Dose-response relationships for these actions are in the high nanomolar range. Similar 'steroid sensing' mechanisms may prevail for other SF1-regulated genes and predict alternative pathways by which environmental endocrine disruptors might influence the functioning of steroid-producing organs and hence indirectly the steroid-dependent control of physiology and development.

1. Introduction – the steroid paradox

The molecular biology of steroid hormone physiology is well described and conforms in general to a model involving the ligand-dependent activation of specific transcription factors, which as homo- or heterodimers within the cell nucleus interact directly with specific palindromic or repeat DNA sequences usually within the upstream regulatory regions of genes (Fig. 1A). Such a classical genomic mechanism is postulated for the actions of estrogens, androgens and progesterone. Historically, such mechanisms have all been elucidated for steroid-dependent gene expression in peripheral tissues such as the uterus or breast. In vitro molecular pharmacology studies further confirm that EC50 values for ligand – nuclear receptor binding as well as for receptor-dependent gene promoter activation all lie in the subnanomolar concentration range, which is well in accord with estimated and available local steroid concentrations in peripheral tissues. There is a major paradox, however, in that firstly, microarray studies to assess global gene activation by specific steroids, such as estradiol, all suggest that only a small proportion of genes with significant quantitative alteration have a canonical steroid response element within the upstream regulatory region of those genes. In fact, a recent study, using a global ChIP-Seq approach, has shown that maximally 40% of estrogen up-regulated genes in the uterus have conventionally binding estrogen receptors anywhere within a large distance of those genes (Hewitt et al., 2012). Such results, also for other steroid hormones, emphasize that most steroid-regulated genes must make use of non-classical mechanisms of gene activation. A second paradox is that there appears to be expression of specific and functional steroid receptors, as well as genes being regulated by such receptors, also within the ovary, testis and adrenal gland, where steroid hormones are synthesized and local concentrations can be in the micromolar range, or even higher. At such concentrations, classical mechanisms of steroid action would not only always be activated, but would offer no opportunity for concentration-dependent modulation of gene activity. Yet it is a feature of several steroid-dependent experimental systems that specific effects are evident with a dynamic range in a high nanomolar to micromolar concentration, for example, the effect of progesterone on endometrial decidualisation (e.g. Inoue et al., 1994), or the impact of steroids on follicle transition in the ovary (Kezele and Skinner, 2003; Yang and Fortune, 2006). Finally, the classical mechanism of steroid action does not explain the differential pattern of tissue-specific effects caused by pharmacological agents referred to as SERMs (selective estrogen receptor modulators), SARMs or SPRMs (for androgen and progesterone receptors, respectively), which may act as antagonists in one context (tissue), but as agonists in another.

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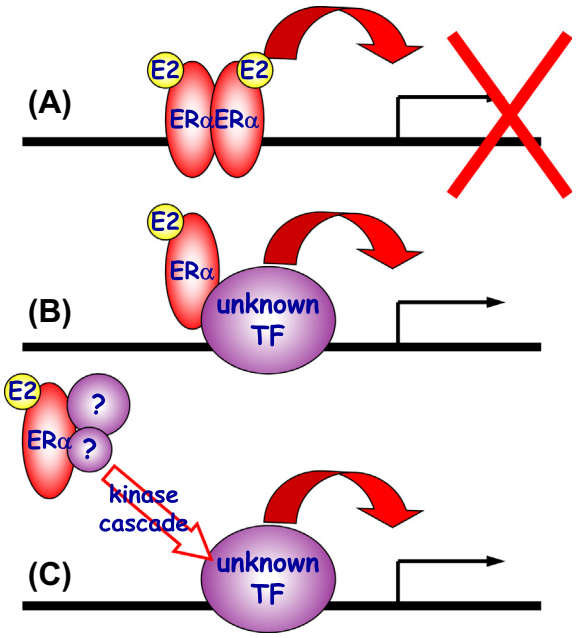

Fig. 1. Scheme to illustrate possible modes of action of ligand-activated estrogen receptors. (A) The classical model of direct binding of a ligand-activated homodimer to a canonical palindromic ERE. (B) A non-classical model employing a 'tethered' receptor, whereby a ligand-activated estrogen receptor does not bind itself to the DNA, but interacts indirectly with a promoter via an unknown intermediate transcription factor. (C) An alternative non-classical model, whereby a ligand-activated estrogen receptor forms part of a larger protein complex, possibly in the cytoplasm, which by activation of a specific kinase cascade targeting other transcription factors is able to trigger a specific transcriptional response. The red cross in the upper panel A indicates that this mechanism does not lead to transcription.

2. Mechanisms of non-classical steroid action

Non-classical actions of steroids can be classified into those involving the classical steroid receptors, though in an atypical fashion, and those which appear to function in a quite different way. Examples of the latter are activation or modulation of alternative receptors, such as GPR30 (GPER; Prossnitz and Barton, 2011) or simple alteration of membrane physiology by intercalation, as is suggested for effects of progesterone or 20alpha-progesterone on the myometrium during pregnancy (Gimpl et al., 2002). Alternatively, effects may be evident consequent on simple enzymological substrate feedback mechanisms resulting in a shift in steroid metabolism (Hillier, 1985). In spite of such effects, however, knockout mouse strategies, involving one or more of the classical nuclear steroid receptors, make it clear that the vast majority of steroid-dependent physiologies indeed depend in one way or another on the activation of the classical steroid receptors (McDevitt et al., 2008; Zhao et al., 2008). Hence, the focus here will be specifically on those non-classical steroid-dependent pathways which make use of the classical steroid receptors in some novel manner.

Briefly, two principally different non-classical pathways have been described, both involving the classical steroid receptors. In the first, or 'tethered receptor' model, a steroid receptor interacts at a specific non-canonical DNA sequence in a gene promoter by forming a complex with another transcription factor, which itself is interacting conventionally with the DNA (Fig. 1B). An example of this is the role of estradiol to activate the oxytocin receptor in the sheep endometrium by a direct binding of the ligand-activated steroid receptor to an Sp1 transcription factor (Fleming et al., 2006). In the second or 'indirect' model, activation of a steroid receptor occurs within the cytoplasm, and this in turn interacts with and activates other cytoplasmic components, leading to the stimulation of a kinase cascade whose end-point includes specific gene activation in the nucleus (Fig. 1C). Examples of this kind of pathway are described for androgen and estrogen effects in breast cancer cells (Migliaccio et al., 2005), involving synergies with IGF1 and EGF signaling pathways. Similarly, many effects attributed to the steroid progesterone in the uterus can be accounted for by cytoplasmic interactions involving the active receptor PR-B, its shorter inactive partner PR-A, and a variety of cytoplasmic kinases and other signaling molecules (Maruyama and Yoshimura, 2008; Gellersen et al., 2009).

3. Steroid sensing within the bovine ovary

The cow represents one of the best worked physiological models for ovarian function. Assuming that steroid concentrations within follicular fluid might be considered representative of the general levels influencing ovarian cells in the sexually mature cow, then values of up to 300 nM have been observed for estradiol and testosterone in both non-pregnant and pregnant situations, and up to 500 nM for progesterone (Fortune and Hansel, 1985; Einspanier et al., 1993; De los Reyes et al., 2006; Nimz et al., 2009). These values are comparable to what has been observed also in human follicular fluids from hyperstimulated patients (E2, 1200 nM; P4, 18,000 nM; T, 20 nM; Δ4A, 150 nM; E1, 4000 nM; Costa et al., 2004). Steroid receptors have also been identified and characterized within cells of the bovine ovary. ERalpha appears to be expressed more in the ovarian stroma than follicles or corpora lutea (D'Haeseleer et al., 2006), though is definitely also recorded from theca interna, granulosa, and luteal cells (Amrozi et al., 2004; Schams and Berisha, 2002; Salvetti et al., 2007; Shibaya et al., 2007). The opposite appears to be true for ERbeta, which was originally cloned from bovine granulosa cells (Walther et al., 1999) and appears to have its highest level of expression there (Walther et al., 1999; Salvetti et al., 2007). Progesterone receptors have been detected in granulosa and theca cells, as well as in corpora lutea (Lioutas et al., 1997; Schams and Berisha, 2002; Salvetti et al., 2007), with high relative expression particularly in granulosa cells (Salvetti et al., 2007). Less is known about androgen receptors in the bovine ovary, though there is good evidence for their expression also in granulosa cells, particularly within growing follicles (Hampton et al., 2004).

Unlike what is observed in cell systems using classical receptor-dependent activation of promoter–reporter gene constructs, where steroids have EC50s in the low or sub-nanomolar range, cell or tissue culture studies suggest that up to 1 μM progesterone is required to promote, for example, primordial follicle assembly (Nilsson and Skinner, 2009) or induce a specific elevation in LH receptor expression in luteal cells (Jones et al., 1992). Similarly, looking at the effects of testosterone to promote primary to secondary follicle transition, Yang and Fortune (2006) showed that maximal effect was not reached even at 0.1 μM, implying that even higher concentrations of androgen would be needed for full physiological response. Few studies have looked at estradiol effects in culture, though here again most responses appear to occur in the high nM or even μM range (Fortune and Hansel, 1979; Voss and Fortune, 1993).

Therefore, for steroids to have physiological relevance within the tissues that produce them there need to be specifically responsive systems present which allow modulation of specific effects within a dynamic range of hormone at high nanomolar concentrations. Such systems are acting at source, i.e. in those tissues where steroidogenesis is occurring, and hence can be considered as "local steroid sensing" systems, which will form part of local feedback loops regulating these tissues. They thus differ from the peripheral