Review

Why two endothelins and two receptors for ovulation and luteal regulation?

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Abstract

The ovary is a dynamic organ that undergoes cyclic structural and functional changes. Structurally, the internal architecture of the ovary constantly changes as follicles grow, rupture and transform into corpora lutea in a cyclical manner. Functionally, a variety of regulatory ovarian hormones are sequentially produced, and eggs are periodically released. As a highly vascularized organ, the ovarian structures and functions change in response to external stimuli that include but are not limited to pituitary gonadotropins. Following stimulation, the ovary synthesizes and releases autocrine and paracrine signals that play unique roles in regulating its function. Recent studies have identified endothelins as local regulators in the ovary that modulate multiple cyclic events, such as follicle growth, steroidogenesis, oocyte maturation, ovulation, corpus luteum formation and luteolysis. Interestingly, in all mammalian species examined to date, a common observation has been made: the ovary produces two pharmacologically similar endothelins (ET-1 and ET-2) but expresses two functionally different endothelin receptors (ETα and ETβ) that often give rise to opposite physiological outcomes following activation by an endothelin. In this review, the physiological significance of the presence of the two ligand-two receptor endothelin system in the ovary will be discussed.

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Introduction: Ovarian endothelin system

The ovary contains follicles that increase in size, release eggs, transform into corpora lutea and finally regress. These cyclic events begin with the onset of puberty and diminish when the follicular pool becomes depleted. The two pituitary hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH), play key roles in maintaining the continuity of these cycles by triggering biochemical and structural changes within the ovary. Changes such as these are initiated by the pituitary hormones and are mostly driven by signaling molecules that are synthesized locally in the ovary (Richards and Pangas, 2010). Among the ovarian signaling molecules are endothelins.

Our understanding of the role of endothelins in regulating ovarian function has increased markedly in recent years (Bridges et al., 2011; Meidan and Levy, 2007). Lately, discoveries in the ovary using both pharmacological and genetic approaches have generated excitement in the field of endothelin research not only because of discoveries specific to this reproductive organ but also because of the potential impact on other endothelin fields of study. Because the ovary is a highly vascularized organ, dynamic changes in blood flow occur under the influence of a variety of locally produced factors, such as prostaglandins and endothelins. Different members of these groups of hormones are involved in ovulation and the corpus luteum functions (Bridges et al., 2011; Duffy et al., 2005; Kurusu et al., 2009). The dynamic nature of
the ovary makes this reproductive organ an excellent model for studying the mechanism of the endothelin system.

Endothelins regulate many ovarian functions including folliculogenesis, steroidogenesis, oocyte maturation, ovulation, luteal formation and regression (Apa et al., 1998; Klipper et al., 2010; Ko et al., 2006; Li et al., 2011; Mancina et al., 1997; Palanisamy et al., 2006; Usuki et al., 1993). Therefore, it is not surprising that all the components of the endothelin system, such as endothelins, endothelin-converting enzymes and endothelin receptors, are expressed in all of the mammalian ovaries examined to date. The ET-1 and ET-2 endothelin isomers, but not ET-3, and at least two forms of endothelin-converting enzymes (ECE1 and ECE2) are expressed in this female gonad. Similar to other organs, two G protein-coupled receptors, endothelin receptor type A (ET_A) and type B (ET_B), which have different molecular and pharmacological characteristics, are present in the ovary and are believed to be responsible for the actions of these ligands (Bridges et al., 2011; Choi et al., 2011; Ko et al., 2006). In general, the two dissimilar endothelin receptors are constitutively expressed but are spatially confined to specific cell types in the ovary; a number of cells express both receptors, whereas other cells express one form or the other. For example, in the human ovarian follicle, the smooth muscle cells of the theca externa express the ET_A receptor, whereas ET_B receptor expression is confined to theca interna that surrounds the entire follicle (Choi et al., 2011). The theca interna is a highly vascularized cell layer that contains steroidogenic cells and separates the theca externa from the core of the follicle. The endothelin receptor expression is also apparent in the granulosa cells of the follicle (Gentili et al., 2001) as well as the corpus luteum. In the corpus luteum, the ET_A receptor is expressed in the small and large luteal cells (Mamluk et al., 1999), and both receptor subtypes are localized in the endothelial cells of this highly vascularized tissue (Karam et al., 1999; Mamluk et al., 1999).

The synthesis of the two functionally similar endothelin isomers, ET-1 and ET-2, is temporally controlled regardless of the cell type. Although the level fluctuates, ET-1 expression in granulosa cells is somewhat constitutive. In contrast, ET-2 is transiently and abundantly expressed only in the granulosa cells of ovulating follicles (Ko et al., 2006; Palanisamy et al., 2006). Endothelin-converting enzymes are expressed throughout the reproductive cycles with minimal changes in their expression levels. Dramatic changes in endothelin receptor expression are not observed during the course of follicular development; however, specificity is observed at the sites of expression (Ko et al., 2006; Palanisamy et al., 2006). Therefore, it is likely that the endothelin-mediated regulation of ovarian events is manifest by both the temporal and the spatial control of endothelin synthesis and the distribution of endothelin receptors.

ET-1 and ET-2 bind to the ET_A receptor with equivalent affinities and induce prolonged vasoconstriction when activated. The ET_B receptor also binds both ET-1 and ET-2 with equal affinity; however, the binding of either endothelin to this receptor elicits physiological responses that are largely opposite to those elicited by ET_A receptor binding in that endothelin binding of the ET_B receptor in endothelial cells causes vasodilation by inducing nitric oxide (NO) signaling pathways. Because of their slow turnover rate, ET_B receptors can act as scavengers, removing bound endothelins from the external milieu (Bremnes et al., 2000; Inoue et al., 1989; Yanagisawa et al., 1988). The physiological significance of co-expressing these two receptors in the ovary is not known, and the biological mechanism underlying how the receptors cooperate to induce a physiological outcome is of great interest not only to understand ovarian endothelin function but also for the general understanding of the activity of endothelins in other organs and systems.

Most of the organs/tissues examined to date express both types of endothelin receptors. Therefore, a physiological outcome of endothelin activity in an organ or tissue should be a result of its binding to both endothelin receptors. It is highly plausible that the activation of one receptor may elicit more obvious or discernable physiological responses than the other. However, while less prominent, the role the other receptor plays may be 'essential' for the correct regulation of the physiological function. This hypothesis suggests that when this proposed 'essential' regulation is lost, an extreme physiological outcome may follow, leading to the hyper-stimulation or hypostimulation of the endothelin-regulated physiological event; for example, the ovulatory process may continue instead of being terminated by this transient event, or a corpus luteum may persist when it is expected to demise. Currently, controversy surrounds a number of studies of the ovarian endothelin system, whereas the results of other studies are consistent (Hinchley and Milvae, 2001; Ko et al., 2006; Palanisamy et al., 2006; Weems et al., 2009). The controversies include whether the ET_A receptor or the ET_B receptor is responsible for regulating a particular ovarian function. Because both receptors are constitutively expressed in the ovary, each of the endothelin-regulated ovarian functions may require the activation of both receptors, or a single receptor may play a dominant role. In this review, the physiological significance of the two endothelin receptors expressed in the ovary will be discussed with respect to the regulation of ovulation and the corpus luteum formation.

The components of the endothelin system and the major biosynthetic processes involved have been described by others (Kawanabe and Nauli, 2011; Khimji and Rockey, 2010; Kid0 et al., 1998; Masaki et al., 1999), and the ovarian endothelin system and its known functions have been summarized previously (Bridges et al., 2011; Meidan and Levy, 2007); therefore, these aspects of the endothelin system will receive only minimal attention in this review.

**Ovulatory regulation**

The main function of an ovary is to produce eggs, which is a critically important biological event for the propagation of life. Ovulation, ending with the rupture of the follicular wall and the expulsion of the oocyte, is a central event and the hallmark of ovarian function. The mechanism governing follicle rupture has challenged researchers for decades and is still revealing its secrets. In the past, increased intrafollicular pressure was considered to be the driving force in follicle rupture (Espey and Lipner, 1963; Schroeder and Talbot, 1982). However, this concept has been challenged because the presumed pressure increase at the time of follicle rupture has never been documented (Espey and Lipner, 1963; Rondell, 1964). Instead, more attention has focused on the view that ovulation results from the proteolytic degradation of the follicular wall, which is supported by the functional presence of matrix metalloproteinases/plasminogen activators and inflammatory leukocytes in the ovary (Brannstrom and Enskog, 2002; Curry and Osteen, 2003; Oakley et al., 2010, 2011; Richards et al., 2002).

Electron microscopy and immunohistochemical examination revealed that the follicles are surrounded by layers of smooth muscle cells. In effect, follicles are interwoven with their neighboring counterparts (Choi et al., 2011; Ko et al., 2006; Schroeder and Talbot, 1982). This structural connectivity makes the entire ovarian smooth muscle network resemble a "spaghetti". In fact, isometric examination verified that the ovary as a whole exhibits a dynamic contractile response following its exposure to stimulants (Martin and Talbot, 1981; Schroeder and Talbot, 1982; Talbot and Chacon, 1982). These findings led to the hypothesis that follicle rupture may be a result of active follicular constriction, whereas the above-mentioned increased follicular tension and the protease-driven weakening of the follicle wall may help to rupture this structure when the follicle wall constricts.

The search for an intra-ovarian factor that induces follicular constriction resulted in an extraordinary discovery: ET-2 is synthesized by the ovulating follicles during an extremely narrow temporal window (~2 h) prior to follicle rupture (Ko et al., 2006; Ling et al., 2011; Palanisamy et al., 2006). Subsequent functional investigations determined that ET-2 induces a rapid and sustained constriction in ovarian strips. The constriction was shown to be induced via endothelin
receptor-mediated pathways because pre- or post-treatment of the strips with tezosentan, a dual endothelin receptor antagonist, abolished the ET-2-induced constriction (Ko et al., 2006). When administered in vivo, this dual endothelin receptor antagonist caused a marked decrease or delay in ovulation, demonstrating the importance of the endothelin receptor(s) in regulating ovulation. Notably, although a number of discrepancies have been reported among different species and by different laboratories, ET-1, the ET_{A} receptor and the ET_{B} receptor are expressed constitutively during the peri-ovulatory period, and dramatic changes in the expression levels are not observed (Ko et al., 2006; Ling et al., 2011; Palanisamy et al., 2006).

When exposed to either ET-1 or ET-2, the ovarian strips display almost identical contractile responses. Why then does the ovary express both ET-1 and ET-2? When considering this question, it is worth noting that ET-1 is constitutively expressed at a relatively low level that does not change dramatically during the entire pre- and post-ovulatory period. In contrast, ET-2 is transiently and highly expressed at the time of ovulation; otherwise, its expression is either absent or is undetectable (Ko et al., 2006; Ling et al., 2011; Palanisamy et al., 2006). Whereas there is a possibility that ET-1 and ET-2 may elicit different physiological outcomes (Ling et al., 2011), this is not likely for the regulation of ovarian contractility because both isoforms induce a similar contractile physiological response (Al-Alem et al., 2007; Bridges et al., 2010; Ko et al., 2006). It will be interesting to examine if the constitutively produced ET-1 is required for maintaining the basal contractile tone of the ovary, and if the transiently and abundantly produced ET-2 instantly increases the total ovarian endothelin content above the threshold level that is required for triggering the ovary to contract.

The ET_{A} receptor and the ET_{B} receptor are expressed in the ovary. Isometric measurements using strips of rat ovaries in vitro showed that endothelins (ET-1 or ET-2) trigger ovarian constriction via the ET_{A} receptor (Bridges et al., 2010). In this study, the ovaries contracted in the presence of an ET_{A} receptor antagonist (3 \mu M of BQ788) but did not contract in the presence of an ET_{B} receptor antagonist (3 \mu M of BQ123). Conversely, the endothelin-induced ovarian constriction was relieved by the subsequent treatment with the ET_{B} receptor antagonist (10 \mu M of BQ123) but not the ET_{A} receptor antagonist (10 \mu M of BQ788). This result appears to support the general concept that endothelin induces vasoconstriction via the ET_{A} receptor, whereas vasodilation is induced by an ET_{B} receptor-mediated pathway. The experimental results clearly demonstrate that the ET_{A} receptor is responsible for the endothelin-induced constriction. However, it is presumptive to conclude from this experiment that the activation of the ET_{A} receptor leads to ovarian relaxation (similar to vasodilation in vessels) because the relaxation induced by BQ123 in the constricted ovary may be a result of the loss of ET_{A} receptor activity rather than the ‘presumed’ relaxation-inducing activity of the ET_{B} receptor in the absence of ET_{A} receptor signaling. Therefore, the assessment of a dose-dependent contractile/relaxation response to endothelins in the presence or absence of ET_{A} receptor antagonism is warranted. In addition, it is important to determine if the ovarian ET_{A} receptor passively antagonizes ET_{A} receptor-mediated endothelin activity by reducing the total ovarian endothelin content via the well-established role of the ET_{A} receptor in endothelin clearance (Bremnes et al., 2000). A comparative measurement of the endothelin clearance rates in the ovaries of rescued ET_{A} receptor knockout animal models (Nishida et al., 2002; Riechers et al., 2004) versus control animals, followed by assessing the functional significance of the ET_{A} receptor deficiency on the ovarian contractile response, will address further the role of the ET_{A} receptor in regulating ovarian contractility.

With emphasis on the ET_{B} receptor expression in granulosa cells and in the theca interna, an ET_{B} receptor-mediated pathway leading to follicle rupture has also been proposed (Palanisamy et al., 2006). This hypothesis suggests that the ET-2 produced by granulosa cells acts on either the granulosa cells or the endothelial cells of the blood vessels in the theca interna via the ET_{B} receptor to induce nitric oxide signaling, local vasodilation and an increase in follicular pressure. In support of this hypothesis, Palanisamy et al. (2006) reported that the treatment with an ET_{B} receptor antagonist inhibited ovulation in gonadotropin-treated mice. In the same experiments, an ET_{A} receptor antagonist did little to inhibit ovulation. Interestingly, Bridges et al. were not able to block ovulation using these receptor-specific antagonists (Bridges et al., 2010). These contrasting results may be caused by differences in the pharmacokinetics of the antagonists (slight differences in the times of treatment were noted between the two studies) and/or subtle differences in the animal models used. Therefore, further studies are warranted, including the ovary-specific deletion of the ET_{A} receptor using conditional ET_{A} receptor knockout mice (Ruest et al., 2005) or rescued ET_{A} receptor knockout animal models (Nishida et al., 2002; Riechers et al., 2004).

**Regulation of the corpus luteum life span**

The ovulatory surge of gonadotropins triggers extensive structural and molecular changes in the pre-ovulatory follicle, leading not only to ovulation but also to luteal formation (Rohrer et al., 2000). Luteal formation is a complex process involving mechanisms similar to wound healing and tumor formation, and robust angiogenesis is involved in the process (Nakhuda et al., 2005). Because of the development of an elaborate network of blood vessels in the corpus luteum, this gland exhibits one of the highest blood flows per unit mass of tissue in the body (Niswender et al., 2000; Stocco et al., 2007), which is essential for supplying nutrients and hormones for proper functioning. The primary function of the corpus luteum is to produce progesterone, which is required for establishing and maintaining pregnancy. Progesterone exerts its critical role by inducing a state of quiescence in the myometrium, suppressing the maternal immune response to the fetal antigens, reducing cyclic ovarian activity during pregnancy, and it is responsible, at least in part, for mammary development (Nardo and Sallam, 2006; Ouzounian et al., 2008). Because of this unique role and its requirement for pregnancy, the formation and maintenance of the corpus luteum are tightly regulated. As such, luteal defects in women are often the cause of early miscarriage (Nardo and Sallam, 2006; Tavanitou et al., 2001). In forming a corpus luteum, a large number locally produced agents complement the action of gonadotropins (Korzekwa et al., 2010; Schams and Berisha, 2004; Stocco et al., 2007). However, inadequate luteal formation or function despite what appears to be a normal LH surge has been documented. Endothelins are an important component of the local factors that are intricately involved in regulating luteal function (Meidan and Levy, 2007). Indeed, all members of the EDN system are expressed in the corpora lutea of various mammalian species (Choi et al., 2011; Flores, 2000; Hinckley and Milvae, 2001; Mamluk et al., 1999; Zorrilla et al., 2010).

Endothelins exhibit a unique profile in the corpus luteum (Klipper et al., 2010; Ko et al., 2006). ET-2 mRNA expression is transiently induced in luteal steroidogenic cells during the early luteal phase and subsequently decreases to basal levels by the mid-luteal phase. In contrast, ET-1 mRNA remains constant during this period. Hypoxia is a strong inducer of ET-2 transcription in all species examined (Kim et al., 2009; Klipper et al., 2010; Na et al., 2008). It is noteworthy that hypoxia also stimulates VEGF expression and therefore luteal angiogenesis (Berisha et al., 2008; Tesone et al., 2005). Interestingly, ET-2 induces VEGF in the granulosa cells (Klipper et al., 2010), suggesting that elevated ET-2 in the newly formed corpus luteum, triggered by hypoxia, may facilitate luteal formation by promoting angiogenesis. Unfortunately, the endothelin receptor(s) responsible for these activities and their downstream signaling pathways have yet to be described. It will be interesting to determine how the ET_{A} and/or ET_{B} receptors are expressed in the different luteal cell types and how
the two receptor pathways interact to regulate correct luteal formation and function.

In the absence of an embryonic signal, the corpus luteum regresses, which is necessary for the initiation of a new reproductive cycle. Prostaglandin F2α (PGF2α) is the principal luteolytic hormone (Niswender et al., 2000; Schams and Berisha, 2004). In a non-fertile cycle, PGF2α is secreted from the uterus to induce luteal regression (McCracken et al., 1972). Initially, luteal steroidogenic function is impaired, and progesterone production declines (Niswender et al., 2000). This process is followed by the apoptosis of luteal cells and the structural demise of the corpus luteum (Niswender et al., 2002; Yadav et al., 2005). Based on these well-established findings, PGF2α is routinely used to induce luteolysis and for synchronizing estrous cycles in domestic species. Studies performed over the past two decades have identified ovarian ET-1 as a mediator of the luteolytic cascade in many species (Girsh and Dekel, 2002; Meidan and Levy, 2002; Meidan et al., 1999; Weems et al., 2009; Wijayagunawardane et al., 2001; Wright et al., 2001). However, consistent with the alternate hypotheses noted above, a number of findings suggest a contrasting role for ET-1 in ovulation. It has been reported that the infusion of ET-1 prevents the decrease in luteal weight and the decline in progesterone that occurs during luteal regression in ewes (Weems et al., 2009), and the authors propose that ET-1 plays an anti-luteolytic role. Interestingly, these authors also report that ET-1 increases the secretion of anti-luteolytic PGE2 by pieces of caruncular endometrium (Weems et al., 2005). In contrast, and in support of a luteolytic role for ET-1, a marked and rapid increase in luteal endothelin concentrations occurs soon after endogenous PGF2α secretion and after the administration of a luteolytic dose of PGF2α (Girsh et al., 1996; Hinckley and Milvae, 2001; Ohtani et al., 1998). Furthermore, ET-1 inhibits basal and gonadotropin-stimulated progesterone synthesis in a dose-dependent manner in bovine (Girsh et al., 1996), ovine (Doerr et al., 2008; Hinckley and Milvae, 2001), rodent (Girsh and Dekel, 2002) and human (Apa et al., 1998) luteal cells. The ETα receptor is likely responsible for this ET-1 activity because co-treatment with an ETα receptor-specific antagonist, but not an ETβ receptor antagonist, abolishes the anti-steroidal activity of ET-1 (Girsh and Dekel, 2002; Meidan and Levy, 2002).

Because ET-1 is involved in luteal regression, efforts have been made to manipulate ovarian cyclicity using this peptide. Intra-luteal ET-1 injections were shown to reduce progesterone secretion and facilitate the luteolytic process during the mid-luteal phase in cows (Shirasuna et al., 2006). In addition, following injection of the ETα receptor antagonist LU 135252 into the corpora lutea of naturally cycling animals, structural luteolysis and progesterone synthesis were delayed significantly (Watanabe et al., 2006). In a different study, using Alzet mini-osmotic pumps implanted in the ewe’s ovary, treatment with the ETα receptor antagonist (BQ-610) alone or in combination with the ETβ receptor antagonist (BQ-788) markedly reduced luteal progesterone content. In contrast, treatment with BQ-788 alone had no effect on progesterone content (Doerr et al., 2008). These in vivo studies support the previous in vivo and in vitro observations showing that ET-1, acting through the ETα receptor, is an endogenous mediator of the luteolytic effect of PGF2α on luteal progesterone secretion as well as on the structural demise of the CL; however, the evidence supporting an anti-luteolytic role for ET-1 must also be considered. Although, the data presented indicate that the ETβ receptor does not play an obvious role in this process, the possibility remains that this receptor acts as a facilitator of endothelin clearance. Therefore, it is likely that important new information will be obtained regarding luteolysis and/or endothelin clearance in CL by assessing the effect of the luteal cell-specific deletion of the ETα receptor using conditional ETα receptor knockout mice (Ruest et al., 2005) and/or measuring the loss of function of ETβ receptor using rescued ETβ receptor knockout animal models (Nishida et al., 2002; Riechers et al., 2004).

Summary and further considerations

The endothelin system has been recognized as a critical regulator of ovarian function (Bridges et al., 2011; Meidan and Levy, 2007). However, in stark contrast to the large amount of information that points to the pivotal role endothelins play in regulating each ovarian function, the mechanism underlying how endothelins regulate ovarian functions is not well understood. It is particularly important to understand the physiological roles of the ETα receptor. Currently, a role for the ETα receptor in the ovary has not been defined; the majority of endothelin-regulated ovarian processes appear to be regulated by the ETβ receptor. The absence of ETβ receptor-regulated ovarian functions suggests that this receptor may not mediate an endothelin-driven event but may act as a terminator of individual events by facilitating the removal of endothelins (Fig. 1). The known role for the ETα receptor as a facilitator of endothelin clearance fits this scenario. As a clearance facilitator, the ETβ receptor might contribute to the maintenance of endothelin homeostasis; the endothelins that are continuously or transiently synthesized must be removed for an endothelin-regulated event to be terminated. Although these presumptive roles for the ETβ receptor require experimental verification, it would be interesting to re-evaluate the existing experimental data to investigate a possible role for the ETβ receptor as a facilitator that terminates endothelin-induced ovarian processes.

During the preovulatory period, ET-1 and ET-2 are expressed in completely different manners; whereas ET-2 expression is induced abundantly and transiently in the granulosa cell layer, ET-1 is minimally and constitutively expressed (Fig. 1). It is not known if ET-1 and ET-2 exhibit the same biological activities for the induction of ovulation. If they do have equivalent biological activities, the transient and abundant expression of ET-2 suggests that the ET-2 gene is only stimulated when increased levels of ovarian endothelin are required. If this fascinating hypothesis is true, there is a possibility that ET-2 expression may be induced in other organs where endothelins play important regulatory roles. During the luteal period, however, the specificity of the ET-1 and ET-2 activities in the corpus luteum lies in their distinct temporal and cell-specific expression patterns. ET-1 and ET-2 are induced in different cell types in the corpus luteum (endothelial vs. luteal, respectively) and are controlled by different factors (PGF2α vs. LH/hypoxia, respectively). Therefore, these two very similar peptides appear to act at discrete luteal stages: ET-1 in the luteal regression stage and ET-2 in the luteal formation stage (Fig. 1).

Because of the diversity of the spatial expression of the endothelin receptors and the biological effects of ligand binding, consideration of the various subtle and additional ways that a receptor-mediated response is induced is warranted. Indeed, it is prudent to think beyond the classical model of an endothelin isoform binding to one of two independent G protein-coupled receptors to stimulate a well-characterized response. The differential binding affinity of the ETα receptor for ET-1 and ET-2 over EDN3 has been established (Davenport and Kuc, 2002), and the ETβ receptor appears to bind each ligand with similar affinity; however, homono- and hetero-dimerization of these receptors (Boesen, 2008), alternate splice variants (Hatae et al., 2007; Shyamala et al., 1994) and post-translation modifications (Horstmeyer et al., 1996) have been reported. Changes in the rate of receptor internalization (Evans and Walker, 2008; Gregan et al., 2004) and calcium signaling (Evans and Walker, 2008) are only two of the consequences that could potentially affect the biological response of receptor binding; therefore, these changes should also be considered from a functional viewpoint. Clearly, the field of endothelin research has the potential to yield fruitful results in the coming years.

Conflict of interest statement

The authors declare that there are no conflicts of interest.
Fig. 1. Hypothetical interactions between endothelins and receptors in regulating ovulation and luteal formation/luteolysis. ET-1 is constitutively expressed at a low level throughout the entire ovulatory cycle. ET-2 is abnormally and transiently expressed during the period of ovulation to luteal phases. In spite of the dissimilar expression patterns, the structural similarity and the extensive experimental evidence indicate that ET-1 and ET-2 likely elicit the same physiological responses following binding to either form of the ET receptors in the ovary. Therefore, it is the total ET content that determines the physiological outcomes of ET-mediated regulation of ovarian events. Unlike their ligands, the two functionally dissimilar ET receptors, ET\(_A\) and ET\(_B\), are constitutively expressed in the ovary. Ovulation and luteal formation, two independent but simultaneous events, and luteolysis are likely stimulated by ET\(_A\) receptor activation that may require high concentrations of ETs. The activation of the ET\(_A\) receptor induces follicular constriction leading to ovulation, increases VEGF secretion (therefore angiogenesis), promoting luteal formation and stimulates PGE2 and P4 (progesterone) synthesis causing luteolysis. The ovulatory surge in ET-2 and the luteolytic increase in ET-1 are induced by LH-induced hypoxia and uterus-derived PGF2\(\alpha\), respectively. Meanwhile, the ET\(_B\) receptor may actively or passively antagonize these events via an unknown pathway or by reducing ovarian ET content, causing the termination of ET\(_A\) receptor-initiated events.

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