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Endothelins in regulating ovarian and oviductal function

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1. ABSTRACT

In the last 30 years, remarkable progress has been made in our understanding of the biological role of endothelins in the regulation of reproductive function and fertility. A peptide hormone identified for its ability to regulate blood pressure has now been shown as a potent mediator of several reproductive pathways. Ligand- and receptor-specific roles have been identified and/or postulated during follicular development and ovulation as well as in the function and regression of the corpus luteum. In this review we have attempted to organize endothelin-mediated ovarian processes in a process-specific manner, rather than compile a review of ligand- or isoform-specific actions. Further, we have included a discussion on "post-ovarian" or oviductal function, as well as the future directions that we believe will increase our understanding of endothelin biology as a whole.

2. INTRODUCTION

2.1 Endothelins

Endothelins were first identified in a hallmark paper by Yanigasawa *et al.* (1) as potent vasoactive peptides, regulating vascular tone and blood pressure. However, diversity in biological function became readily apparent with many other roles for these peptides now described. Three isoforms of endothelins (EDN1, 2 and 3) coded by three different genes (2) have been identified to date (3). These 21 amino acid peptides are derived by cleavage of the biologically inactive "big-endothelin" by the action of at least 2 endothelin converting enzymes (ECE1 and ECE2). The biological effects of endothelin production are then determined via activation of one of two G-protein coupled receptors, endothelin receptors A and B (EDNRA and EDNRB). EDNRA has a high affinity for

Table 1. Ovarian expression of endothelins, their receptors and converting enzymes

Species	Types	Localization	References
Human	EDN1	GC, EC, LC	(87-89)
	EDN2		
	EDNRA	SC, GC, TC	(87, 90)
	EDNRB	LC	(87, 91, 92)
	ECE-1	GC, LC, TC	(91, 92)
	ECE-2		
Mouse	EDN1	GC	(46, 93)
	EDN2	GC	(46, 49)
	EDNRA	GC,	(46, 93)
	EDNRB	GC, EC	(46)
	ECE-1		(94)
	ECE-2		(94)
Rat	EDN1	GC	(14)
	EDN2	GC	(14)
	EDNRA	SC, GC	(95, 13)
	EDNRB	GC	(13)
	ECE-1	GC	(14)
	ECE-2	GC	(14)
Bovine	EDN1	GC, LC, EC	(96, 97)
	EDN2	LC	(51)
	EDNRA	LC	(97)
	EDNRB		
	ECE-1	GC, TC, LC, EC	(70, 96, 98)
	ECE-2		

GC, granulosa cells; TC, theca cells; LC, luteal cells; EC, endothelial cells; SC, smooth muscle cells

both EDN1 and EDN2 (and a low affinity for EDN3) whereas EDNRB has a similar affinity for all the isoforms (4).

Genetic deletion of components of the endothelin system has highlighted the importance of these peptides rather than defining tissue specific effects. Endothelin-1 knockout mice die of respiratory failure at birth (5), EDN2 knockout mice exhibit growth retardation and juvenile lethality (6), EDN3 knockout mice exhibit aganglionic megacolon and die young (7), EDNRA knockout mice are embryonic lethal (8) and EDNRB knockout mice die close to the age of puberty (9). Researchers have therefore relied heavily on the use of agonists and antagonists to study the function of these peptides, however conditional transgenic models are now becoming more readily available. As expected from their structure, the three purified peptide ligands are available for commercial purchase, albeit not always in a species-specific state. Similarly, a plethora of antagonists can be purchased, selective (or not) in their antagonism to either EDNRA or EDNRB. While several of the studies described in this review have utilized the "BQ compounds", receptor-specific antagonists such as BQ123 (an EDNRA-antagonist) and BQ788 (an EDNRB-antagonist), there are many non-selective or dual-receptor antagonists that have been developed for therapeutic applications. Unfortunately, the unavailability of some of these has hampered their use to the reproductive biologist.

2.2. Expression

At the level of the whole body, low amounts of endothelins are universally present. Each isoform, however, shows a tissue type-dependent pattern of expression. Endothelin-1 is widely expressed in endothelial cells, vascular smooth muscles, the central nervous system (CNS), and reproductive tissues. Levels of EDN2 are high in the intestine and kidney, whereas EDN3 is mainly expressed in the brain (3). Endothelin converting enzymes are predominantly found in the same, or in close proximity to, those cells expressing each endothelin. Endothelin receptor subtypes are also distinctive in their localization, with co-localization to various tissues signifying the presence of autocrine and paracrine functions. At the level of the ovary, all the components of the endothelin system with the exception of EDN3 are expressed. The expression sites of EDN1, EDN2, EDNRA, EDNRB, ECE1 and ECE2 in the human, mouse, rat and bovine are summarized in Table 1. It should be noted though that throughout the different species, a high level of similarity is seen in their spatial localization. EDN1 expression is apparent in the granulosa cells, luteal cells and endothelial cells of the ovary, whereas EDN2 appears exclusively expressed in the granulosa cells. Endothelin receptor A is found in a variety of cells that include granulosa cells, theca cells, smooth muscle cells and luteal cells and EDNRB is found in the granulosa cells, luteal cells and endothelial cells. Furthermore, both the converting enzymes (ECE1 and ECE2) are expressed in the granulosa cells, luteal cells and theca cells.

3. OVARIAN FOLLICLE

Not long after the identification of endothelins by Yanagisawa *et al.* (1), a role for these peptides within the ovarian follicle emerged. The effects during follicular development appear to be mediated by EDN1, rather than EDN2 and EDN3, although a role(s) for these other isoforms during folliculogenesis should not be discounted. Overall, levels of EDN1 within the ovary are regulated with

stage of follicular development. Kamada *et al.* (10) reported that the concentration of EDN1 in large porcine follicles was higher than that in small to medium sized follicles and Flores (11) expanded on this using *in situ* hybridization and immunohistochemistry, also utilizing tissues collected from swine. Those authors reported that EDN1 was absent in primordial and primary follicles and that less than 5% of secondary follicles expressed this isoform. EDN1 immunoreactivity was then reported to increase in the granulosa cells of follicles as the antrum developed, with EDN1 present in 2/3 of large antral follicles.

Continuing through follicular development and into the periovulatory period, Shimizu *et al.* (12) evaluated the expression of mRNA for EDN1 in follicular samples collected from cows after gonadotropin releasing hormone (GnRH) was administered to induce the preovulatory surge of luteinizing hormone (LH) and follicle stimulating hormone (FSH). In their study, mRNA for EDN1 remained relatively stable after treatment with GnRH, while mRNA for both EDNRA and EDNRB decreased. Of course, the role for endothelins during the next process, rupture of the follicle or ovulation, has become a topic of intense interest that will be discussed later in this review. To turn to receptor binding, Iwai *et al.* (13) localized mRNA for EDNRB to the granulosa cell layer of healthy dominant follicles collected from rats by *in situ* hybridization. Although only low levels of mRNA for EDNRA were detected in their study, other studies using tissues collected from rodents indicate relatively constitutive expression of both isoforms (14, 15). Overall, expression of endothelin receptors does not appear to be a limiting factor to their biological function within the ovary, however Usuki *et al.* (16) reported that FSH up-regulates the expression of both isoforms in granulosa cells collected from rats, suggesting some regulation of function at the level of receptor expression.

From a functional perspective, the role for endothelins within the follicle centers around the regulation of steroidogenesis, however the stimulation of granulosa cell growth and DNA synthesis by EDN1 have also been described (10). Endothelin-1 inhibits LH- (17), FSH- (18) and hCG- (10) stimulated progesterone secretion by granulosa cells, suggestive of a function for this peptide in the control of premature luteinization. Tedeschi *et al.* (19) demonstrated that this inhibition of progesterone secretion was due to the down-regulation of progesterone-forming enzymes (cholesterol side-chain cleavage and 3 beta-hydroxysteroid dehydrogenase (HSD)/isomerase) as well as the up-regulation of those involved in progesterone metabolism or degradation (20 alpha-HSD and 5 alpha-reductase). Several authors also examined signaling pathways in their studies. EDN1 inhibits cAMP accumulation by granulosa cells (17, 18) and consistent with this, EDN1 also inhibited forskolin-stimulated progesterone accumulation by granulosa cells (20). Kamada *et al.* (21) took this line of research further, provided evidence that EDN1 may also act at sites distal to the generation of cAMP as a second messenger. The requirement for endothelins in the regulation of follicular steroidogenesis is, however, more than a simple steroidogenic brake as FSH has been shown to increase, while LH decrease, the specific binding for EDN1 to cultured rat granulosa cells (22).

It is worthy to note that non-progestogenic attributes have also been described for EDN1, including acting as an inhibitor in the synthesis of estradiol (23). However, the regulation of estradiol by EDN1 is not as clearly defined as for progesterone. Interestingly, using a perfusion system where preovulatory follicles were collected from cows and implanted with capillary dialysis membranes, Acosta *et al.* (24) observed the now expected inhibition of progesterone secretion by EDN1, however EDN1 stimulated the secretion of estradiol. These authors also reported the release of EDN1 from the theca layer of these follicles and an interaction of endothelins with prostaglandins (PGE_2) and cytokines. Given this, it appears that the local regulation of follicular function by these peptides must be considered inclusive to both steroids, the theca layer and with respect to the cross-talk that occurs between these follicular compartments.

3.1. Ovulation

The ovulatory process, ending with rupture of the follicular wall and expulsion of the oocyte, is a central event in the reproductive cycle. Perhaps due to human curiosity concerning the core process of egg production, the mechanism governing ovulation has challenged researchers for decades. Up to the middle of the 1960s, it had been generally accepted that increased intrafollicular pressure was a driving force of rupture (25). However, the consensus was challenged by reports that there was no significant increase in pressure before ovulation and that artificially increasing intrafollicular pressure by the injection of saline into preovulatory follicles of rabbits did not induce rupture (26, 27). These reports steered researchers to the view that decreasing tensile strength of the follicular wall was a main biochemical event required for ovulation, thus emphasizing the importance of proteolytic enzymes during this cascade. Since then, numerous studies have demonstrated the involvement of matrix metalloproteinases (MMPs) and the plasminogen activators (PAs) in the degradation of the collagenous tissues, which is present in the basement membrane, theca externa, and tunica albuginea of the follicular wall (28). Subsequent findings showed that both ovarian cells and leukocytes are involved in the tissue degradation process (28-30). In addition to the increased intrafollicular pressure and proteolytic activity, in the early 1980s, Talbot and her colleagues indicated that follicular contraction might be associated with follicle rupture (31-33). This idea was supported by the identification of the presence of smooth muscle cells in the follicular wall and evidence of their contraction in the gonadotropin-stimulated hamster ovary using electron microscopy (32). Interestingly, not knowing a direct trigger of the contraction, a neuronal regulation was suggested as a controlling mechanism of the follicular constriction.

We recently sought to identify an endogenous molecule that was produced immediately prior to ovulation

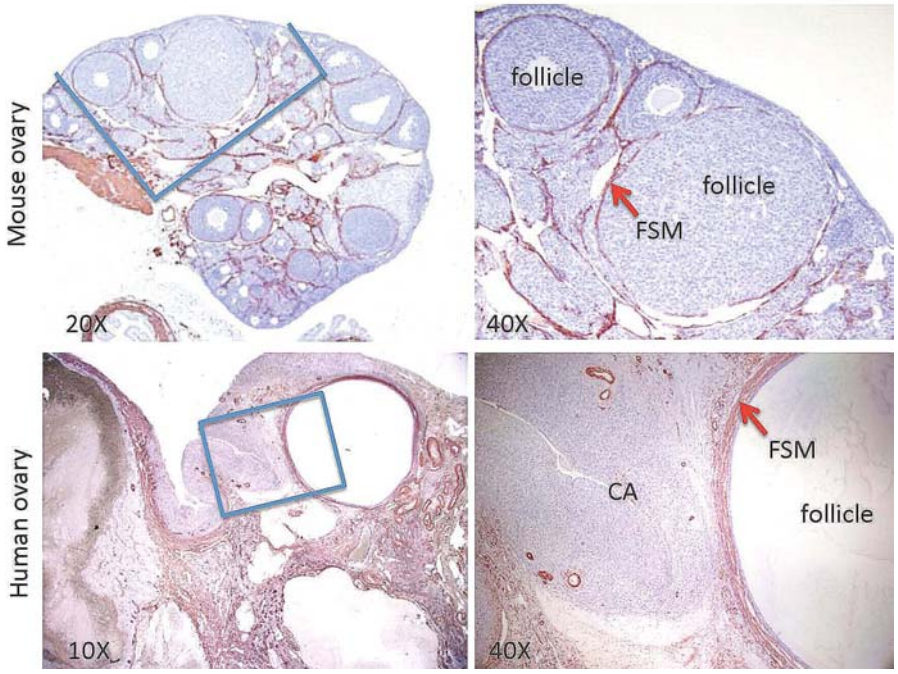

Figure 1. The ovarian smooth muscle network. (Top panels) A section through a 5-week old mouse ovary. (Bottom panels) A section through a 47-years old woman's ovary. The sections were stained with an antibody for α-SMA (reddish staining) followed by a counter staining with Mayer's hematoxylin (blue). The high magnification images (400X) were taken in the boxed areas. CA, corpus albicans; FSM, follicular smooth muscle.

and which induced follicular constriction. A genome-wide gene expression profiling approach was employed and we gave attention to vasoconstrictors that were expressed prior to and/or at the time of ovulation. This approach led us to the identification of a potent vasoconstrictor, EDN2. In striking contrast to most of the components that are expressed constitutively in multiple cell types, the pattern of EDN2 expression is quite unique; mRNA for EDN2 is dramatically increased within hours of ovulation and specifically in granulosa cells of the periovulatory follicle. At all other times, follicular expression of mRNA for EDN2 appears to be maintained at a basal level. The physiological properties of this ligand and unique expression pattern made EDN2 a strong candidate as an endogenous molecule that could induce follicular constriction at the time of ovulation. We therefore examined whether this peptide induced contraction of ovarian tissue. For this purpose, strips of ovarian tissue were dissected from the ovaries of gonadotropin-primed rats and the isometric contraction response recorded after treatment with the peptide ligands (14). Using this approach we found that EDN2 induces a rapid and sustained contraction of ovarian strips and that the addition of tezosentan, a dual endothelin receptor antagonist, decreased the contractile force (14). When administered *in vivo*, treatment with tesozentan resulted in a marked decrease or delay in ovulation.

In addition to EDN2, several other vasoconstrictive molecules have been identified in the preovulatory follicle, including prostaglandins (PGs), pituitary adenylate cyclase activating peptide (PACAP) and vasoactive intestinal peptide (VIP) (34, 35). Their pattern of ovarian expression dramatically increases after stimulation by the pre-ovulatory surge of gonadotropins or by injection of hCG. However, it is unlikely that these molecules play a direct role in inducing the contraction involved in follicular rupture because the time of peak expression of the relevant genes regulating their production is temporally distant from the time of follicle rupture. For example, the peak in the expression of mRNA and/or protein for PACAP and VIP are 6 to 9 hours from the actual time of follicular rupture. In addition, their contraction-inducing abilities are weaker than those of EDN2 (unpublished data). With respective to prostaglandins, studies in domestic ruminants indicate that both vasoconstrictive and vasodilatory prostaglandins and their receptor subtypes are present at ovulation (36, 37). This, along with the temporal regulation observed in the expression of mRNA for the specific receptor subtypes throughout the entire periovulatory period, would suggest that prostaglandins are not acting as the primary contractile signal at the time of follicle rupture.

In regard to the mechanism that EDN2 utilizes in inducing follicular constriction, it is likely that EDN2 released from the granulosa cells travels across the follicular wall to stimulate the contraction of smooth muscle cells in the theca externa as well as in the ovarian interstitium. In fact, the presence of smooth muscle cells in the ovary has been reported by multiple groups of researchers (38-45). As shown in Figure 1, each follicle is surrounded by a layer of smooth muscle cells, with neighboring smooth muscle layers directly contacting or

interweaving with each other. This forms a "sponge-like" smooth muscle network at the level of the ovary. The contraction of individual smooth muscle cells should then generate a synergic constriction force at the level of the ovary, leading to simultaneous follicle rupture and, thus, rupture of weakened follicles and release of their oocytes. This may also explain why dozens of oocytes are released within a narrow window of time when follicular development and ovulation are stimulated and synchronized by treatment with exogenous gonadotropins.

It should be noted that functionally, both EDNRA and EDNRB are present within the ovary and upon activation by ligand binding, these receptors often elicit opposite physiological responses in their target tissues. Activation of EDNRA leads to vasoconstriction and the contractile forces that we believe are required for ovulation, whereas EDNRB has other actions including that of vasodilation. With emphasis on the expression of EDNRB in granulosa cells and the theca interna, an EDNRB-mediated pathway to follicle rupture has also been proposed (46). This hypothesis speculates that EDN2 produced by granulosa cells acts on either granulosa cells or the endothelial cells of the blood vessels in the theca interna via EDNRB to induce nitric oxide signaling, local vasodilation and an increase in follicular pressure. In support of this hypothesis, Palanisamy *et al.* (46) reported that treatment with an EDNRB antagonist inhibited ovulation in gonadotropin-primed mice. In their same experiments, an EDNRA antagonist showed little activity in inhibiting ovulation. Interestingly, we were not able to block ovulation using these receptor-specific antagonists (47). These contrasting results may be caused by differences in the pharmacokinetics of the antagonists (slight differences in the time of treatment were noted between the two studies) and/or subtle differences in the animal models utilized. Further studies are therefore warranted, including the use of conditional gene targeting approaches, which should clearly characterize the role(s) of endothelin receptor signaling during ovulation.

With respect to the mechanism regulating EDN2 expression in granulosa cells, Palanisamy *et al.* (46) reported that the progesterone receptor (PR) and Kim *et al.* (48) that PPAR were the upstream transcription regulators of EDN2 gene expression. We have reported that hypoxia is a main inducer of EDN2 (49). In our studies, we first observed that granulosa cells cultured under hypoxic condition expressed significantly higher level of mRNA for EDN2. We then conducted a promoter analysis which revealed that when presumptive hypoxia-inducing factor binding sites were deleted from the promoter, its activity significantly decreased. Interestingly, in this same study we also demonstrated that neither RU-486 (a PR antagonist) nor indomethacin (a cyclooxygenase inhibitor) altered EDN2 gene expression, suggesting no involvement of those pathways in the regulation of EDN2 within the ovary. Meanwhile, the importance of hypoxia in the regulation of EDN2 has been reported by others. Using mice, Kim *et al.* (50) reported that injection of echinomycin, an inhibitor of hypoxia inducible factors, suppressed both EDN2 expression and ovulation. Consistent with this, Klipper *et al.* (51) reported that treatment with cobalt chloride, a hypoxia-mimicking agent, elevated EDN2 in bovine granulosa cell cultures. Intrafollicular oxygen concentrations are also believed to decrease with development of the preovulatory follicle (52). Overall, it should be not surprising to find consensus for hypoxia as an inducer of EDN2 expression.

Taken together, these findings have led us to formulate an 'inclusive' model of follicle rupture which incorporates the three main factors described above: intrafollicular pressure, degradation of collagenous tissue, and follicular constriction. Base upon this model, the preovulatory surge of gonadotropins induces the sequential activation of proteolytic enzymes causing partial digestion of the extracellular matrix, including that of the follicular basement membrane. Concurrently, gonadotropin-induced ovarian inflammation brings more fluid into the follicular antrum, increasing follicular size, decreasing oxygen content and further weakening the structure of the follicular wall. While the integrity of the intrafollicular structure becomes loose due to the degradation of the extracellular matrix of the follicular wall, the overall follicular structure is maintained because of the balance between the pressure from intrafollicular fluid and the tensile force of theca externa. Finally, as a final trigger for ovulation, EDN2 causes a rapid and sustained follicular constriction via EDNRA at the time when the follicle is mature and ready to release its oocyte, breaking the balance and therefore resulting in rupture of the follicular wall and ovulation.

4. CORPUS LUTEUM

An ovulating follicle then goes through a complete transformation to become the endocrine organ known as the corpus luteum (CL). This transformation includes the formation of new vasculature or angiogenesis, leading to the formation of an extensive capillary network within the CL. This is required to support these endocrine cells, supplying oxygen and precursors of steroid production, as well as releasing progesterone in preparation for potential implantation and the maintenance of pregnancy (53-55). VEGF (vascular endothelial growth factor) plays a central role in angiogenesis. In the ovary, luteinizing granulosa cells secrete VEGF proteins that in turn activate two VEGF receptors (VEGFR-1 and -2) that are localized to luteal endothelial cells and steroidogenic cells (56, 57). It is well established that hypoxia is a key factor in inducing VEGF in the luteinizing granulosa cells. Interestingly, Klipper *et al.* (51) reported that EDN2 directly induces VEGF in granulosa cells of the bovine ovary. It is also noteworthy that we have shown that the HIF-1α binding site in the proximal promoter region of the EDN2 gene is required for the induction of EDN2 expression by hypoxia (49). Taken together, it is very likely that the well-known hypoxia effect on VEGF expression and angiogenesis, and therefore CL formation is achieved by inducing EDN2 via activating HIF-1α. In the meantime, the EDNRA is believed to mediate EDN2 action as this receptor has been shown to be responsible for endothelin's action in a variety of angiogenic pathologies (56, 58, 59).

Endothelin-1 is a CL-peptide (60) that can bind locally to this transient endocrine organ (61) and it is now well established that luteal-derived EDN1 plays a role during luteolysis. However, the precise role(s) for this peptide during luteal regression remains controversial, in part due to the variety of techniques and experimental models that have been utilized to address this paradigm. Within the confines of a normal estrous cycle, luteal and systemic concentrations of EDN1 have been well characterized in the cow. Ohtani *et al.* (62) reported increasing plasma concentrations of EDN1 during the final days of the estrous cycle, concomitant with decreasing progesterone as the corpus luteum regressed. Interestingly, peripheral concentrations of EDN1 continued to rise well after the demise of the CL with concentrations of this peptide peaking around the time of estrus. In a later study by Shirasuna *et al.* (63), microdialysis (MDS) lines were implanted into the corpora lutea of naturally cycling cows and the local release of several hormones of interest, including EDN1, determined during spontaneous luteolysis. These authors observed a pulsatile pattern in the local release of EDN1 by the regressing CL, truly suggestive of a regulated, physiological role. From the timing and pattern of release, it is therefore tempting to conclude that EDN1 acts in a luteolytic manner, in concert with PGF2α to ensure the demise of this steroidogenic tissue, however an alternate hypothesis has been proposed. With EDN1 reported to stimulate *in vitro* secretion of the luteotropic prostaglandin, PGE, but not PGF2α, by slices of luteal tissue collected from cows (64), a protective role for this endothelin on luteal function has been postulated and must be considered.

Weems *et al.* (65) went on to install catheters into naturally cycling ewes and infused EDN1 into either the uterine lumen or ovarian pedicle adjacent to the luteal-bearing ovary and their results again indicated a luteotropic, not luteolytic, role for this peptide. In ewes infused with vehicle, the expected spontaneous luteal regression was observed. However, by Day 18 of the cycle, luteal weights remained 2.5 to 3-fold greater and progesterone secretion remained above 1 ng/ml (the benchmark of luteal function) in animals that had been infused with this peptide. That being said, several experiments utilizing receptor-specific antagonists and/or models of induced luteal regression have produced a contrasting result. Doerr *et al.* (66) utilized osmotic minipumps filled with several of the commercially available receptor-specific antagonists to directly infuse the CL during PGF2α-induced luteolysis. Infusion of the EDNRA-specific antagonist BQ610 maintained serum concentrations of progesterone, suggesting a luteotropic role of the antagonist and hence, luteolytic role of the endogenous peptide. Consistent with this, intrauterine infusion of BQ610 on Days 16 to19 of the estrous cycle appeared luteotropic in a study from Connecticut, delaying luteal regression in cattle for ~24 h (67). Unfortunately, a compilation of all the literature describing the pharmacological manipulation of luteal function *in vivo* will not answer the most pertinent question of whether EDN1 is luteolytic or luteotropic. With a thorough analysis of the process of luteal regression beyond the scope of this paper, and with several comprehensive reviews readily available (68-70), the authors urge readers to follow this line of inquiry with an open mind and an understanding of the physiology behind spontaneous versus PGF2α-induced luteal regression and the pharmacokinetics of the various compounds used for experimental purposes.

5. OVIDUCT

Like the ovary, the oviduct has presented itself as a tissue with function(s) mediated by this family of peptide hormones. Also consistent with the ovary is spatial and temporal regulation in the expression of functional isoforms of endothelins and their receptors, with luminal epithelial cells consistently reported as the dominant site of endothelin production (71-73). Cyclic regulation of endothelin gene expression is reported to occur during the estrous cycle in cattle (74), a result consistent with our recent findings in mice (75) and the oviduct ipsilateral to the ovulating ovary appears to produce more peptide than the contralateral side (76). From a ligand perspective, the focus of oviductal endothelins has centered around EDN1 (73, 74) with estradiol, tumor necrosis factor-α (TNFα) and angiotensin II all shown to stimulate this isoform (77, 78). Reinhart *et al.* (72) did report an inhibitory effect of estradiol on the synthesis of this peptide *in vitro* though. Overall, the role of endothelins in the regulation of oviductal function is well established, however the role(s) attributed to this family of hormones should not be considered specific to EDN1. We have postulated a role for ovarian-produced EDN2 (79) and most recently, oviductal produced EDN3 (75), which is described in more detail below.

Studies on the localization of endothelin receptor subtypes within the oviduct show some inconsistency in the literature, however the presence of both receptor subtypes within this tissue has now become relatively well documented. Sakamoto *et al.* (73) noted dominant localization of EDNRB to the tubal epithelium of the human oviduct, with some stromal expression apparent, which is consistent with the pattern of expression that we observed in the mouse (75). However, they identified EDNRA to be located more specifically to the muscle layer of the oviduct, in contrast to our immunohistochemical findings which pointed again to the luminal epithelium as a major site of action for EDNRA. We did still detect some stromal expression of this isoform though. From a functional viewpoint, the majority of reports on endothelins within the oviduct focus on the ability of these peptides to affect smooth muscle tone, modulating oviductal contractility and transport of the gametes (71, 74, 79-81). There is a strong physiological basis for the regulation of oviductal tone by these peptides, with receptor subtype-specific contractile responses reported (80) as well as the temporal regulation of contractile forces over the course of an estrous cycle (74).

Given the notable role of endothelins in the regulation of vascular tone and hence blood pressure, a role in contractility within the oviduct is intuitive. However it is still interesting to note, given the indication of both epithelial and stromal receptor localization, that the concurrent (82) regulation of epithelial function by these

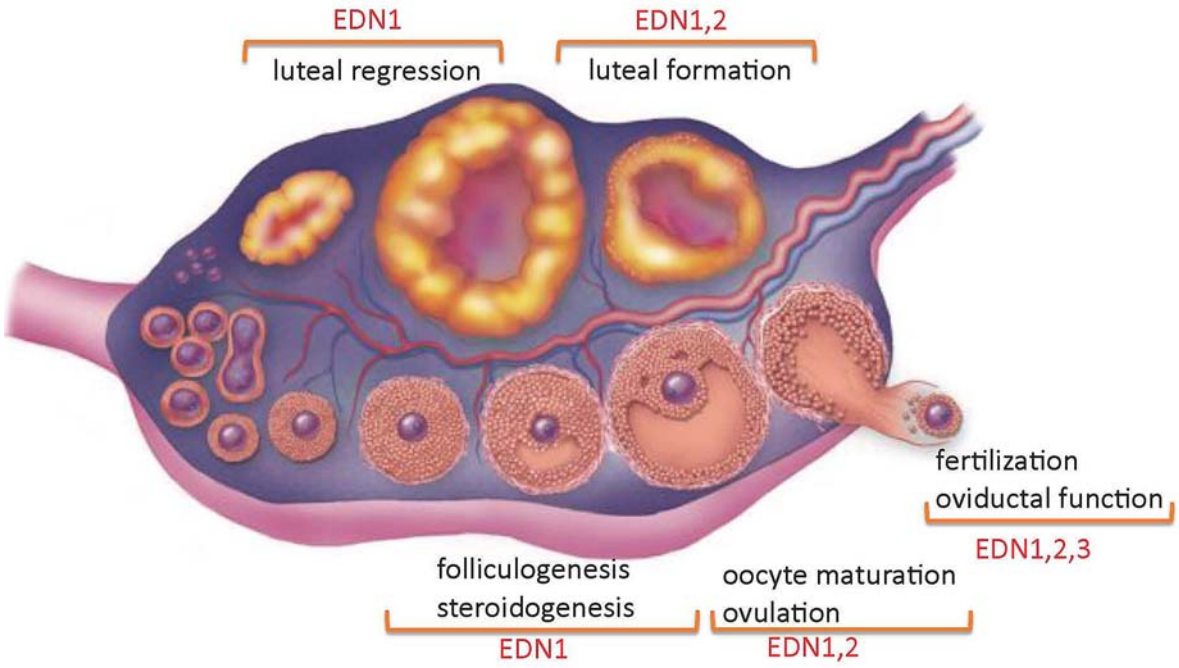

Figure 2. Endothelin types responsible for ovarian and oviductal function. Different stages of follicles, oocytes and CL are shown. The biological target of endothelin actions and responsible endothelin subtypes are listed.

peptides is not as well defined. One focus of our research has shifted in that direction, and we have now proposed an independent, non-contractile role for endothelins within the oviduct that we believe is acting concomitant to this established role in contraction. Using mice, we reported a gonadotropin-induced increase in mRNA for EDN3 within the luminal epithelial cells of the oviduct (75) and hypothesized that this isoform was regulating the epithelial cell secretions that are known to affect fertilization and early embryonic development (83, 84). With EDN3 perhaps the least recognized isoform, at least from the viewpoint of a reproductive biologist, this was an exciting discovery for us. We also observed the specific induction of mRNA for EDN3 within the isthmus of the oviduct around the time of ovulation, which is again consistent with the cyclic regulation of secretions by this tissue (85, 86). In this report, we also provide evidence for a dramatic requirement for endothelins during early embryonic development. We treated mice with the dual-receptor antagonist tezosentan around the time of breeding and observed a marked decrease in the number of 2-cell embryos that we could collect from within their oviducts.

6. FUTURE STUDIES

Over the last 20 years, EDN1 has been the major subject of study within the ovary, leading to recognition of the importance of these peptides in folliculogenesis, steroidogenesis and luteal function. Excitement to the study of endothelins in female reproduction was added by the identification of EDN2 as one of the final triggers of follicle rupture and as a critical regulator of luteal formation. Further excitement is brewing as the third endothelin, EDN3, has been identified as a major endothelin in the oviduct (Figure 2). In the next few years, it is expected that endothelin research in the ovary and oviduct will lead to a better understanding of mechanisms involved, with an emphasis on the receptor-type specific endothelin regulated pathways. Conditional gene targeting approaches are anticipated to be used heavily and a significant effort made to translate the findings to clinical applications.

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