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Endothelin Expression in the Uterus

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The endothelins (ETs) comprise a family of 21 amino acid peptides, ET-1, ET-2 and ET-3, first demonstrated as products of vascular endothelium. Subsequent work showed that they are also found in non-endothelial cells from a variety of tissues such as breast, parathyroid and adrenal gland. At first, the ETs were recognized for their pressor effects. However, ET administration *in vivo* initially caused hypotension at low concentrations by triggering the paracrine release of endothelial-derived vasodilators. The ETs exert powerful contractile actions on myometrium and other types of smooth muscle and are mitogenic, or co-mitogenic for fibroblasts, vascular smooth muscle and other cells. Demonstration of extravascular ET in endometrium has revealed a powerful vasoconstrictor which might act on the spiral arterioles to effect a powerful and sustained contraction of vascular smooth muscle. ETs might also contribute to the process of endometrial repair. In addition, the ETs appear to play a fundamental role in the control of uterine function in pregnancy. Effects on myometrial contractility have been implicated in the mechanisms governing the onset of normal and pre-term labour, and the peptides are likely to be key determinants of placental blood flow by binding to vascular smooth muscle receptors in the placenta.

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INTRODUCTION

The endothelins (ETs) comprise a family of 21 amino acid peptides, ET-1, ET-2 and ET-3 (Fig. 1), first demonstrated as products of vascular endothelium [1, 2]. Subsequent work has shown that the ETs are also found in non-endothelial cells from a variety of tissues such as breast, parathyroid and adrenal gland [3–5].

A wide range of physiological properties and pharmacological actions have been attributed to the compounds. Initially, the ETs were recognized for their vascular effects, being described as potent vasoconstrictors. However, ET administration *in vivo* initially caused hypotension at low concentrations by triggering the paracrine release of endothelial-derived vasodilators [1, 6]. In addition to effecting vasoconstriction by binding to vascular smooth muscle, the ETs exert powerful contractile actions on myometrium and other types of smooth muscle [7, 8]. Furthermore, the peptides are mitogenic, or co-mitogenic for fibroblasts, vascular smooth muscle and other cells [9, 10].

ET actions are mediated by binding to distinct cell surface receptors. Two ET receptor subtypes have been described in the human, ET_A and ET_B [11]. The ET_A receptor is selective for ET-1 and ET-2; binding of peptide to ET_A receptor on vascular smooth muscle is thought to be the mechanism by which the ETs exert their pressor actions. On the other hand, the ET_B receptor binds all three ET isoforms with similar affinity. Binding of ligand to ET_B may permit the ETs to release prostaglandins or nitric oxide in a paracrine fashion [12].

This paper will review the evidence for a role for the ETs in the local control of both the uterine vascular bed and myometrial contractility (Fig. 2).

ETs IN THE UTERUS

ET-like immunoreactivity (ET-IR) has been demonstrated in the endometrium of different species including that of the rabbit, sheep and human [13–18]. Using the ET-1 polyclonal antibody RAS 6901 (Peninsula Laboratories), ET-IR was measured in conditioned medium in which collagenase-dispersed rabbit endometrial cells had been grown to confluence. The time-dependent release of ET-IR reached a plateau after 12 h, and was stimulated 7-fold by the oxytocin analogue [Thr^4, Gly^7] oxytocin [13]. It was suggested that oxytocin and vasopressin might potentiate their own

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stimulatory action on myometrium by releasing local prostaglandins and ET-1.

Subsequent studies using RAS 6901 antibody determined the immunocytochemical localization of ET-IR in rabbit endometrium, and assessed the presence of specific binding sites for iodinated ET-1 and ET-3 [14]. In immature animals, ET-IR was localized to endometrial surface epithelium, but after priming with estradiol, either alone or with progesterone, staining was less apparent in epithelial cells, and was localized to the stroma, with the greatest intensity seen in stromal cells surrounding endometrial glands. In sheep, ET-IR was detected in endometrium and myometrium throughout the estrus cycle and in increasing concentrations in early pregnancy [15]. ET-IR was localized to most cell types, and appeared to be under ovarian steroid control, the intensity of staining being reduced by estradiol plus progesterone in all tissues examined [15].

The distribution of ET-IR in human endometrium differs. ET-IR was sought in sections of endometrium and myometrium from women undergoing hysterectomy for benign disease. Using primary antibody raised in rabbits against the C-terminal heptapeptide of ET-1, ET-IR was localized to vascular endothelium in both endometrium and myometrium, and endometrial glandular epithelium [16]. A similar pattern of staining was seen using primary antibody raised against the cyclized N-terminal, ET-$1_{(2-13)}$, or using commercially obtained antibodies against whole ET-1 (Peninsula, RAS 6901), or ET-3 (Peninsula, RAS 6911). Immunocytochemical localization of ET-IR using another non-isoform-selective polyclonal antibody from Cambridge Research Biochemicals revealed low levels of stromal staining throughout the cycle, with the strongest staining in luminal epithelium in the secretory phase and in glandular epithelium in the late secretory phase [17]. Variation in results between different studies may be accounted for in part as polyclonal antibodies raised against either whole ET or part of the peptide may recognize different ET isoforms by cross-reacting with different portions of the ET molecule itself.

ET-IR was detected in the supernatant of enriched endometrial epithelial and stromal cell preparations after 5–8 and 1–4 days in culture, respectively [18]. These studies also demonstrated that the concentration of mRNA for the ET precursor prepro-ET-1 was greatest in premenstrual and menstrual endometrium, compared with proliferative and secretory tissues [18].

ET isoforms

Antibody directed against the common C-terminus cannot determine the exact nature of endometrial ET-IR because of cross reactivity between the various ET isoforms. This problem was addressed using high performance liquid chromatography and immunocytochemistry with primary antibody raised against the precursor big ETs (pro-ETs) [19]. Endothelin-1 was detected in seven of eight samples. ET-2 and ET-3 were seen in four and five specimens respectively. Big ET-IR was localized predominantly to endometrial glandular and luminal epithelium in a similar pattern to that of the biologically active "mature" 21 amino acid ET peptides themselves [19].

That the different ET isoforms are present in human endometrium was further supported by the demonstration of mRNA for ET-1, ET-2 and ET-3 using the polymerase chain reaction. Messenger RNA for all three ET isoforms was found in tissues obtained throughout the menstrual cycle [20]. Furthermore, the detection of spliced variants of big ET-2 and ET-3 in endometrium suggested that alternative splicing might be utilized to control gene expression or the function of the mature peptide [20, 21].

Synthesis and metabolism

ET bioavailability is determined by the balance between the synthesis and release of the mature 21 amino acid peptide and its degradation. The ETs are thought to be synthesized and stored as their big (prepro- and pro-) forms. Release of the mature peptide

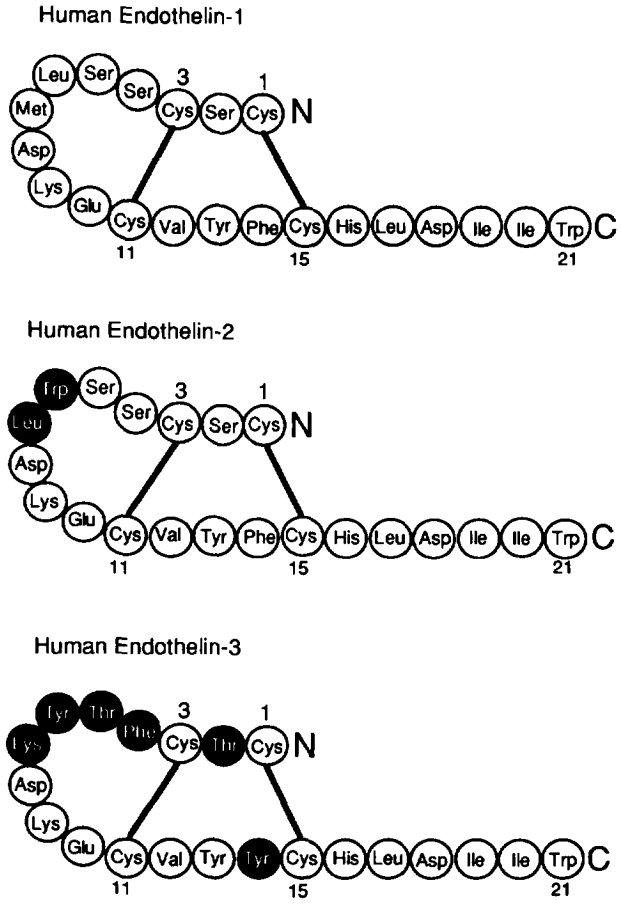

Fig. 1. The amino acid sequence of the human ET isoforms, ET-1, ET-2 and ET-3. Note the common C-terminal. Differences in the amino acid constitution of the N-terminal are shown by the filled circles.

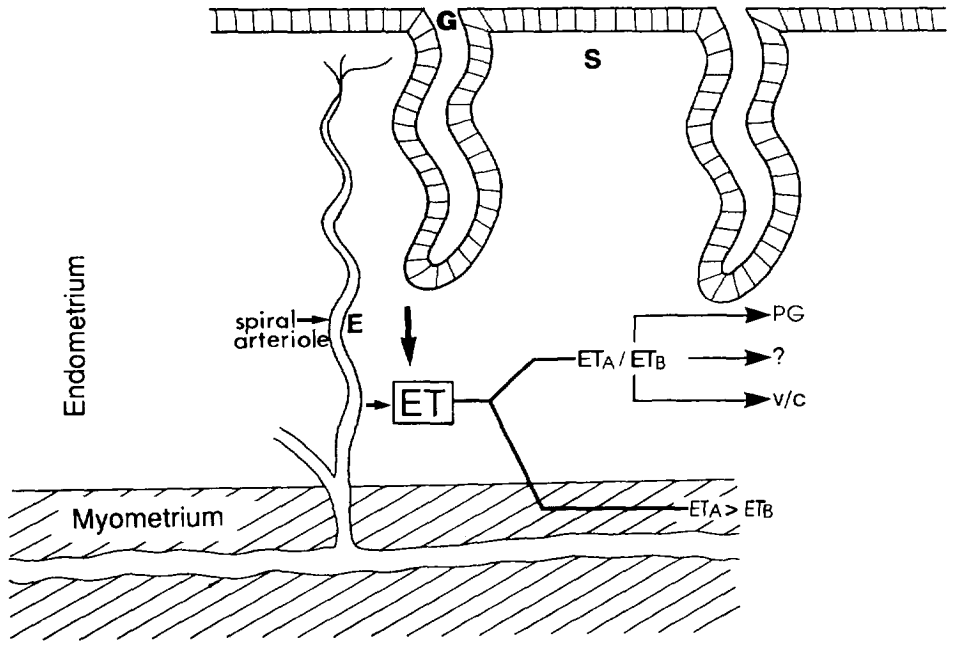

Fig. 2. ET expression in the human uterus. In endometrium, ET is present in vascular endothelium (E) and glandular epithelium (G). Binding to ET_A or ET_B receptors may cause vasoconstriction (v/c) and the release of prostaglandins (PG) or other agents (?). The predominant ET receptor in human myometrium is ET_A. S, stroma.

is effected following cleavage, catalysed by one or more ET converting enzymes [1, 22a, 22b]. A variety of factors have been shown to stimulate ET release *in vitro*, including the mechanical stimulus of shear stress, thrombin, angiotensin II, vasopressin, transforming growth factor(TGF)β and oxyhaemoglobin [23, 24].

In human endometrium, ET release into the supernatant of epithelial cell cultures was not stimulated by agents known to be effective in other cell types, but was stimulated by fetal calf serum [25], an action which might be attributable to a TGFβ-related peptide [26]. Others showed that TGFβ stimulated ET release from stromal cell cultures, but release was greater from endometrial stromal cells maintained in serum-free medium than from cells grown in serum-supplemented conditions [18]. Fundamental differences in culture conditions might explain these apparent discrepancies.

ETs are rapidly inactivated *in vivo* by encephalinase (membrane metalloendopeptidase—EC 3.4.24.11) [27, 28]. Encephalinase activity has been localized to stromal cells in human endometrium [29]. There was a 6-fold increase in specific activity of the enzyme in endometrium between early proliferative and mid secretory phases of the menstrual cycle, with a decline in activity paralleling the fall in progesterone concentrations after luteolysis [30]. Encephalinase activity was low during menstruation itself, suggesting that bioavailability of ET may be increased at this time, enabling the peptide to play a role in the local control of menstrual haemostasis.

ET RECEPTORS IN THE UTERUS

Besides the factors controlling synthesis, release and metabolism of the ETs, the physiological action of the peptides is determined by the concentration and type of ET receptor expression. Binding experiments demonstrated the presence of two classes of ET receptor in rabbit myometrium [14]. The first site showed low capacity and no selectivity for the ET isoforms, whereas the second was selective for ET-1. The concentration of ET receptors in myometrium was 100-fold greater than that in aorta; receptor density was increased after estradiol priming, and this effect was reversed following the sequential administration of estradiol and progesterone [14].

In the human, the distribution of binding sites for iodinated ET-1, ET-2 and ET-3 was similar to that of the ET peptide itself, predominantly to endometrial glandular epithelium and vascular smooth muscle [31]. The density of binding sites for each ET isoform was 2–3-fold greater in endometrium than myometrium. Subsequent saturation binding assays revealed that iodinated ET-1 bound with a single affinity to receptors in endometrium and myometrium (dissociation constant, K_D; mean $\pm$ SEM $= 1.4 \pm 0.5$ and 1.2 ± 0.2 nM respectively) [32]. The maximum number of binding sites (B_{max}) was reported as higher in myometrium from pre-menopausal and pregnant women compared with that from post-menopausal individuals, again suggesting that sex steroids may be involved in the control of ET receptor density [33].

Following the demonstration of mRNA for ET_A and

ET_B in both endometrium and myometrium, competition binding assays and autoradiography were used to determine the differential localization of the two receptor subtypes in human uterus [20, 32]. Receptors of ET_A subtype predominated in myometrium, and a population of ET_B receptors was detected in endometrium, localized to glandular epithelium. Whereas activation of ET_A receptors mediates myometrial contractility [32, 34, 35], the function of ET_B receptors in the uterus remains unknown.

ACTIONS

Contractile effects

The ETs exhibit powerful contractile effects on vascular and myometrial smooth muscle in the uterus. ET (the isoform was not specified) caused a potent long-lasting contraction of human uterine artery and vein [36]. The threshold for the establishment of contractions was 3×10^{-9} mol/l, and the subsequent dose–response curve was two orders of magnitude to the left of that seen with noradrenaline. Similar experiments showed that ET, along with arginine vasopressin, oxytocin and noradrenaline was more potent at stimulating contraction of small branches of the uterine artery, in comparison with the main stem of the vessel [37].

ET-stimulated myometrial contractions have been demonstrated *in vitro* in the rat, rabbit and human [7, 8, 14, 38, 39]. A biphasic response to ET (isoform not specified) was seen in the rat. The response was abolished in the absence of calcium, and the eradication of rhythmic, but not developing contractions by nifedipine suggested the activation of two distinct mechanisms [7]. In the rabbit, however, the stimulatory effect of ET-1 was completely abolished by verapamil and nicardipine [14]. In human uterine circular and longitudinal smooth muscle strips, nifedipine inhibited both phasic uterine activity, and the amount of force generated in response to ET. In addition, ET resulted in an increase in intracellular calcium and stimulated myosin light chain phosphorylation [39].

Paracrine actions

Besides smooth muscle contractile effects, the ETs may play an important role in the paracrine control of uterine function. As shown in vascular smooth muscle cells [40, 41], ET activated phospholipase A_2 and phospholipase C in human endometrium, resulting in the generation of prostaglandins and other second messengers [42]. Using a porcine coronary artery model, the contractile response to ET-1 was attenuated by the concomitant release of prostaglandin E_2 [43]. Whether such a mechanism occurs in the human uterus remains to be determined.

ET-induced vasodilatation has been shown to be the result of the paracrine release of prostaglandin I_2 (prostacyclin) and nitric oxide [6]. That nitric oxide release is thought to be mediated by binding to the ET_B receptor implies that the physiological response to ETs may be determined by differential localization of ET receptor subtypes [12, 44, 45]. The demonstration of mRNA and protein for nitric oxide synthase in human uterus might support the existence of such a paracrine loop in endometrium [46].

Mitogenesis

The ETs exhibit growth factor-like actions for a variety of cell types including vascular smooth muscle cells and fibroblasts [9, 10, 47, 48]. A growth-promoting role for the ETs in the uterus has not been described, however, both alone and in combination with insulin-like growth factor (IGF)-1, ET-1 stimulated the incorporation of tritiated thymidine into placental fibroblasts [49].

CONCLUSION

The production of a powerful vasoconstrictor in basal endometrium is crucial for the mechanism of menstruation. Intense vasoconstriction of the endometrial spiral arterioles occurs prior to the onset of menses [50]. Furthermore, most of the functional endometrium is shed within the first 20–24 h of bleeding, and subsequent haemostasis is achieved by vasoconstriction, until blood loss is definitively controlled by repair of vessels and the surrounding epithelium. Demonstration of extravascular ET in endometrium reveals a powerful vasoconstrictor which could be available either before or during menstruation, and which would be released as the endometrium breaks down, to act on the abluminal aspect of the spiral arterioles to effect a powerful and sustained contraction of vascular smooth muscle. ETs might also contribute to the process of endometrial repair, either alone or in concert with other locally released growth factors.

The ETs appear to play a fundamental role in the control of uterine function in pregnancy. Their effects on myometrial contractility have been implicated in the mechanisms governing the onset of normal and preterm labour [51], and by binding to vascular smooth muscle receptors in the placenta the peptides are likely to be key determinants of placental blood flow [52, 53].

A better understanding of the function of the ETs in uterine physiology might permit exploitation of ET antagonists for the management of a variety of clinical problems including disorders of menstruation and implantation, and pre-term labour.

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