Steroid receptors in blood vessels of the rhesus macaque endometrium: a review

Robert M. Brenner and Ov D. Slayden

Division of Reproductive Sciences, Oregon National Primate Research Center, Oregon Health & Sciences University, Beaverton, OR, USA

Summary. Estradiol (E) and progesterone (P) act on the primate endometrium to induce dramatic changes in the vascular system during the menstrual cycle. These changes include vessel breakdown and bleeding during menstruation, heightened angiogenesis during the early proliferative phase, and extensive growth of the spiral arteries in the luteal phase of the cycle. Because steroid hormone action is dependent upon the presence of specific nuclear receptors in target tissues, we used immunocytochemistry with receptor-specific monoclonal antibodies to characterize the spatial and temporal expression of estrogen receptor alpha (ERα), estrogen receptor beta (ERβ), progesterone receptor PR and androgen (A) receptor (AR) in the endometrial vessels of rhesus macaques (Macaca mulatta). The only sex steroid receptor that was present in the endothelium and smooth muscle walls of endometrial vessels was ERβ. ERα, PR, and AR were not detectable in either the endothelium or vascular smooth muscle cells of primate endometrial vessels. However, all of these receptors were strongly expressed by the perivascular stroma, and in these cells, all were modulated by the changes in levels of E and P during the cycle. We concluded that any direct effects of E on endometrial vessels would be mediated by ERβ, and that the actions of P and A, and possibly some of E, were indirectly mediated through perivascular stromal cells.

Introduction

The endometrium undergoes extraordinary changes each menstrual cycle, and during pregnancy this tissue changes even more dramatically (Brenner and Slayden, 1994). At the end of a nonpregnant cycle, severe declines in progesterone (P) levels lead to vasoconstriction, bleeding, sloughing and surface repair. The proliferative phase that follows is marked by vascular, glandular and stromal growth, all dependent on estradiol (E) (Nayak and Brenner, 2002). The subsequent luteal phase, when P levels rise, is marked by further growth of the spiral artery system (Brenner and Slayden, 1994). If implantation occurs, P and E levels rise further, and the spiral arteries grow to eventually become the major vessels that provide oxygenated blood to the fetus (Ramsey, 1973). Clearly the vascular system of the endometrium plays a major role in fertility and reproductive success.

However, the precise role that the genomic receptors (R) for estradiol (ER) and progesterone (PR) play in these vascular events is poorly understood. The roles of other hormone receptors, including the androgen (AR) and glucocorticoid receptors (GR) in endometrial vascular physiology is equally uncertain. Great difficulties exist in analyzing the biochemical and physiological properties of vascular systems, because key interactions within the microenvironment are disrupted once blood vessels are separated from their in situ locations. Consequently, in situ techniques are essential for analyzing the vascular physiology of the endometrium. We have used immunocytochemistry to localize steroid receptors in the different cellular components of blood vessels in the nonhuman primate endometrium (Critchley et al., 2001b). The results suggest that indirect paracrine mechanisms, as well as direct, receptor mediated ones, play important roles in the hormonal control of endometrial vessels.
Materials and Methods

Animal treatments

All procedures on animals were approved by the Institutional Animal Care and Use Committee of the Oregon National Primate Research Center/ Oregon Health Sciences University. In order to more precisely control hormonal conditions and to facilitate timed sampling during the menstrual cycle, we treated ovariectomized macaques with Silastic implants of estradiol (E) for 14 days and then added a progesterone (P) implant for 14 days to complete a 28 day cycle. To induce menstruation, the P implant was removed and the E implant left in place. The removal of P induces menstruation, which occurs over 2-3 days, after which the endometrium regenerates under E influence (proliferative phase) until a P implant is reinserted 14 days later to induce a secretory phase. Periodic insertion and removal of the P implant every 2 weeks on a constant background of E induces endometrial cycles indistinguishable from normal. For most of the studies reviewed here, endometrial samples were obtained by hysterectomy or necropsy in either the late proliferative or late secretory phase.

Immunocytochemistry

Immunocytochemistry (ICC) was conducted on samples that were microwave stabilized and frozen in liquid propane (Slayden et al., 1995). Cryostat sections (5 μm) were mounted on Superfrost Plus (Fisher Scientific Pittsburgh, PA) slides and processed for steroid receptors and markers of cell proliferation (Ki-67) as recently described (Slayden et al., 1998; Slayden et al., 2001). The monoclonal antibodies used included: anti-ER (1D-5; Biogenex, San Ramon CA), anti-AR (F-39; Biogenex), anti-PR (PR Ab-8; Neomarker Inc., Fremont CA), and anti-ER β (NCL-ER β, clone EMR02, Novocastra, Laboratories, LTD). Localization of AR was confirmed by in situ hybridization as previously described (Slayden et al., 2001). Antibodies against VEGFR2 were provided courtesy of Schering, AG (Nayak et al., 2000). Photomicrographs of ICC preparations were prepared from digital images captured with an Optronics DEI-750 CCD camera through Zeiss planapochromatic lenses.

Results

Vascular patterns and proliferation

The endometrium is extremely vascular. Its blood vessels ramify extensively throughout the mucosa and are closely associated with the glandular tree (Fig. 1). The radial arteries of the myometrium give rise to two types of arteries, basilar and spiral. The basilar arteries vascularize the deepest endometrial zones, and the spiral arteries feed the uppermost zones. The extensive capillary network of the endometrium is drained by veins that parallel the arterial tree and exit through the main uterine veins. The anatomy and histology of the blood vessels of the primate endometrium are fully described in the older literature (Daron, 1936; Bartelmez, 1957).

As noted in the introduction, vascular growth occurs in the proliferative phase under the influence of E when the endometrium regenerates after menstruation. In a recent paper from our laboratory we reported that this growth was heavily restricted to the upper functionalis zone and that there was a peak of DNA synthesis in the endothelium in this zone on day 8 of the cycle, as detected by uptake of bromodeoxyuridine (BrdU) (Nayak and Brenner, 2002). Throughout the remainder of the cycle there was a continuous, low level of endothelial proliferation without obvious peaks.
Fig. 2. Steroid receptor distribution in rhesus macaque uterus during the proliferative phase. The figure is arranged in 4 rows and 3 columns. Row 1-4 depicts ERα, ERβ, PR and AR in order, top to bottom. Columns 1-3 depict Capillaries, Spiral Arteries and Myometrium in order, left to right. In the capillary endothelium, only ERβ is expressed; ERα, PR and AR are found only in the perivascular stroma. In the spiral arteries, ERβ is expressed in the endothelium, smooth muscle and perivascular stroma, but ERα, PR and AR are only expressed in the perivascular stromal cells. In the myometrium, ERβ is present in the myometrial smooth muscle cells as well as the arterial endothelial, smooth muscle and perivascular stromal cells. ERα, PR and AR are only expressed by the myometrial muscle and perivascular stromal cells. ×250
**Glandular and stromal steroid receptors**

We and others have noted that dramatic cyclic changes occur in ER, PR and AR in the glands and stroma of the primate endometrium during the menstrual cycle (Critchley et al., 2001a). To briefly summarize, the glands and stroma are both rich in ER and AR during the proliferative phase under E influence. AR is also elevated but is only expressed in the stroma. Once P levels rise after ovulation, ERα declines to nondetectable levels in both glands and stroma while ERβ diminishes less, PR becomes nondetectable in the glands but remains in the stroma, and AR is moderately suppressed but remains detectable in the stroma.

**Estrogen receptor α and β:** Figure 2 depicts the immunocytochemical staining patterns seen during the proliferative phase in the endometrium and myometrium. In the endometrium, ERα is strongly expressed by the perivascular stroma around capillaries and spiral arteries but is minimally detectable in the arterial musculature and completely absent from the endothelium. Similarly, in the myometrial arteries, ERα is strongly expressed by the perivascular smooth muscle cells of the myometrial muscle fibers but is absent from the endothelium and muscular walls. On the other hand, ERβ is very strongly expressed in the endothelium of capillaries and arteries and in the muscular walls of the arteries. It is also strongly expressed by the perivascular stroma in the endometrium and the smooth muscle cells of the myometrium. The glucocorticoid receptor, not illustrated here, is also expressed by the endothelium and muscular walls of endometrial blood vessels (Henderson et al., 2003; Bamberger et al., 2001).

**Progesterone and androgen receptors:** Figure 2 also shows that PR and AR are absent from endothelial cells of capillaries and the muscular cells of the artery walls, in both the endometrium and myometrium. Both receptors are easily detected in the perivascular stroma and myometrial smooth muscle.

**Lack of cyclic changes in vascular steroid receptors:** The pattern of vascular steroid receptor expression in the endothelium and muscular walls, as detected by ICC, does not change greatly during the cycle. The only hormonally induced changes were in the perivascular stroma, as noted.

**Discussion**

These data lead to the following conclusions. First, the only steroid receptor that was strongly expressed in the endothelium and muscular walls was ERβ. Therefore any direct effects of E on the endometrial vascular system would be mediated by this isoform rather than ERα. Second, the vessels in the myometrium express the same steroid receptors as those in the endometrium, indicating that myometrial and endometrial vessels could respond in parallel to the same hormonal shifts. Third, there were no obvious changes in vascular ERβ expression during the cycle, and no expression of ERα, PR or AR in blood vessels at any time. Because the neighboring perivascular stromal cells do express all these receptors, and because these receptors do undergo cyclic changes, the hormonal shifts that affect endometrial blood vessels may be mediated by these cells through paracrine mechanisms.

There are several times in the cycle when paracrine signaling is likely to modulate vascular physiology. One is the demise of the corpus luteum at the end of the cycle. This demise is accompanied by a severe decline in serum levels of P which induces the menstrual cascade. A key part of this cascade is a severe vasoconstriction of the spiral arteries that leads to hypoxia in the uppermost endometrial zones and the eventual sloughing of this zone along with the menstrual blood. Severe constrictions of the radial arteries in the myometrial/endometrial border occur in parallel with the vasoconstrictions in the spiral arteries (Daron, 1936). Because the only cells that express genomic PR at this time in the cycle are the perivascular stromal and myometrial cells, it follows that P withdrawal could directly signal these perivascular cells to secrete factors that act indirectly to induce vasoconstriction. In the endometrium, known vasoconstrictive factors including prostaglandins (Eldering et al., 1990; Kelly et al., 2002; Hapangama et al., 2002) and endothelins (Economos et al., 1992; Cameron et al., 1992; Kubota et al., 1995) are known to be induced by P withdrawal in perivascular, stromal, glandular and endothelial cells. P withdrawal could initiate several different cell:cell signalling pathways that initiate in PR-positive stromal cells and link to PR-negative glandular and endothelial cells, and finally to PR-negative vascular muscle cells. Studies are needed to determine the precise nature of the cellular and molecular pathways induced by P withdrawal which culminate in severe vasoconstriction in the primate endometrium.

Once menstruation has ceased and the luminal surface healed, glandular regeneration begins. By day 8 of the proliferative phase a burst of accelerated capillary growth occurs that is known to be E-dependent (Nayak and Bren-
In summary, although the blood vessels of the primate endometrium are highly responsive to estrogens and progesterins, they lack immunocytochemically detectable ERβ and PR in the endothelium and muscular walls. They also lack AR, and androgens can inhibit the proliferative effects of estrogens on endometrial cells (Grody et al., 1995; Tuckerman et al., 2000). Aside from GR, ERβ is the only steroid receptor that is expressed in all the vessel components, but this isoform changes little during the cycle. Consequently, our working hypothesis is that the genomic effects of sex steroids on endometrial vessels are regulated in at least two ways: directly through ERβ and indirectly through paracrine signalling from perivascular cells. Glucocorticoids may act directly through GR, which is expressed in vascular endothelium, but the role of GR in the vascular physiology of the endometrium is unknown. Finally, the possibility that nongenomic effects may be involved in steroid hormone actions on endometrial blood vessels remains to be explored. Hopefully, continued research on these and related mechanisms will lead us to a more complete understanding of how the complex vascular network of the primate uterus is hormonally regulated.

References


Critchley HOD, Brenner RM, Henderson TA, Williams K, Nayak NR, Slayden OD, Millar MR, Saunders PT: Estrogen receptor beta, but not estrogen receptor alpha, is present in the vascular endothelium of the human and


