Regulation of the cGMP-cPKG pathway and large-conductance \( Ca^{2+} \)-activated K\(^+ \) channels in uterine arteries during the ovine ovarian cycle

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Khan LH, Rosenfeld CR, Liu X, Magness RR. Regulation of the cGMP-cPKG pathway and large-conductance \( Ca^{2+} \)-activated K\(^+ \) channels in uterine arteries during the ovine ovarian cycle. Am J Physiol Endocrinol Metab 298: E222–E228, 2010. First published November 17, 2009; doi:10.1152/ajpendo.00375.2009.—The follicular phase of the ovine ovarian cycle demonstrates parallel increases in estrogen and uterine blood flow (UBF). Although estrogen and nitric oxide contribute to the rise in UBF, the signaling pathway remains unclear. We examined the relationship between the rise in UBF during the ovarian cycle of nonpregnant sheep and changes in the uterine vascular cGMP-dependent pathway and large-conductance \( Ca^{2+} \)-activated K\(^+ \) channels (BK\(_{Ca}\)). Nonpregnant ewes (\( n = 19 \)) were synchronized to either follicular or luteal phase using a vaginal progesterone-releasing device (CIDR), followed by intramuscular PGF\(_2\alpha\), CIDR removal, and treatment with pregnant mare serum gonadotropin. UBF was measured with flow probes before tissue collection, and second-generation uterine artery segments were collected from nine follicular and seven luteal phase ewes. The pore-forming \( \beta \)-and regulatory \( \beta \)-subunits that constitute the BK\(_{Ca}\), soluble guanylyl cyclase (sGC), and cGMP-dependent protein kinase G (cPKG) isoforms (cPKG\(_1\), and cPKG\(_{Ca}\)) were measured by Western analysis and cGMP levels by RIA. BK\(_{Ca}\) subunits were localized by immunohistochemistry. UBF rose \( >3 \)-fold (\( P < 0.04 \)) in follicular phase ewes, paralleling a 2.3-fold rise in smooth muscle cGMP and 32% increase in cPKG\(_{1}\), (\( P < 0.05 \)). sGC, cPKG\(_{1}\), and the BK\(_{Ca}\) \( \alpha \)-subunit were unchanged. Notably, expression of \( \beta_1 \)- and \( \beta_2 \)-regulatory subunits rose 51 and 79% (\( P \leq 0.05 \)), respectively. Increases in endogenous ovarian estrogens in follicular-phase ewes result in increases in UBF associated with upregulation of the cGMP- and cPKG-dependent pathway and increased vascular BK\(_{Ca}\), \( \beta/\alpha \)-subunit stoichiometry, suggesting enhanced BK\(_{Ca}\) activation contributes to the follicular phase rise in UBF.

estrogen; uterine blood flow; protein kinase G; guanylyl cyclase
METHODS

Animal preparation and experimental protocol. Tissues obtained from 19 nonpregnant, intact multiparous ewes (50–65 kg) of mixed Western breed were used in the present studies. Animals were studied between September and May; those studied between September and March had evidence of estrous cycles, whereas those studied between April and May exhibited cycles. Animals were randomly assigned to either follicular (n = 10) or luteal (n = 9) phases of the ovarian cycle; all were implanted with a vaginal regimen causing a rapid fall in P4; a rise in plasma estrogens and the prost Tromethamine-Lutalyse; Upjohn, Kalamazoo, MI) 4 h apart to lyse existing corpora lutea. On the 10th day following CIDR placement, the CIDR was removed, and each animal received one dose of 500 IU pregnant mare gonadotropin intramuscularly. This regimen causes a rapid fall in P4; a rise in plasma estrogens and the estrogen-to-P4 ratio (E/P), with levels peaking at 36–48 h; a surge in plasma 17 beta-estradiol at 36–48 h (9, 43). The above procedures were performed at the breeding farm under natural lighting conditions, and animals were transported to the laboratory before termination where similar lighting conditions were maintained. Follicular phase ewes were studied 48 h after CIDR removal, designated as day 0 of the ovarian cycle. Luteal phase ewes were studied 12–13 days after CIDR removal, designated as day 10–11 of the cycle. Ovaries were collected from all animals at the time of termination and mapped to confirm the status within the cycle (Table 1). Ovaries from follicular phase ewes had more total follicles and large follicles 4–6 mm than luteal phase ovaries as well as avascular regressing corpora luteum (CL) vs. large vascular CL in luteal phase ewes. Measurements of P4 in jugular venous blood were taken at 42 h (9, 38). These studies demonstrated that actin was unaffected (P > 0.1; data not shown) after 7 days of daily E2 and P4 exposure (1 μg/kg iv) in nonpregnant castrated ewes; thus, we chose to use α-actin as a loading control (1:8,000; monoclonal α-actin; Sigma) on immunoblots performed with cPKGIα, and the BKCa, α-subunit (1:1,000; Calbiochem, San Diego, CA), or cPKGIβ (1:750; a gift from HK Surks, Tufts-New England Medical Center). To determine what protein could be used as a loading control, we performed preliminary studies in which we measured uterine artery smooth muscle actin contents. These studies demonstrated that actin was unaffected (P > 0.1; data not shown) after 7 days of daily E2 and P4 exposure (1 μg/kg iv) in nonpregnant castrated ewes; thus, we chose to use α-actin as a loading control (1:8,000; monoclonal α-actin; Sigma) on immunoblots performed with cPKGIα, and the BKCa, β-subunit in luteal (n = 4) and follicular (n = 4) phase arteries and reprobed for α-actin at 42 kDa. There was no difference in α-actin on either immunoblot (P > 0.8; Fig. 1). The repeat immunoblots performed to demonstrate equal loading the two proteins of interest are available as Supplemental Fig. 1 (Supplemental data for this article can be found on the American Journal of Physiology: Endocrinology and Metabolism website.). After 1 h of incubation with anti-rabbit immunoglobulin G conjugated with horseradish peroxidase (1:2,000), immunoreactive protein was visualized by enhanced chemiluminescence. Samples were compared by scanning densitometry in arbitrary units (TotalLab Software Package; Biosystematica, Wales, UK) as reported (23, 29, 33, 38). Ovine cerebellum served as a positive internal control for BKCa, α-subunit. The cGMP content of uterine arteries harvested from follicular and luteal phase animals was measured by radioimmunoassay (cGMP [125I] RIA KIT; NEX-133; Perkin-Elmer Life Sciences, Boston, MA) as previously reported (33, 39). Briefly, 50 mg of frozen uterine artery were homogenized in 1 ml of 6% TCA and centrifuged at 3,000 g to remove bulk proteins. TCA was added to the homogenate and extracted from the supernatant with water-saturated dimethyl-ether three times. The supernatant was precipitated by hypophosphilization, dissolved in 0.05 M sodium acetate, and used in the assay. All samples were measured in a single assay. Immunohistochemistry. At the time of tissue collection, intact segments of second-generation uterine arteries were washed in PBS, fixed in 4% paraformaldehyde for 6 h at room temperature, and subsequently embedded in paraffin as previously described (29, 39). Sections were mounted on slides, deparaffinized, hydrated, incubated with avidin-biotin blocking agent for 30 min, and incubated overnight at room temperature with polyclonal antibodies to the BKCa, α-, β1-, horns and placed in sterile chilled physiological saline solution; the adventitia and intraluminal blood were removed. Samples were frozen in liquid nitrogen and stored at −80°C until the time of assay (21, 29, 39). Additional uterine artery samples were prepared for immunohistochemistry as described below. Tissues from both groups of animals were used in other studies; therefore, samples were not available from each animal for each assay, resulting in a difference in the n values noted in the RESULTS.

Western immunoblots. Samples of second-generation uterine arteries (30 mg) were weighed and homogenized in 40× volumes of SDS buffer as previously described (23, 29, 33, 38). Homogenates were centrifuged at 10,000 g for 2 min, the supernatant was removed, and an aliquot from each animal was used to measure cellular or soluble protein by bichinonic acid reagent (Pierce, Rockford, IL) as previously reported (23, 33, 38). Bromphenol blue and 2-mercaptoethanol were added to the aliquots representing each animal studied, and equal amounts of soluble protein were loaded (20 μg) on a single 7.5–10% polyacrylamide minigel and subjected to SDS-PAGE followed by transfer to nitrocellulose paper at 100 volts for 1 h (23, 29, 33, 38). Blotting all samples on a single gel takes into account differences in protein transfer; because similar amounts of soluble protein are loaded, comparisons can be made between samples. The immunoblots were incubated overnight with antisera to BKCa, α-subunit (1:300; AB5228–200UL; Chemicon International, Temecula, CA), β1-subunit (1:400; ab3587, Abcam, Cambridge, MA), β2-subunit (1:200; APC-034; Alomane Labs, Jerusalem, Israel), soluble guanylyl cyclase (sGC; 1:1,000; G 4405; Sigma, St. Louis, MO), cGMP-dependent protein kinase G1α (cPKGIα; 1:1,000; PK10; Calbiochem, San Diego, CA), or cPKGIβ (1:750; a gift from HK Surks, Tufts-New England Medical Center). To determine what protein could be used as a loading control, we performed preliminary studies in which we measured uterine artery smooth muscle actin contents. These studies demonstrated that actin was unaffected (P > 0.1; data not shown) after 7 days of daily E2 and P4 exposure (1 μg/kg iv) in nonpregnant castrated ewes; thus, we chose to use α-actin as a loading control (1:8,000; monoclonal α-actin; Sigma) on immunoblots performed with cPKGIα, and the BKCa, β-subunit in luteal (n = 4) and follicular (n = 4) phase arteries and reprobed for α-actin at 42 kDa. There was no difference in α-actin on either immunoblot (P > 0.8; Fig. 1). The repeat immunoblots performed to demonstrate equal loading the two proteins of interest are available as Supplemental Fig. 1 (Supplemental data for this article can be found on the American Journal of Physiology: Endocrinology and Metabolism website.). After 1 h of incubation with anti-rabbit immunoglobulin G conjugated with horseradish peroxidase (1:2,000), immunoreactive protein was visualized by enhanced chemiluminescence. Samples were compared by scanning densitometry in arbitrary units (TotalLab Software Package; Biosystematica, Wales, UK) as reported (23, 29, 33, 38). Ovine cerebellum served as a positive internal control for BKCa, α-subunit.

Measurement of cGMP. The cGMP content of uterine arteries harvested from follicular and luteal phase animals was measured by radioimmunoassay (cGMP [125I] RIA KIT; NEX-133; Perkin-Elmer Life Sciences, Boston, MA) as previously reported (33, 39). Briefly, 50 mg of frozen uterine artery were homogenized in 1 ml of 6% TCA and centrifuged at 3,000 g to remove bulk proteins. TCA was added to the homogenate and extracted from the supernatant with water-saturated dimethyl-ether three times. The supernatant was precipitated by hypophosphilization, dissolved in 0.05 M sodium acetate, and used in the assay. All samples were measured in a single assay.

Table 1. Ovarian structures in nonpregnant ewes synchronized in either the follicular or luteal phases of the ovarian cycle as described in methods

<table>
<thead>
<tr>
<th>Diameter, mm</th>
<th>Follicular Phase (n = 9)</th>
<th>Luteal Phase (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corpora lutea</td>
<td>6.9 ± 0.6 (avascular)*</td>
<td>10.1 ± 0.3 (vascular)</td>
</tr>
<tr>
<td>Medium follicles (3–5 mm)</td>
<td>3.8 ± 0.2 (28)</td>
<td>4.0 ± 0.3 (8)</td>
</tr>
<tr>
<td>Large follicles (≥6 mm)</td>
<td>8.2 ± 0.4 (14)*</td>
<td>6.9 ± 0.6 (4)</td>
</tr>
</tbody>
</table>

Values are means ± SE for the no. of specific ovarian structures for all ovaries from each ewe; n, no. of ewes in each group. The total no. of structures is in parentheses. *P < 0.05 by nonpaired t-test.
RESULTS

UBF. UBF measurements did not differ in the two uterine horns \( (P > 0.1) \); therefore, we used the average of the two values for each ewe. UBF was 3.2-fold higher in the follicular phase animals \( (n = 5) \) at the time of tissue collection compared with those allowed to progress to the luteal phase \( (n = 3) \) (Fig. 2; \( P < 0.04 \)).

Signaling pathway. sGC was detected in all samples of uterine artery smooth muscle examined, but there was no difference in protein expression in the follicular and luteal phases of the ovarian cycle \( (P = 0.9, \text {data not shown}) \). In contrast, uterine arteries obtained from follicular phase ewes had cGMP contents 2.3-fold greater than vessels collected from luteal phase ewes \( (P < 0.02; \text {Fig. 3}) \). To further define the NO-cGMP-cPKG pathway, we examined cPKG\(_{1}\) isoform expression using immunoblot analysis. cPKG\(_{1}\) expression did not differ in follicular and luteal phase uterine arteries \( (P = 0.22; \text {Fig. 4B2}) \), however, cPKG\(_{1\alpha}\) protein was 32% higher in follicular vs. luteal phase tissues \( (P = 0.047; \text {Fig. 4B1}) \). In the secondary analysis to assess protein loading, there was no difference in \( \alpha \)-actin \( (P = 0.9) \), but cPKG\(_{1\alpha}\) increased 21% \( (P = 0.008; \text {see Supplemental Fig. 1}) \).

\( \text{BK}_{\text{Ca}} \) expression. The density of \( \text{BK}_{\text{Ca}} \) channels is determined by measuring the expression of the pore-forming \( \alpha \)-subunit \( (17, 23, 33, 38) \). There was no difference in \( \alpha \)-subunit protein in uterine artery smooth muscle from follicular and luteal phase ewes \( (P = 0.2; \text {Fig. 5B1}) \). In contrast, \( \beta_{1} \)- and \( \beta_{2} \)-subunit protein expression was 51% \( (P = 0.009) \) and 79% \( (P = 0.05) \), respectively, greater in arteries obtained from follicular phase animals vs. luteal phase ewes \( (P = 0.8; \text {Fig. 5B2 and B3}) \). As above, \( \alpha \)-actin did not differ in luteal \( (n = 4) \) and follicular \( (n = 4) \) phase arteries \( (P = 0.8), \) and \( \beta_{1} \)-subunit increased 37% \( (P = 0.1); \) see Supplemental Fig. 1). Immunohistochemistry. To determine the tissue localization of the \( \alpha \)- and \( \beta \)-subunits comprising the \( \text{BK}_{\text{Ca}} \) channel within the intact uterine artery and to further assess the pattern of expression during the ovarian cycle, we performed immunohistochemistry on randomly selected samples of uterine arteries from three follicular and three luteal phase animals. Nonimmune rabbit serum did not show immunostaining in any vessel examined (Fig. 6, A and E). Although \( \alpha \)-subunit immunostaining was observed throughout the uterine smooth muscle in each sample examined, this was not seen in the endothelium of either follicular or luteal phase arteries (Fig. 6, B and F). There also was diffuse immunostaining for the regulatory \( \beta_{1} \)- and \( \beta_{2} \)-subunits in the smooth muscle of all arteries assessed, but not in the endothelium. Although densitometric measurements were not made, \( \beta_{1} \) (Fig. 6G) and \( \beta_{2} \) (Fig. 6H)-subunit immunostaining appears to be greater than \( \alpha \)-subunit immunostaining in the media of follicular phase uterine arteries.

DISCUSSION

Propagation of any species depends on the successful progression through each portion of the reproductive cycle, which in eutherian mammals includes the ovarian cycle, implantation
or attachment, placentation, and growth of the maternal uteroplacental and fetal umbilicalplacental vascular beds (28). UBF changes during the reproductive cycle and is responsible for maintaining uterine and placental oxygen and nutrient delivery (28). Although our understanding of UBF regulation in pregnancy has expanded, only modest attention has been given to its regulation in the ovarian cycle and, in particular, the rise in UBF in the follicular phase when the uterus is being prepared for implantation (7, 11, 26). This rise is believed to reflect receptor-mediated actions of ovarian estrogens and local NOS activation (9, 18, 21); however, the remainder of the signaling pathway is incompletely described. In the present study, the rise in UBF in follicular phase ewes was paralleled by upregulation of the cGMP-cPKG pathway and increases in expression of BKCa regulatory subunits of the BKCa channel, which contributes to E2-mediated uterine vasodilation and the maintenance of uteroplacental blood flow in pregnancy (23, 30, 36, 37). Thus we now provide evidence that the rise in endogenous ovarian estrogens and UBF in pregnancy have been difficult because of the seasonality of the cycle and variability in the cyclical pattern of ovarian hormones and UBF (6, 7, 9, 11, 27). Gibson et al. (9) developed a chronic ovine model that allows the study of these interactions independent of the season. They observed a fivefold rise in UBF during the follicular phase that paralleled increases in plasma estrogens and the E/P. In the luteal phase, UBF fell in concert with decreases in the E/P and increases in P4. Sprague et al. (43) reported similar observations using this model. In our study, UBF also rose three- to fivefold in follicular phase ewes and was associated with low P4 values. The rise in UBF is also associated with activation of an estrogen- and NO-mediated pathway, since the estrogen receptor (ER) antagonist ICI-182,780 and the NOS inhibitor NG-nitro-L-arginine methyl ester attenuate the rise in UBF (9, 18, 21). Similar changes occur in ovariectomized nonpregnant ewes after exposure to exogenous E2 (18, 29, 31, 39, 48, 50), demonstrating that responses to endogenous and exogenous estrogens are very similar. However, ER and NOS inhibition only partially decrease UBF in follicular phase ewes; thus, alternative pathways may also contribute to the rise in UBF. These pathways are unclear as are the changes in vascular cGMP and other aspects of the cascade that contribute to NO-mediated increases in UBF.

Studies of the interaction of these endocrine changes and UBF in sheep have been difficult because of the seasonality of the cycle and variability in the cyclical pattern of ovarian hormones and UBF (6, 7, 9, 11, 27). Gibson et al. (9) developed a chronic ovine model that allows the study of these interactions independent of the season. They observed a fivefold rise in UBF during the follicular phase that paralleled increases in plasma estrogens and the E/P. In the luteal phase, UBF fell in concert with decreases in the E/P and increases in P4. Sprague et al. (43) reported similar observations using this model. In our study, UBF also rose three- to fivefold in follicular phase ewes and was associated with low P4 values. The rise in UBF is also associated with activation of an estrogen- and NO-mediated pathway, since the estrogen receptor (ER) antagonist ICI-182,780 and the NOS inhibitor NG-nitro-L-arginine methyl ester attenuate the rise in UBF (9, 18, 21). Similar changes occur in ovariectomized nonpregnant ewes after exposure to exogenous E2 (18, 29, 31, 39, 48, 50), demonstrating that responses to endogenous and exogenous estrogens are very similar. However, ER and NOS inhibition only partially decrease UBF in follicular phase ewes; thus, alternative pathways may also contribute to the rise in UBF. These pathways are unclear as are the changes in vascular cGMP and other aspects of the cascade that contribute to NO-mediated increases in UBF.

E2 enhances type I and III NOS expression in uterine arteries from nonpregnant ewes, and this is associated with increased cGMP synthesis and uterine vasodilation (29, 31, 39, 48, 50).
Although NO contributes to the rise in follicular phase UBF (9), it is unclear if guanylyl cyclase and cGMP are altered. Using the model of Gibson et al. (9), we observed equal fold increases in UBF and uterine artery cGMP in follicular phase animals. However, sGC was unchanged, suggesting enzyme activity increases after exposure to endogenous estrogen. Similar changes are also seen in uterine arteries from late pregnant ewes, which are exposed to endogenous estrogens of placental origin (33). Although endothelial NOS mRNA and protein increase in follicular phase uterine arteries (21, 48), type 1 NOS has not been examined. This is of interest, since daily E2β increases type I NOS in uterine arteries of 48, 50).

**Fig. 5.** Effect of the ovarian cycle on BKCa subunit expression in the uterine artery during the luteal (n = 4) and follicular (n = 6) phases. A: immunoblot analyses for α-, β1-, and β2-subunits. B: densitometry in arbitrary units from the immunoblots for α (B1)-, β1 (B2)-, and β2 (B3)-subunits. Data are means ± SE; *P = 0.009 and **P = 0.05, nonpaired t-test.

**Fig. 6.** Representative immunohistochemistry of BKCa α-, β1-, and β2-subunits in randomly selected uterine arteries during the luteal (A–D) and follicular (E–H) phases of the ovine ovarian cycle. Figures are at ×40 magnification. NIRS, control nonimmune rabbit serum; L, vessel lumen.
ovariectomized ewes (29, 39). Moreover, we have seen increases in uterine artery type I NOS during ovine pregnancy (Rosenfeld, unpublished observations). Future studies should address this.

Smooth muscle contraction and relaxation are regulated by the relative rates of myosin light chain (MLC) phosphorylation by Ca$$^{2+}$$/calmodulin-dependent MLC kinase and dephosphorylation by myosin phosphatase (5, 13, 42). The NO-cGMP pathway activates relaxation via cPKG and Ca$$^{2+}$$-dependent and -independent mechanisms (44). The former involves decreases in intracellular Ca$$^{2+}$$ and the latter activation of MLC phosphatase, which dephosphorylates MLC resulting in SMC relaxation (44, 46). Two cPKG isoforms, cPKG$$\text{1}$$ and cPKG$$\text{2}$$, exist (25, 44); however, cPKG$$\text{1}$$, expressed as cPKG$$\text{1}\alpha$$ and cPKG$$\text{1}\beta$$, predominates in SMC (46). Both cPKG$$\text{1}$$ isoforms are coexpressed in SMC, but their relative levels and specific roles are poorly understood (44, 45). Both modify intracellular Ca$$^{2+}$$, whereas cPKG$$\text{1}\alpha$$ also activates MLC phosphatase (44). Notably, the sensitivity of cPKG$$\text{1}\beta$$ to cGMP is 10-fold greater than cPKG$$\text{1}\alpha$$ (2). We found both isoforms in uterine artery SMC, but only cPKG$$\text{1}\alpha$$ increased in the follicular phase, suggesting endogenous estrogens selectively increase transcription and/or translation. Similar changes are seen in uterine arteries from pregnant sheep, which are exposed to increases in endogenous placental estrogens (1, 33). If cPKG$$\text{1}\alpha$$ is selectively upregulated by estrogen and is 10-fold more sensitive to cGMP than cPKG$$\text{1}\beta$$, amplification of the NO-cGMP-cPKG pathway is likely to occur after estrogen exposure. This will need to be examined, as well as the mechanisms that regulate cPKG$$\text{1}\alpha$$ during the reproductive cycle.

B$$\text{K}_{\text{Ca}}$$ contribute to the vasodilatory effects of exogenous estrogens via the NO-cGMP-cPKG pathway (4, 16, 37, 51) and/or directly through $$\beta$$-subunit activation (49). $$\beta$$- and $$\beta$$-regulatory subunits regulate Ca$$^{2+}$$ and voltage sensitivity and modify B$$\text{K}_{\text{Ca}}$$ activation kinetics (41, 47). The pore-forming $$\alpha$$-subunit and both SMC specific $$\beta$$-regulatory subunits were present in uterine artery SMC of cycling nonpregnant sheep, confirming observations in nonpregnant women and ovariectomized nonpregnant and intact pregnant sheep (23, 30, 33, 38). Channel density was unchanged during the ovarian cycle, but $$\beta$$-subunit expression increased at the time of maximum UBF, thereby increasing B$$\text{K}_{\text{Ca}}$$ stoichiometry. Similar alterations occur in E$$\beta$$-treated nonpregnant castrated ewes in which basal UBF increases and responses to acute E$$\beta$$ exposure are enhanced (23, 39). The change in B$$\text{K}_{\text{Ca}}$$ stoichiometry occurred in concert with increases in uterine vascular NOS expression (29, 31, 39, 48, 50) and cGMP synthesis. Thus upregulation of the NO-cGMP-cPKG pathway in the follicular phase could enhance B$$\text{K}_{\text{Ca}}$$ phosphorylation and activation, resulting in uterine vasodilation. It is notable that nonspecific ER and NOS inhibition do not completely reverse the rise in follicular phase UBF (9, 18). Thus estrogen might also directly activate B$$\text{K}_{\text{Ca}}$$ via the $$\beta$$-subunit and contribute to the rise in UBF (37). The role of P$$\text{A}_{\text{2}}$$ in the luteal phase reversal of UBF and attenuation of the NO-cGMP-cPKG-B$$\text{K}_{\text{Ca}}$$ pathway requires further study.

This is the first report showing that increases in endogenous estrogens during the follicular phase of the ovine ovarian cycle contribute to changes in uterine artery B$$\text{K}_{\text{Ca}}$$ expression and possibly function by increasing B$$\alpha$$ stoichiometry and thus channel sensitivity and kinetics without altering channel density. This is paralleled by increased uterine artery cGMP contents and cPKG$$\text{1}\alpha$$ expression; thus, the entire NO-cGMP-cPKG-B$$\text{K}_{\text{Ca}}$$ pathway is upregulated by endogenous estrogen. It is unclear how each segment of the pathway is regulated and if the concertmaster is indeed estrogen or the E/P. If B$$\text{K}_{\text{Ca}}$$ regulate uterine vascular tone during the follicular phase of the ovarian cycle and if increases in B$$\alpha$$ stoichiometry modify channel function, the B$$\text{K}_{\text{Ca}}$$ may be a pharmacologic target in conditions associated with impaired uterine vascular function, e.g., diabetes and chronic hypertension.

REFERENCES


