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Gap Junction Regulation of Vascular Tone: Implications of Modulatory Intercellular Communication During Gestation

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Abstract

In the vasculature, gap junctions (GJ) play a multifaceted role by serving as direct conduits for cell–cell intercellular communication via the facilitated diffusion of signaling molecules. GJs are essential for the control of gene expression and coordinated vascular development in addition to vascular function. The coupling of endothelial cells to each other, as well as with vascular smooth muscle cells via GJs, plays a relevant role in the control of vasomotor tone, tissue perfusion and arterial blood pressure. The regulation of cell-signaling is paramount to cardiovascular adaptations of pregnancy. Pregnancy requires highly developed cell-to-cell coupling, which is affected partly through the formation of intercellular GJs by Cx43, a gap junction protein, within adjacent cell membranes to help facilitate the increase of uterine blood flow (UBF) in order to ensure adequate perfusion for nutrient and oxygen delivery to the placenta and thus the fetus. One mode of communication that plays a critical role in regulating Cx43 is the release of endothelial-derived vasodilators such as prostacyclin (PGI_2) and nitric oxide (NO) and their respective signaling mechanisms involving second messengers (cAMP and cGMP, respectively) that are likely to be important in maintaining UBF. Therefore, the assertion we present in this review is that GJs play an integral if not a central role in maintaining UBF by controlling rises in vasodilators (PGI_2 and NO) via cyclic nucleotides. In this review, we discuss: (1) GJ structure and regulation; (2) second messenger regulation of GJ phosphorylation and formation; (3) pregnancy-induced changes in cell-signaling; and (4) the role of uterine arterial endothelial GJs during gestation. These topics

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integrate the current knowledge of this scientific field with interpretations and hypotheses regarding the vascular effects that are mediated by GJs and their relationship with vasodilatory vascular adaptations required for modulating the dramatic physiological rises in uteroplacental perfusion and blood flow observed during normal pregnancy.

Keywords

Connexins; Nitric oxide; Endothelium; Cyclic nucleotides; Vasodilation; Uterine blood flow

1 Introduction

Cell-to-cell communication is essential for normal multicellular tissue and organ function. Vascular cell responses rely on coordination and synchronization to elicit physiological processes such as changes in vascular tone, cell growth and differentiation, as well as the coordinated contractions of cells. Comparable analogous models have been described in cardiac and myometrial tissue [1–5], however, we will focus this review only on the blood vessels. Vascular cell-to-cell communication is in part accomplished by an intricate system involving endothelial cell regulation of vascular smooth muscle (VSM) cell tone partly via the release of signaling molecules that move through gap junctions (GJ). In addition, endothelial–endothelial cell communication also contributes to vascular tone regulation via GJ-modulated vasodilator production [6–9].

GJs are tightly packed clusters of intercellular channels between endothelial–endothelial cells and endothelial–VSM cells that couple cells both electrically and metabolically [10, 11]. GJ-mediated intercellular communication (GJIC) directly connects the cytoplasm of adjacent cells in order to facilitate the passive and directional diffusion of ions, small signaling molecules (e.g. Ca^{2+} and IP3) and second messengers (cAMP and cGMP) from one cell interior to another (Fig. 11.1) [1, 3, 12]. The coupling of neighboring cells permits relatively fast acting, coordinated intercellular signaling important for vascular homeostasis [13], e.g. paracrine interactions through an extracellular pathway.

Normal pregnancy-induced hemodynamic changes are associated with vascular adaptations in the maternal cardiovascular system including profound reductions in systemic and uterine vascular resistance with the most dramatic changes seen in the uterine vascular beds [14–16]. These vascular adaptations are dependent upon endothelial cell adaptations that play a critical role in the modulation of vascular resistance and thus uterine blood flow (UBF) through elevations in the potent endothelial-derived vasodilators prostacyclin (PGI_2) and nitric oxide (NO) [15–18]. Additionally, it was recently recognized that cell-signaling processes that were shown to regulate sustained elevations in these vasodilators include the enhancement of cell–cell connectivity via GJs in pregnancy [19, 20]. These mechanisms may have clinical relevance since endothelial cell dysfunction has been shown to be a critical and central event in the pathophysiology and progression of preeclampsia (PE) [21–24]. Moreover, impaired and mutational alterations in the expression and/or function of GJs are related to a wide variety of pathological conditions including hypertension [25, 26]. We hypothesize that if these negative changes in GJs manifest during gestation, this will be

associated with gestational diseases such as PE with intrauterine growth restriction (IUGR). Therefore, identifying factors that regulate GJIC and their physiological mechanisms of action are relevant and significant to understanding the function of GJs and GJ-related diseases.

In this review, we integrate the available evidence for connexin hemichannels acting as a pathway for paracrine intercellular communication and their role in signaling. We evaluate and summarize information related to: (1) GJ structure and regulation; (2) second messenger regulation of GJ phosphorylation and formation; (3) pregnancy-induced changes in cell-signaling; and (4) the role of uterine arterial endothelial GJs in gestation.

2 GJ Structure and Regulation

GJs are formed by members of the connexin (Cx) gene family that is composed of 21 iso-forms in humans [27, 28]. Connexins are four pass integral membrane proteins that hexamerize to form a GJ hemichannel called a “con-nexon” (Fig. 11.1). A GJ is formed when a collection of connexons meet head-to-head with connexons in an adjacent cell forming cell-to-cell channels. Different connexin iso-forms yield channels with different conductance, permeability, and regulatory control properties [3, 29, 30]. There are four different connexin isoforms Cx37, Cx40, Cx43, and/or Cx45 found in the vasculature depending on the vessel type [30, 31]. The expression of connexins also is not always uniform within blood vessels since they are made up of several cell types. For example Cx45 is observed only in the VSM cells [32] whereas Cx37, Cx40, and Cx43 have been reported to be present in both VSM and endothelial cells [33–35]. However there are conflicting data on the localization and expression of Cx37 in the aorta, pulmonary artery, coronary artery, and uterine artery smooth muscle cells [6, 34, 36–38]. A consistent observation is that Cx43 is the predominate connexin isoform found in more than 34 tissues and 46 cell types [39] including uterine tissues [30, 31, 40]. Understanding the distribution and regulation of GJ proteins is important in elucidating their conductive and gating properties for cell–cell communication.

Connexins are highly regulated on multiple levels both by alterations in gene expression [41], epigenetic mechanisms [41], and also post-translation modifications via phosphorylation on the C-terminal domain where sites for protein–protein interaction are present [3]. Phosphorylation of Cx43 is seen at multiple specific regulatory sites (serine, threonine, and tyrosine residues) that can control trafficking, assembly, and degradation [3, 27, 42, 43]. Throughout its life cycle, Cx43 is differentially phosphorylated [44–48], with most phosphorylation events on serine regulatory sites [48–51], which affects the rapid turnover of connexins and drives channel gating [3, 52]. The activation of cyclic nucleotides (cAMP and cGMP) and kinases such as protein kinase A (PKA) [53–55], protein kinase C (PKC) [56, 57], p34cdc2/cyclin B kinase (p34cdc2) [50], casein kinase 1 (CK1) [58], mitogen-activated protein kinase (MAPK) [59, 60] and pp60src kinase [45, 61] as well as protein kinase inhibitors such as proto-oncogene tyrosine-protein kinase Src, can alter Cx43 phosphorylation and lead to assembly or degradation of Cx43 GJ channels [52, 58, 62–66]. For example, Cx43 channels conductive states can be driven via phosphorylation by either PKA or by PKC, which respectively increases [54] or decreases [42] gap junction

communication. However, the identification of which protein kinase(s) specifically mediates cyclic nucleotide-induced signaling and the characterization of GJ-related signaling events downstream from kinase activation remains incomplete.

3 Second Messengers Regulation of Gap Junction Phosphorylation and Formation

The cyclic nucleotides, cAMP and cGMP, are second messengers that are involved in a variety of cellular processes including increasing GJ protein assembly [54, 55, 67–70] (Fig. 11.2). it is widely accepted that cAMP-mediated signaling in endothelial cells, osteocytes, and osteoblast cells are involved in the up-regulation of Cx43 [19, 55, 67–71]. cAMP may regulate Cx43 expression acutely by the rapid phosphorylation and trafficking of Cx43 to the cell membrane or chronically by increasing Cx43 gene transcription [41]. Elevated cellular cAMP typically leads to increased GJIC and the number and size of GJ plaques [53, 72] in a process termed “enhanced assembly” in which GJ channels are formed [54]. These cAMP events are mediated via cAMP-dependent protein kinase (PKA) mechanisms that are associated with an increased phosphorylation state of the Cx43 protein that regulates increased connexin export to the plasma membrane, assembly into GJs, and gating. Although cAMP-mediated signaling in endothelial cells are involved in the up-regulation of the level of Cx43 within gap junctions [55, 67–70], virtually nothing is known about the role of cGMP on GJIC. In many tissues, cAMP and cGMP levels are interrelated and the effects of either cAMP or cGMP elevations can be difficult to differentiate due to their regulation by phosphodiesterase activity and cross-talk [73]. Only in one report was there an indication that 8-Br-cGMP decreased gap junctional conductance in cardiac tissue [74], suggesting cGMP may be working to inhibit GJ function. While PKA has been shown to function to increase GJIC, whether cGMP-dependent protein kinase PKG has a direct, an indirect (potentially via cAMP levels) or no effect on Cx43 phosphorylation in the endothelium is unknown.

3.1 PKA Phosphorylation of Cx43 Enhances GJIC

Inhibition of PKA via H89 (PKA inhibitor) eliminated enhanced GJ assembly [54]. Therefore the assembly of GJs that are enhanced by cAMP is thought to be mediated via cAMP-dependent protein kinase (PKA). Studies by TenBroek and coworkers have indicated that serines 364/365 (S364/365) at the C-terminal domain of Cx43 [69] are important phosphorylation sites for cAMP-enhanced GJ assembly. Since PKA-mediated phosphorylation at S364 increases the total number and conductance of GJs [75–77] and GJ assembly was abrogated by Cx43 mutations at S364, these data clearly suggest that phosphorylation of S364 may be a prerequisite for enhanced assembly [69]. However, because Cx43 is a poor substrate for PKA as demonstrated in vitro, there is considerable controversy as to whether PKA directly phosphorylates Cx43 or whether it activates other kinases that perform this task [70, 78, 79]. The possibility of direct PKA phosphorylation cannot be excluded because purified PKA was able to phosphorylate the C-terminal region of wild-type Cx43 to a low level in vitro, but not a Glutathione *S*-transferases (GST)-construct in which S365, S368, S369 and S373 were mutated to alanine. It is also known that PKA can partly act independently of cAMP [80–82]. cAMP-independent PKA effects

are thought to occur via the “Exchange Protein Directly Activated by cAMP” (Epac) signaling pathway that leads to activation of Rap, a small molecular weight GTPase of the Ras family, which has also been implicated in GJ regulation [80–82].

4 Pregnancy-Induced Changes in Cell-Signaling

Endothelium-dependent relaxations of the systemic and uterine vasculatures during gestation rely on the up-regulation of the biosynthetic processes for the potent vasodilators, PGI_2 and NO, during gestation. These profound gestational increases in endothelial production of PGI_2 and NO are responsible for the downstream induction of the respective substantially elevated levels of arterial cAMP [83–89] and cGMP [18, 85, 87–96]. The importance of cAMP and cGMP, as well as GJs, in mediating cell-signaling events and thus cell-to-cell communication were described in detail above, however herein we focus on their role(s) in vasodilation and cardiovascular adaptations seen during gestation. Pregnancy-induced endothelial cell adaptations help to maintain the dramatically increased UBF that facilitates ample oxygen and nutrient delivery to the growing fetus.

4.1 Prostacyclin and cAMP

Vasodilatory prostanoids (i.e. PGI_2 and PGE_2) normally function in an autocrine or a paracrine fashion and exert their physiological effects on various vascular cell types. The endothelium is the primary source of PGI_2 production in uterine vessels, however it is also produced by VSM cells [13]. Pregnancy is directly associated with mechanisms that contribute to the significant rise in uterine secretion of PGI_2 including elevations in endothelium-derived enzymes [13, 17, 97–99] such as the prostanoid producing enzymes $cPLA_2$ [100], cyclooxygenase [13, 97], and PGIS [101–104]. Vascular-derived PGI_2 and PGE_2 is synthesized under basal conditions and in response to various stimuli, such as cytokines, growth factors, mechanical strain, as well as estrogens, and regulate multiple functions including smooth muscle contraction/relaxation [17, 24, 71, 101–103]. However, the central role of endogenous vasodilator prostanoids (PGI_2 and/or PGE_2) in acutely maintaining UBF during gestation is questionable since in vivo experiments infusing the cyclooxygenase inhibitor, indomethacin, lowered prostaglandin production by 70–80 % without significantly decreasing UBF [103, 105, 106]. Another interpretation of these results is that there are multiple other vasodilators (e.g. NO, CNP, EDHF, etc.) [92, 94] elevated in pregnancy and through redundant mechanisms perfusion is maintained by them as the prostaglandins are reduced, suggesting a coordinated co-regulation servo-mechanism. Redundant vasodilatory mechanisms therefore would confer an evolutionary advantage for the developing fetus. Previous studies have shown the role of the prostanoids produced by the utero-placental unit and its vasculature during pregnancy are functioning to reduce the uterine and systemic vasoconstrictor effects of angiotensin II [103, 107] and also norepinephrine [103, 105, 106, 108, 109]. The effects of PGI_2 in vascular cells are mediated by the classic PGI_2/cAMP/PKA pathway (Fig. 11.2). Similarly, the prostanoid PGE_2 activates adenylate cyclase to increase cAMP and has been shown to stimulate GJ function and Cx43 expression in osteoblast-like cells [19, 20].

From a clinical and translational perspective, PGI_2 is quite important. There are decreased PGI_2 plasma concentrations and urinary metabolite concentrations [110–113] prior to the onset of clinical symptoms of PE. Placental PGI_2 is present early in the gestation and increases considerably between 6 and 12 weeks of human pregnancy during the time when a dramatic decrease in systemic and uterine vascular resistance are observed. Women with PE are also considerably more sensitive to infusions of exogenous angio-tensin II compared to normal pregnant women showing elevated pressor responses [114, 115]. It has also been reported that the normal pregnancy insensitivity to angiotensin II is abrogated by treatment with a cyclooxygenase inhibitor [88, 103, 107, 115, 116]. This further illustrates the role PGI_2 plays in uterine vasculature in normal pregnancy.

4.2 Nitric Oxide and cGMP

The vascular effects that increase UBF during gestation are partly mediated by the rapid production of the potent vasodilator NO via elevations in endothelial nitric oxide synthase (eNOS) expression and its activation [18, 93, 99, 117, 118]. Consistent with previous reports [17, 18, 98, 99, 118–120], we have recently shown that uterine artery endothelium (UAendo) total eNOS was elevated during the follicular phase and pregnancy [6]. The reported stimulatory phosphorylation site serine 635 (S635), an index of enzyme activity in UAendo [121, 122], was also elevated by pregnancy suggesting that both expression capacity and activity of eNOS are increased to accommodate increases in UBF. Additionally, we reported a pregnancy-associated adaptation in nonreproduc-tive OAendo which showed increases in both P ^{635}eNOS and total eNOS [6], the latter confirming our previous observation [18]. These data and others suggest that systemic and uterine resistance vessels show greater endothelium and NO-mediated vasorelaxation in pregnant vs. non-pregnant animals [90, 123], and in women [124]. Similarly, elevations in shear stress, which is the most powerful physiologic mechanical stimulus of endothelial NO production [125], is an additional mechanism that is associated with rises in UBF and likely exacerbate the rises in eNOS and NO [6, 125]. Shear stress, which is the tangential frictional force exerted on the surface of endothelial cells, acutely regulates vascular tone by altering the production of vasoactive mediators by endothelial cells through simulating the expression and phosphorylation activation state of eNOS, the latter controlled via posttranslational modifications [126–128]. NO exerts its physiological effects through the NO/cGMP signaling cascade. The NO/cGMP/PKG signaling cascade is increased in response to growth factors, vasoactive peptides, and other stimuli and have been shown to effect differentiation/proliferation of VSM [129–131]. Additionally, Yao et al. have reported that activation of the NO/cGMP pathway increases GJIC and Cx43 expression acting through PKA activity [132] suggesting possible cross-talk between cyclic nucleotides and their respective protein kinases via phosphodiesterases.

Endothelium-dependent relaxation acting via the NO-cGMP pathway is inhibited in systemic aortic vessels of IUGR in rat pregnancies [133]. Likewise, placental expression of eNOS is reduced in various pregnancy conditions associated with IUGR in humans [134]. Studies have reported correspondingly reduced urinary nitrite/nitrate (NO metabolites) in subjects with PE [135], although plasma levels were not different from controls. Still, in pregnancies with associated IUGR, Schiessl et al. also observed reduced plasma nitrite/

nitrate and cGMP [136]. A dramatic elevation of this cyclic nucleotide is observed in normal pregnancy occurring in the uterine venous drainage of pregnant sheep, i.e. adjacent to the uterine horns with placentation [94], suggesting uterine vascular NO output in particular is profoundly elevated at this time.

5 Uterine Arterial Endothelial Gap Junctions in Gestation

Since, endothelial cells express various combinations of Cx37, Cx40, Cx43, and/or Cx45[30, 31], which provide the potential of diversity in GJ conduction and regulation [3], it is important to study the changes in expression and distribution of connexins during pregnancy. We recently reported the expression of both Cx37 and 43 in the uterine artery. However, Cx43 protein is much more abundant within the uterine artery [6, 7, 37, 125] and myometrium where it accumulates until parturition and is necessary for the synchronization of electrical and metabolic activities of uterine smooth muscle contractions during delivery [40]. Thus the distribution of connexins in reproductive tissues may play a critical role in the conductance and gating of signaling molecules.

5.1 Distribution of Connexin Proteins in the Uterine Artery Endothelial Cell Model In Vitro

In our previous in vitro culture studies using a validated ovine uterine artery endothelial cell (UAEC) model derived from nonpregnant (NP-UAEC) or pregnant ewes (P-UAEC), we detected Cx43, but not Cx37 or Cx40 in UAECs [7]. Using the same ovine UAEC passage 4 culture model, we demonstrated that the presence of functional Cx43 is specifically required for ATP-induced Ca^{2+} associated eNOS activation in vitro [7] (Fig. 11.3). Using short peptide mimicking sequences that correspond to specific short Cx sequences, GJ channels were blocked with the inhibitory Gap peptide GAP27 specific to Cx43 [(43,37) Gap27], which selectively blocked the normal pregnancy enhanced Ca^{2+} responses to ATP [7]. However this was only seen in the P-UAECs, but not in NP-UAECs, thereby demonstrating Cx43 is vital to pregnancy specific vasodilatation programming as previously defined [17].

5.2 Distribution of Connexin Proteins in the UA Endothelium and VSM In Vivo

Recently we also determined if the pregnancy specific changes observed in our in vitro model [7] were also seen in vivo [6] and thus have physiologic relevance in pregnancy. In this study, we utilized an in vivo ovine model restricting pregnancy to a single uterine horn [6, 137–139]. In Fig. 11.4 we show that Cx37 and 43 were both expressed and upregulated by the physiologic state of pregnancy in UAendo and/or uterine artery vascular smooth muscle (UAvsm) [6]. Moreover, UAvsm Cx37 and Cx43 were elevated by the follicular phase, but even more so by pregnancy; a physiologic state of high estrogen [15, 120, 125, 140, 141]. The nongravid unilateral side also showed elevations in UAvsm Cx37 and Cx43, demonstrating that systemic circulating hormones (e.g. estrogen) may partly regulate VSM connexins. This is consistent with another smooth muscle cell type in the myometrium, in which Cx43 is up-regulated when endogenous estrogen is elevated in labor [142]. Cx43 is classically categorized as a “contraction associated protein” elevated in labor when the estrogen/progesterone ratio is elevated [40, 142]. The pregnancy-associated fold changes of Cx37 were similar in UAendo and UAvsm suggesting that Cx37 synthesis may be co-regulated and we proposed this is the basis for the formation of myoendothelial GJs for

heteromeric cell communication between UAendo and UAvsm [8, 9]. By contrast, pregnancy-induced Cx43 increases were much greater in UAendo than in UAvsm [8, 9] and specifically support a role for endothelial–endothelial homomeric cell communication.

5.3 Expression of Cx43 in Systemic vs. Uterine Arterial Endothelia

The physiologic importance of GJ expression is perhaps best evaluated when examining the specificity of the local uterine vs. systemic effects of pregnancy. We previously reported that the uterine vasculature is affected in many aspects to a much greater extent by pregnancy and estrogen treatments than nonreproductive vasculatures [17, 18, 98, 99, 118–120]. In studying the omental arteries (OAs) and renal arteries (RAs) as prototypic vessels important for blood pressure regulation, we found that both OAendo and RAendo Cx37 and Cx43 levels remained unchanged to both treatments, demonstrating that gestational endothelial adaptations were specific to uterine, but not systemic vasculature [6].

5.4 The Role of Pregnancy-Enhanced eNOS Activity/NO Production in Association with Gap Junction Function

As described above, it is known that cAMP and possibly cGMP up-regulate Cx43 expression and the enhanced production of NO by the UAendo is critical to vascular adaptations to pregnancy. However, what is not known is if the corresponding local UAendo changes in the expression and distribution of connexins play a role in further reinforcing enhanced in vivo eNOS activation and NO production during normal gestation. We demonstrated that Cx43, but not Cx37, specifically modulate ATP-stimulated Ca^{2+}-mediated eNOS activation evident in ex vivo UAendo, demonstrating physiologic functional significance [6]. These data are consistent with our observations shown in passage 4 cultured P-UAECs, but not NP-UAECs (Fig. 11.3), that Cx43 was a prerequisite requirement for ATP-mediated Ca^{2+} bursts-associated NO production [7]. We showed using ex vivo isolated UAs that this ATP-stimulated pregnancy programmed burst pattern for Ca^{2+}-mediated NO production were specific to Cx43 and are seen ex vivo and thus under physiologic conditions [6], rather than in vitro conditions [7] (Fig. 11.3). In addition, [43,37] Gap27 pretreatment of UAendo in vessels from pregnancy and P-UAECs converted the ATP-stimulated Ca^{2+} and NO response to ones that were identical to those we had reported from nonpregnant Luteal or Follicular UAendo and NP-UAECs [143] (Fig. 11.3b).

6 Discussion and Perspectives

Collectively, we have presented data supporting the notion that Cx43 expression and function are involved in the endothelial adaptations needed to increase cell–cell communication during pregnancy. We have shown that Cx43 is crucial for local Ca^{2+}-mediated eNOS activation and NO production by the UAendo [6]. In addition, we suggest a role for prostanoids such as PGI_2 in stimulating Cx43 expression [19, 20]. We also suggest an important physiologic mechanistic role for connexins and elevations in UA shear-stress to maintain uterine perfusion via NO and PGI_2 during ovine pregnancy [6, 125]. These pregnancy-induced endothelial cell adaptations are critical in gestation since their dysfunctions are found in disorders of pregnancy such as PE with IUGR which is seen in 5–13 % of all pregnancies [21]. Notably, PE with IUGR are associated with reduced UBF

causing significant maternal and fetal morbidity and mortality as well as greater susceptibility and earlier onset of future cardiovascular disease in both the mother and baby [21].

GJs have a significant role in regulating vasodilatory pathways that modulate numerous cardiovascular functions including increasing and maintaining UBF during gestation. The connection between endothelial dysfunction, reduced PGI_2 and NO biosynthesis, and reduced UBF in PE has been previously reported [110–113, 132]. In this review we suggest that the normal physiologic rises in UBF and thus UA shear stress, Cx43 and eNOS phosphorylation states are increased via local mechanisms only in the uterine vessels adjacent to the uterine horn that contain a feto-placental unit. Understanding the mechanisms regulating UA function gives us greater understanding of the specific mechanisms controlling normal UBF during gestation which may function abnormally in PE. Local steroid hormones or growth factors produced by the placenta may modulate mechanisms controlling UBF. These are locally secreted into the uterine venous blood and reach the tissues via arterial-venous shunts [144] or the lymphatic drainage [145, 146] in order to cause unilateral vasodilation and vascular remodeling. We recently reported that UAendo ATP-induced eNOS activation and NO production is Ca^{2+}-mediated and has an obligatory requirement for Cx43 [6]. Additionally, the local production of prostanoid enzymes as well as PGI_2 by the endothelium may play a role in increasing UBF through the stimulation of cAMP associated Cx43 expression and assembly as well as through the regulation of the responses to vasoconstrictors that are elevated in gestation [103, 105, 106, 108, 109]. Thus even under conditions of uterine space limitations and placental insufficiency, uterine perfusion is partly maintained at control levels via the co-regulation of Cx43, prostanoids, and eNOS for more robust PGI_2 and NO production. The end result is to maintain UBF for nutrient and oxygen delivery and thus fetal growth in an albeit comprised *in utero* environment.

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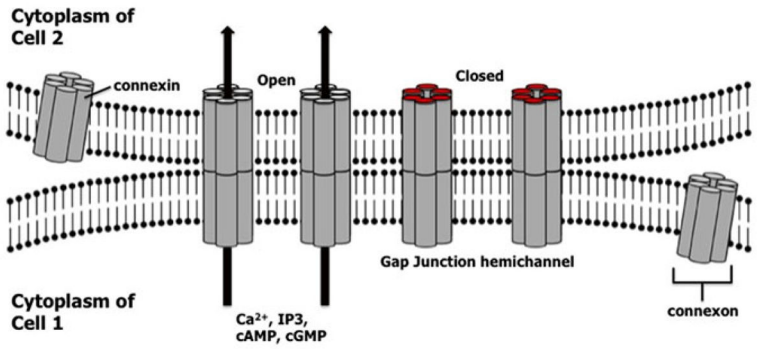

Fig. 11.1.

GJ structure and intercellular communication. Illustration of GJ channels, which connect the intercellular environments of two neighboring cells and facilitate the passive and directional diffusion of small ions, signaling molecules, and secondary messengers. Membrane proteins called connexins hexamerize to form a GJ hemichannel called a connexon. Connexins configure on adjacent cell membranes and connect, permitting cell–cell communication

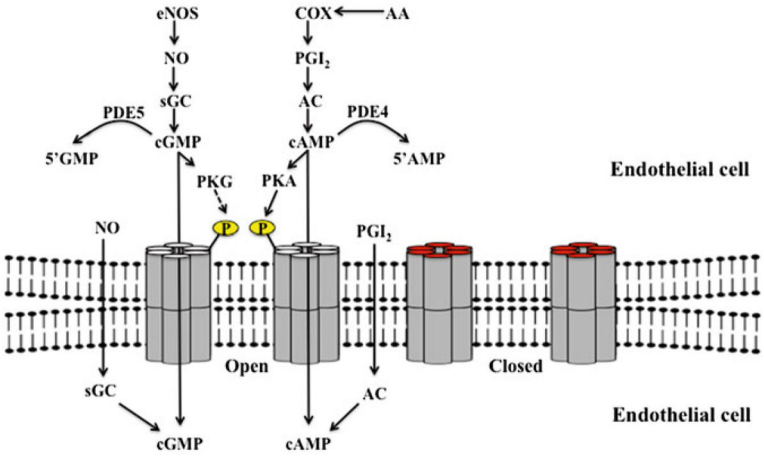

Fig. 11.2.

Signal transduction pathway for PGI_2/cAMP/PKA and NO/cGMP/PKG systems for endothelium–endothelium communication. In the vascular system, PGI_2 and NO are produced by the endothelium. PGI_2 is synthesized from AA by the COX pathway. PGI_2 activates adenylate cyclase, leading to increased production of cAMP, whereas the degradation of cAMP is catalyzed by phosphodiesterase 4 (PDE4), which converts cAMP to inactive 5′ AMP. cAMP activates PKA to phosphorylate and open gap junction channels to enhance cell–cell communication. NO is synthesized from eNOS and activates sGC, yielding increased levels of cGMP, whereas the degradation of cGMP is catalyzed by PDE5, which converts cGMP to inactive 5′GMP. cGMP activates PKG to phosphorylate and may open gap junction channels and enhance cell–cell communication. Adenylate Cyclase (AC); Arachidonic Acid (AA); Cyclooxygenase (COX); cyclic AMP (cAMP); cyclic GMP (cGMP); endothelial NO synthase (eNOS); Phosphodiesterase 4 (PDE4); Phosphodiesterase 5 (PDE5); Prostacyclin (PGI_2); soluble guanylatecyclase (sGC)

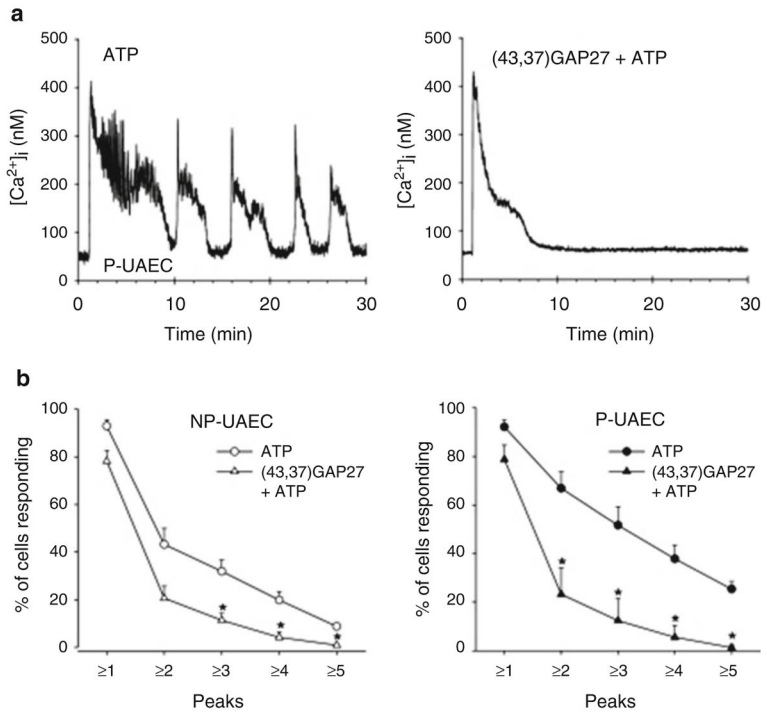

Fig. 11.3.

GAP27 inhibition of [CA2+]$_i$ burst activity in confluent uterine artery endothelial cells from the pregnant state (P-UAECs). (**a**) P-UAECs treated with 100 μM ATP show the expected [Ca $^{2+}$]$_i$ burst activity (*left*), but pretreatment of 300 μM [43,27] GAP27 prevents all burst activity except for the initial [Ca $^{2+}$]$_i$ response (*right*). (**b**) Quantification of [Ca $^{2+}$]$_i$ burst activity shows that although P-UAECs (*right*) initially demonstrate a greater percentage of cells with burst activity than NP-UAECs (*left*), application of [43,37] GAP27 inhibits both down to a common level. Data are means ± SEM n = 5–7 dishes, with approximately 60 observations per dish. Significant difference between ATP alone and [43,37] GAP27 plus ATP response is shown by $*P < 0.05$ [7]. Data originally reported by Yi et al. [7] (Reprinted with permission from the Society for the Study of Reproduction). Inhibitory Gap peptide GAP27 specific to Cx43 [(43,37) GAP27]; Uterine Artery Endothelial Cells from the pregnant sheep (P-UAECs); Uterine Artery Endothelial Cells from non-pregnant sheep (NP-UAECs)

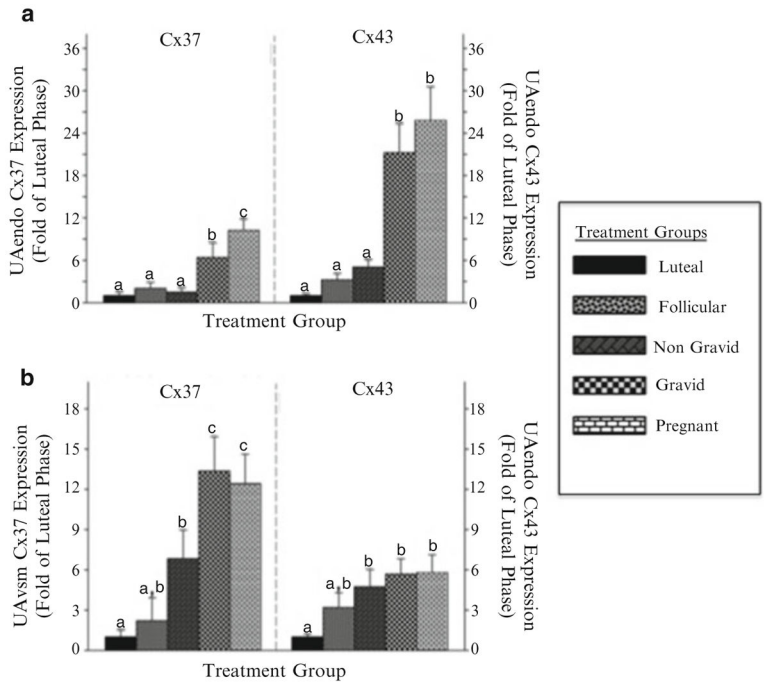

Fig. 11.4.

Connexin 37 and Connexin 43 protein expression in ovine (**a**) uterine artery endothelium (UAendo) and (**b**) vascular smooth muscle (UAvsm) from luteal, follicular, nongravid/ gravid unilateral pregnant, and control pregnant ewes. Uterine horns were ligated laterally before breeding, therefore restricting pregnancy to a single uterine horn yielding one horn that was gravid while the other was nongravid [6, 137–139]. Western Blot analysis comparing relative levels of connexin 37 and 43 in (**a**) UA endo and (**b**) UAvsm obtained from luteal (n = 8), follicular (n = 8), unilateral (nongravid vs. gravid; n = 15), and pregnant (n = 23) sheep. Data shown from these treatment groups are expressed as means ± SEM fold of Luteal. Different letters denote differences ($P < 0.05$). This figure is partly adapted from data originally reported [6]