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Roles of CLR/RAMP Receptor Signaling in Reproduction and Development

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Abstract

Adrenomedullin (ADM), calcitonin gene-related peptides (α - and β -CGRPs), and intermedin/adrenomedullin 2 (IMD/ADM2) are major regulators of vascular tone and cardiovascular development in vertebrates. Recent research into their functions in reproduction has illuminated the role of these peptides and their cognate receptors (calcitonin receptor-like receptor/receptor activity-modifying protein (CLR/RAMP) receptors) in fetal–maternal blood circulation, feto-placental development, female gamete development, and gamete movement in the oviduct. Although ADM family peptides function in a temporally and spatially specific manner in various reproductive processes, they appear to act via a similar set of second messengers, including nitric oxide, cyclic GMP, cyclic AMP, and calcium-activated potassium channels in different tissues. These discoveries supported the view that CLR/RAMP receptors were recruited to perform a variety of newly evolved reproductive functions during the evolution of internal reproduction in mammals. These advances also provided insight into how CLR/RAMP receptor signaling pathways coordinate with other physiological adaptations to accommodate the extra metabolic needs during pregnancy, and captured some important details as to how fetal–maternal vascular communications are generated in the first place. Furthermore, these findings have revealed novel, promising opportunities for the prevention and treatment of aberrant pregnancies such as pregnancy-induced hypertension, preeclampsia, and tubal ectopic pregnancy. However, significant efforts are still needed to clarify the relationships between certain components of the CLR/RAMP signaling pathway and aberrant pregnancies before CLR/RAMP receptors can become targets for clinical management. With this understanding, this review summarizes recent progresses with particular focus on clinical implications.

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CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

Keywords

CGRP; adrenomedullin; intermedin; adrenomedullin 2; pregnancy; preeclampsia; implantation; placentation; cumulus cells; CLR; RAMP1; RAMP2; RAMP3

1. INTRODUCTION

Adrenomedullin (ADM), calcitonin gene-related peptides (α - and β -CGRPs), and intermedin/adrenomedullin 2 (IMD/ADM2) belong to a peptide family that also includes calcitonin and amylin [1, 2]. ADM, CGRP, and IMD are structurally similar and signal through a G protein-coupled receptor system consisting of two transmembrane components, the calcitonin receptor-like receptor (CLR) and one of the three receptor activity-modifying proteins (RAMP1, 2, and 3) [1–5]. Whereas CGRPs mainly act through the CLR/RAMP1 receptor, ADM has high affinity for CLR/RAMP2 and 3 receptors (Table 1) [3, 6]. On the other hand, IMD exhibits no distinct preference for the three CLR/RAMP receptors [1]. These peptides have overlapping biological effects because of their cross-reactivity between receptors. Specifically, these ligands have been associated with the regulation of neurotransmission, vasodilation, the proliferation and differentiation of endothelial cells, vascular development, and inflammatory response [5, 7–10]. Whereas CGRPs play important roles in neurotransmission, ADM and IMD have been characterized as pleiotropic autocrine and paracrine factors in a variety of tissues as well as circulating hormones [5, 11, 12]. Although earlier studies of these peptides tended to focus on the cardiovascular system, studies in the last decade showed that CLR/RAMP receptor signaling also plays important roles in numerous reproductive processes in mammals.

ADM, CGRP, IMD, and their receptors are highly conserved across species [13–15], and are expressed in female reproductive organs including the ovaries, oviducts, and uterus as well as the placenta [16–21]. During the evolution of placental reproduction in therian mammals, these conserved peptides and receptors were recruited to perform new regulatory functions that originated in the cardiovascular system. In female reproductive tissues, these ligand-receptor pairs are dynamically regulated depending on the reproductive stage of the mother and the developmental stage of the fetus [22–24]. Accordingly, in this review, we examine the roles of ADM, CGRP, and IMD based on their target tissues, which include main blood vessels that supply reproductive organs, uteroplacental tissues, the ovaries, and oviducts (Fig. 1). For the readers' benefit, we focus on emerging roles of these peptides and receptors based on recent studies, instead of providing a comprehensive review of the literature. In addition, readers may refer to a recent review by Lenhart and Caron that specifically focuses on the functions of ADM during pregnancy [25].

2. THE ROLE OF CLR/RAMP RECEPTOR SIGNALING IN THE MAIN ARTERIES THAT SUPPLY BLOOD TO THE REPRODUCTIVE ORGANS**2.1. Plasma Levels of ADM, CGRP, and IMD Increase During Pregnancy**

One of the most interesting findings about the role of ADM/CGRP/IMD in reproduction is that plasma concentrations of ADM elevate several fold during pregnancy; this observation

has been the subject of many studies in the last decade [25–27]. Because ADM has a potent vasodilatory effect, this observation suggested that an increase of ADM-CLR/RAMP signaling could be part of the cardiovascular adaptation in response to the increased metabolic burden during pregnancy [5, 26, 28]. Given that ADM is known to regulate endothelial cell development, vasodilation, and smooth muscle contraction in various vascular beds [5, 29, 30], it has been hypothesized that the increased circulatory levels of ADM during pregnancy could be involved in the regulation of uterus contraction, implantation, or maternal-fetal blood flow [5].

Studies of circulating ADM indicated that ADM levels increase gradually during the course of pregnancy [31, 32]. Although there was negligible difference of ADM concentrations between non-pregnant and first-trimester pregnant women, circulatory ADM concentrations in second-trimester and third-trimester were significantly higher than those of non-pregnant women [33–35]. In addition, it was shown that in each trimester, the maternal plasma ADM concentrations of triplet-pregnant women were significantly higher than those in twin and singleton-pregnant women. Furthermore, there was a significant correlation between ADM levels and blood volume per unit body weight in pregnant women [32, 36]. Likewise, plasma ADM levels in rats at 21 days of pregnancy were more than threefold that of non-pregnant animals, and the level returned to pre-pregnancy values post-partum [31]. Studies of CGRP and IMD have documented a similar trend. CGRP concentrations increased in both maternal and fetal circulation during late pregnancy in humans [37]; whereas, circulatory IMD levels were associated with progression of pregnancy in rats [38]. These studies together indicated that the circulatory ADM, CGRP, and IMD could all be up-regulated during pregnancy (Fig. 1).

In mammals, uteroplacental blood flow increases substantially during pregnancy in order to maintain an efficient oxygen and nutrient supply to the developing embryo(s) (1). For example, it was shown that blood flow through the uterus and mesenteric arteries increases 3,500% and 90%, respectively, by term in guinea pig [39]. By contrast, the flow through renal arteries increases by only 10%. To accommodate the increased blood flow, the weight of uterine arteries can increase many fold during pregnancy [40]. In humans, this adaptive response begins soon after the trophoblasts migrate into the lumen of spiral arteries, and the invasive trophoblasts start to displace endothelium to become endovascular trophoblasts. At the same time, the invading trophoblasts actively degrade the muscularis to loosen the endometrium arteries. This conversion process results in a decrease in vessel resistance and an increase in vessel capacitance, thereby allowing the arteries to accommodate the increased blood flow during pregnancy. Because ADM, CGRP, and IMD are among the most potent, known vasodilators in humans, the increased levels of these peptides in circulation may be functionally important for maintaining the high flow/low resistance circulation required during pregnancy [35, 41, 42]. In humans, an elevated plasma ADM level has been associated with various cardiovascular, respiratory, endocrine, and renal disorders [5]. It was generally assumed that the elevated ADM levels under pathological conditions were consequences rather than causes of the diseases. It has also been repeatedly suggested that the elevated ADM levels under pathological conditions could be part of the compensatory mechanism to maintain homeostasis in the face of elevated blood pressure or hypoxic conditions [5, 43–46]. This hypothesis is supported by the finding that the promoter

regions of ADM, IMD, and CLR all contain multiple hypoxia response elements that transactivate with the hypoxia inducing factor-1 [44–47]. Therefore, the pregnancy-induced increases of ADM, CGRP, and IMD in circulation could result from a regulatory mechanism that also contributes to increased plasma ADM concentrations in patients with a variety of cardiovascular disorders in the face of insufficient blood supply [5].

In healthy humans, ADM is expressed in a variety of tissues, including cardiovascular, central nervous, lymphatic, respiratory, skin, gastrointestinal, and reproductive systems [5]. While the exact sources of circulating ADM, CGRP, and IMD during pregnancy have yet to be established, it has been shown that ADM mRNA levels increase in the abdominal aorta, renal artery, and kidneys during pregnancy [41]. Consistently, the immunoreactive ADM in the kidneys, uterus, abdominal aorta, and renal, uterine and superior mesenteric arteries was elevated in pregnant rats (compared to non-pregnant rats) (Table 2) [41]. Thus, the elevated plasma concentrations of, at least, ADM during pregnancy could be partly attributed to an enhanced peptide production by the systemic vasculature.

2.2. Vascular Responsiveness to ADM, CGRP, and IMD Increases During Pregnancy

In addition to increased circulatory ADM, CGRP, and IMD levels, multiple studies have reported that the hypotensive effects of these peptides increase significantly during pregnancy [38, 48, 49]. These data suggested that there could be an increase of CLR/RAMP receptors or downstream effectors in select arteries during pregnancy (Fig. 1).

Because one of the most effective ways to increase blood flow to a select organ is to increase the capacitance of vessels that are in proximity to the target organ, many studies on vascular responses to ADM family peptides during pregnancy have focused on uterine and mesenteric arteries. Analysis of endothelium-intact mesenteric arterial rings that had been precontracted by norepinephrine showed that the potency of ADM is significantly higher in vessels from pregnant rats as compared to those from non-pregnant females at diestrus [48]. The high vasodilatory effects of ADM are associated with increased generation of cGMP and cAMP in the artery [48]. Using a similar approach, it was shown that IMD stimulation leads to a dose-dependent relaxation of endothelium-intact arterial tissues from pregnant rats, and the vascular reactivity to IMD is significantly higher in pregnant rats as compared to that of non-pregnant rats [38]. Because inhibition of soluble guanylate cyclase, endothelial nitric oxide synthase, adenylate cyclase, protein kinase A, or calcium-activated K⁺ (KCa) channels all led to a reduced relaxation of mesenteric arteries, the ADM- and IMD-induced vasorelaxation of endothelium-intact arterial tissue appeared to depend on nitric oxide, cGMP, cAMP, and KCa channel [38, 50]. ADM is also capable of producing concentration-dependent relaxation of endothelium-denuded mesenteric artery rings that have been precontracted with norepinephrine. Thus, ADM-induced relaxation is not strictly endothelium-dependent; this vascular adaptation involves both vascular endothelium and underlying smooth muscle cells [51].

Similar to studies of rat mesenteric arteries, *in vitro* measurements of isometric force of uterine arteries showed that ADM-induced relaxation of uterine arteries is influenced positively by pregnancy [50]. Likewise, CGRP was capable of producing a concentration-dependent relaxation of uterine arteries, and the vascular sensitivity to CGRP was enhanced

during pregnancy [52]. In uterine arteries, the CGRP-induced relaxation was significantly attenuated by inhibitors of guan-ylate cyclase, adenylate cyclase, and potassium channels [52]. The combined results of these studies suggested that there is a significant increase in responsiveness to ADM family peptides in main arteries that deliver blood to fetuses. Here, it is important to note that the increased sensitivity to ADM family peptides during pregnancy is specific given that the well-characterized endothelium-dependent relaxation effect of acetylcholine in the uterine artery is not altered by pregnancy [53].

Other than having direct effects on mesenteric and uterine arteries, ADM and CGRP have a potent relaxation effect on the uterus [54–56]. The uterus is innervated by CGRP immunoreactive neurons, and CGRP inhibits spontaneous and acetylcholine-induced contractions of the uterus. Importantly, the CGRP-induced uterine relaxation has been found to be reduced at parturition compared to earlier stages of pregnancy in mice [55, 57]. Likewise, ADM is capable of inhibiting spontaneous and bradykinin-induced uterine contractions [54]. Because ADM is highly expressed in the uterus during pregnancy [20, 58], these data indicated that circulatory and locally produced peptides could participate in the regulation of both vascular adaptations and uterus tension during pregnancy.

2.3. The Expression of CLR/RAMP Receptor Components in Mesenteric, Uterine, and Umbilical Arteries Increases During pregnancy

To explain the increased vascular sensitivity to ADM family peptides during pregnancy, a number of studies have investigated the expression of CLR and RAMPs in arteries. Analysis of mRNA expression in rat mesenteric arteries indicated that CLR and RAMP1 transcripts increase significantly during pregnancy compared to those of rats in diestrus [59]. Similar to mesenteric arteries, the expression of CLR, RAMP1, RAMP2, and RAMP3 mRNA in the uterus is significantly higher in pregnant compared to nonpregnant rats [20, 60]. On the other hand, immunofluorescent staining of fetal tissues revealed that CLR and RAMP1 are present in the vascular endothelium and the underlying smooth muscle cells in the umbilical artery/vein, chorionic artery/vein, and stem villous vessels during pregnancy [61]. These analyses clearly suggest that (1) increased vascular reactivity to ADM family peptides during pregnancy could be partly attributed to enhanced expression of CLR and RAMPs in select arteries [52, 57], and (2) a concerted elevation of ADM family peptides in circulation and CLR/RAMP receptors in mesenteric, uterine, and umbilical arteries during pregnancy increases the flexibility and capacitance of these blood vessels to accommodate the increased blood flow [62](Fig. 2).

2.4. The Expression of ADM and CLR/RAMP Receptors in the Uterus, and Mesenteric and Uterine Arteries is Regulated by Female Sex Steroid Hormones

In addition to changes in peptide and receptor expression, one active area of investigation of ADM-CLR/RAMP signaling during reproduction is the analysis of the regulatory mechanisms that underlie these changes. Earlier studies of vascular smooth muscle cells, fibroblasts, and macrophages have shown that ADM expression is increased by a range of cytokines, growth factors, and hormones, including tumor necrosis factor (TNF) α and β , interleukin-1 α and β , dexamethasone, cortisol, aldosterone, retinoic acid, and thyroid

hormone [63–66]. In these tissues, progesterone, estradiol, and testosterone had a negligible effect [65, 66].

Importantly, functional analysis in ovariectomized pregnant rats showed that both estrogen and progesterone treatments produce greater decreases in mean arterial blood pressure in response to CGRP as compared to both ovary-intact and ovariectomized nonpregnant rats, suggesting that the decrease in vascular tone during pregnancy may be mediated, at least in part, by a sex-steroid hormone-induced increase in the vascular sensitivity to CGRPs [49]. In addition, it was shown that levels of uterine ADM mRNA are positively correlated with plasma estradiol in cycling rats but are not correlated with plasma progesterone [67]. Of importance, ovariectomy almost completely abrogated uterine ADM expression, and estradiol replacement was able to restore ADM transcript expression [67].

Analysis of uterine expression of CLR, RAMP2, and RAMP3 during the estrous cycle in mice indicated that these receptor components increase prior to the uterine receptive phase, suggesting that CLR/RAMP expression in the uterus could be regulated by female steroid hormones [68]. Indeed, in adult ovariectomized rats, progesterone significantly increased CLR, RAMP1, and RAMP2 mRNA in the uterus whereas treatment with 17 β -estradiol or RU-486 (mifepristone, an antagonist of progesterone and glucocorticoid receptors) significantly decreased the expression of CLR and RAMPs [57]. In addition, the expression of RAMP2 and RAMP3 in the uterus has been shown to be estrogen-dependent [69–71], and the expression of RAMP3 was mediated by estrogen response elements in the gene promoter. In corroboration with these studies, both estradiol and progesterone have been shown to stimulate the expression of CLR and RAMP3 in the mesenteric artery [72]. In addition, analysis of CLR and RAMP1 expression in the mesenteric artery showed that progesterone upregulated these receptor components whereas RU-486 downregulated their expression [59].

Taken together, these data suggest that the expression of ADM and CLR/RAMP receptors in the uterus, and mesenteric and uterine arteries is regulated by estrogens and progesterones in a tissue-specific manner, and these adaptive changes could represent part of the concerted regulation of reproductive processes by gonadotropins and sex steroid hormones during pregnancy.

2.5. Pharmacological Blockage of CLR/RAMP Receptor Signaling *In Vivo* Leads to Aberrant Reproductive Outcomes

In addition to vascular tone regulation, ADM was found to have effects on vascular cell proliferation, differentiation, and angiogenesis soon after its discovery [5]. The increased levels of ADM family peptides in circulation during pregnancy may be important for the regulation of not only vascular tone but also vessel development at the maternal-fetal interface (Table 3). To provide a better understanding of the role of ADM family peptides, researchers have taken a pharmacological ablation approach to investigate their roles at different stages of pregnancy. In a series of analyses of pregnant rats, Yallampalli and colleagues showed that treatment with CLR/RAMP receptor antagonists, including AM22-52, CGRP8-37, and IMD17-47, leads to suboptimal pregnancy outcomes [73–76]. For example, a study of gestational day 15 rats— which had been implanted with osmotic

minipumps that delivered the ADM antagonist AM22-52 on gestational day 8 —showed that blocking CLR/RAMP signaling reduces both placental and fetal weights in a dose-dependent manner [74]. In antagonist-treated rats, active caspase-3 protein was abundant in the placenta and uterus. The levels of apoptotic bax protein increased in mitochondrial fractions whereas the cytochrome c level increased in the cytosol fraction, suggesting that blocking the CLR/RAMP2 and 3 receptors activates bax- and cytochrome c-dependent apoptotic pathways [74].

Another study showed that infusion of CGRP8-37 into pregnant rats from day 17 to 20 of gestation led to increased blood pressure, reduced placental and fetal weights, as well as increased mortality of pups [73]. Similarly, infusion of IMD antagonist IMD17-47 from day 3 to day 9 of pregnancy caused a significant decrease in the weight of placentas and embryos, and serum levels of 17 β -estradiol, progesterone, and nitric oxide as well as serum matrix metalloproteinase 2 and 9 gelatinase activities [76]. Together, these *in vivo* studies showed that impaired CLR/RAMP receptor signaling could lead to cell death, aberrant implantation, and retarded embryo development, perhaps due to reduced blood flow to the uteroplacental tissues. Importantly, these data raised the possibility that exogenous ADM family peptides may be useful for managing aberrant pregnancies that are associated with impaired maternal-fetal blood circulation. Nonetheless, the function of individual peptides (i.e., ADM, CGRP, and IMD) in supporting normal pregnancy remains to be clarified because available CLR/RAMP receptor antagonists cross-react with the three CLR/RAMP receptors [77].

3. ROLE OF CLR/RAMP RECEPTOR SIGNALING IN UTEROPLACENTAL TISSUES

3.1. Both Ligands and Receptors of the CLR/RAMP Signaling System are Widely Expressed in Feto-Placental Tissues

One key step in mammalian pregnancy is proper invasion of trophoblasts into endometrium decidua and subsequent establishment of maternal-fetal circulation. In the early implantation stages, decidual endometrial cells secreted several molecules, such as insulin-like growth factor (IGF) binding protein-1, transforming growth factor- β 1 (TGF- β 1), and osteopontin to facilitate embryo attachment and migration [78, 79]. After blastocyst adhesion, blastocyst-derived trophoblast cells progressively invade and anchor into the uterine wall to establish a placenta and the maternal-fetal vessel communication. Given that ADM family peptides have potent stimulatory effects on the proliferation of endothelial cells that serve as starter materials for blood vessels [80–82], it is conceivable that circulatory and locally derived peptides could facilitate implantation and placentation by promoting vessel development at the maternal-fetal interface [28].

In support of this proposition, ADM was shown to be expressed in endometrium, endometrial stroma, myometrium, feto-placental tissues, and implantation sites in humans [67, 83, 84]. Immunoreactive IMD was found in syncytiotrophoblasts, cytotrophoblasts, and endothelial cells throughout human pregnancy [76, 85]; immunoreactive CGRP, however, was mainly localized in the villous and extravillous trophoblasts, and decidual cells in the

first-trimester villous tissues [86]. Likewise, uterine expression of ADM in mice was found to be localized specifically at the implantation site next to the developing embryo [68, 87]. In addition, studies of fetal-derived preimplanting trophoctoderm cells and differentiated trophoblast giant cells showed that ADM expression is upregulated by more than 30-fold in differentiated trophoblast giant cells as compared to that of undifferentiated trophoblast cells [87]. In rats, the ADM transcript in the implantation sites in mid (day 12) and late pregnancy (day 17) was more than 10-fold higher than that in nonpregnancy, pre-implantation (day 3), or early (day 7) pregnancy [24]. By contrast, the expression of IMD in implantation sites in rats was shown to be significantly higher on day 15 compared to days 18–22 [76, 88].

Studies of receptor components have shown that CLR protein is expressed in human decidua and extravillous trophoblast cells, and vascular endothelial cells in the first-trimester placental chorionic villi as well as human chorio-carcinoma JAr cells and cytotrophoblast HTR-8/SV neo cells [86, 89, 90]. In rat placentas obtained on day 18 of pregnancy, CLR and RAMP1 were specifically concentrated in the cytotrophoblast and syncytium, trophoblastic giant cells, basophilic cells in the trophospongial cell layer, endothelium, and smooth muscle cells in fetal vessels [62]. In pregnant mice, CLR and RAMP2 transcripts were found to be upregulated in uterine luminal epithelial cells, stroma, and glandular epithelium whereas RAMP3 expression was the most robust in the stroma adjacent to the conceptus [68].

Similar to the expression in mesenteric and uterine arteries, the expression of CLR and RAMP1 in placentas was shown to be female-sex-steroid hormone-dependent; treatment with progesterone stimulated CLR and RAMP1 expression whereas 17 β -estradiol and RU-486 inhibited the expression of these receptor components [62]. In addition, studies of cultured human trophoblast cells showed that local environmental conditions (e.g., hypoxia) could stimulate the expression of ADM in trophoblast cells, perhaps mediated by hypoxia response elements in the ADM gene promoter [44, 45, 91]. Furthermore, it has been demonstrated that secretion and expression of ADM in human placental trophoblast cells and in amnion cells of the fetal membranes is regulated by select glucocorticoids [92, 93].

Together, these data implied that (1) the increased plasma concentrations of ADM family peptides during pregnancy could be attributed to increased peptide production in both maternal vasculatures and feto-placental tissues, and (2) CLR/RAMP receptor signaling in feto-placental tissues could be affected by both circulatory and locally derived ligands.

3.2. ADM Signaling is Essential for Normal Placental and Fetal Development

In addition to pharmacological analyses, studies of genetic models have provided much-needed insight into the role of ADM family peptides in implantation, placentation, and early embryo development. Research has indicated that mouse embryos with an ADM deficiency cannot survive due to poor development of blood and lymphatic vasculatures, and formation of hydrops fetalis [94–96]. Homozygous ADM knockout mice (*Adm*^{−/−}) died in utero during midgestation from systemic hemorrhage and interstitial lymphedema [94, 96](Table 3). By contrast, mice deficient for α -CGRP exhibited defects mainly in cardiovascular regulation, but not in reproduction [97–100].

Although female *Adm*^{+/-} mice have normal ovulation and fertilization rates, they exhibit a variety of pregnancy-associated defects, including absence of uterine pinopodes, reduced pregnancy and implantation rates, defective placentation, abnormal implantation spacing, and fetal growth restriction [68, 87]. In addition, placentas from growth-restricted embryos exhibit defects in trophoblast cell invasion [87]. Based on these observations, it has been hypothesized that reduced pregnancy rates of *Adm*^{+/-} female mice may be due to inadequate maternal ADM expression and defects in uterine receptivity. In support of this view, administration of ADM to crossbred *Adm*^{+/-} female mice was able to increase the survival rate of *Adm*^{-/-} embryos at E14.5 [94].

3.3. CLR/RAMP2 Signaling Plays a Critical Role in the Development of Blood and Lymphatic Vascular Systems

Similar to ADM-deficient mice, mouse embryos deficient for CLR (*Clr*^{-/-}) died during mid-gestation, and exhibited hydrops fetalis, thin vascular smooth muscle walls, and disorganized hearts [101]. Consistent with studies of transgenic mice, loss of CLR in zebrafish resulted in delays in inter-somitic vessel development and a loss of arterial endothelial cell identity in dorsal aorta, as evidenced by the lack of arterial markers ephrin-B2a, delta C, and notch5 [102]. Furthermore, because down-regulation of CLR or ADM affected vascular endothelial growth factor (VEGF) expression [82, 102], and because blockage of VEGF receptor signaling abolished the proangiogenic effect of ADM [103, 104], ADM and CLR/RAMP receptors could control vessel development during placentation and embryogenesis partly by regulating the expression of VEGF morphogen.

Among the three RAMPs, RAMP2 appeared to play an essential role in embryo development. Mice deficient for RAMP2 (*Ramp2*^{-/-}) died at midgestation due to severe edema and hemorrhage, similar to *Adm*^{-/-} and *Clr*^{-/-} mice [105]. In these mice, the expression of tight junction, adherence junction, and basement membrane molecules by endothelial cells was diminished, leading to paracellular leakage. In addition, it was shown that the number of live births was markedly reduced when heterozygous *Ramp2*^{+/-} mice were cross-bred [105, 106]. Other reproductive defects of *Ramp2*^{+/-} mice included fetal growth restriction, postnatal mortality, hyperprolactinemia during pregnancy, enlarged pituitary glands, accelerated mammary gland development, and decreased bone mineral density [106]. Importantly, further characterization of ADM-, CLR-, and RAMP2-deficient mice indicated that ADM-CLR/RAMP2 signaling plays a crucial role in the development of lymph sacs and jugular lymphatic trunk during embryogenesis, and the maintenance of normal lymphatic permeability and function [96, 105, 107, 108]. By contrast, mice deficient for RAMP1 or RAMP3 exhibited negligible defects in reproduction and embryo development [97–100].

Overall, these genetic studies indicated that (1) the actions of ADM family peptides are compartmentalized (that is, peptides that originated from both maternal and fetal tissues are needed for normal development of the fetus and feto-placental tissues at various stages of gestation [87]), and (2) the ADM-CLR/RAMP2 signaling pathway at both maternal and fetal compartments is especially important for the regulation of vessel development in placentas and embryos in mice [68]. In addition, the observation that ADM-, CLR-, and RAMP2-

deficient mice vary in their reproductive and developmental phenotypes suggests that other molecules could interact with CLR or RAMP2 and contribute to the variations. Although the exact mechanism is not clear, at least three mechanisms could lead to these differences. First, the restricted defects found in ADM-deficient mice could be a result of compensation by CGRP or IMD. Second, the severe defects in CLR- and RAMP2-deficient mice could be related to the fact that these proteins are anchored proteins whereas ADM is a free-moving ligand. Third, the wide spectrum of defects found in RAMP2-deficient mice could be associated with nonCLR-coupled signaling because RAMP2 is capable of interacting with the calcitonin receptor to form an amylin receptor; it is also able to interact with other type B G protein-coupled receptors, including corticotropin-releasing factor receptor 1 and vasoactive intestinal polypeptide receptors 1 and 2, to modulate their functions [109–112].

3.4. CLR/RAMP Receptor Signaling Supports Implantation and Embryo Development by Promoting Tissue Remodeling and Vascular Development

Although ADM family peptides facilitated implantation and uteroplacental communications in a temporally and spatially specific manner, they could act by mechanisms similar to those found in the adult cardiovascular system [5]. These peptides have been shown to promote capillary formation and hemoglobin content in Matrigel implants as well as migration and tube formation of cultured endothelial cells from a variety of vascular beds [5, 80]. In addition, overexpression of these peptides in the adductor muscles of rats after femoral artery ligation has been shown to increase blood perfusion, and capillary and arteriole density in the ischemic hind limb [80–82, 113, 114]. Mechanically, ADM, CGRP, and IMD could promote tissue remodeling by increasing phosphorylation of mitogen-activated protein kinase, protein kinase B, and endothelial nitric oxide synthase, as well as nitric oxide formation and VEGF receptor 2 synthesis [80–82].

Because trophoblast invasion is accompanied by extensive tissue remodeling, it has been hypothesized that circulatory and locally produced ADM family peptides may facilitate embryo implantation by promoting tissue remodeling and vascular integrity at the uteroplacental interface [89, 90, 115] (Figs. 1 and 2). Indeed, recent studies have revealed that ADM family peptides may facilitate embryo implantation by increasing gelatinolytic activity and reducing plasminogen activator inhibitor-1 expression [85, 88]. In addition, these peptides may enhance vessel development by enforcing normal vascular permeability given that ADM family peptides are capable of suppressing hyperpermeability induced by a variety of pharmacological agents [81, 115–117]. In corroboration with this idea, it has also been shown that endothelial cells with overexpression of RAMP2 exhibit enhanced tight junction integrity and reduced vascular permeability [105]. Furthermore, it has been speculated that IMD may facilitate implantation by stimulating the expression of nonclassical major histocompatibility complex class I molecules (HLA-G antigen) in trophoblast cells, which is important for the inhibition of uterus natural killer cell-mediated cytotoxicity and extravillous cytotrophoblast migration through the maternal decidua [88, 118].

Therefore, the disruption of normal pregnancy by CLR/RAMP receptor antagonists *in vivo* or genetic ablation could be due to the suppression of trophoblast invasion, implantation, and

subsequent vessel development. Nonetheless, the role(s) of individual ligands and receptors in implantation and placentation should be interpreted with caution because the interaction of these ligands and receptors at the maternal-fetal interface could be highly compartmentalized.

3.5. Dysregulated CLR/RAMP Signaling is Associated with Aberrant Pregnancies in Humans

Pregnancy-induced hypertension (PIH) and preeclampsia are disorders that impact 5–8% of pregnancies, and can lead to multi-organ failure and death. Preeclampsia was defined as a raised blood pressure of at least 140/90 mm Hg, or a rise in systolic (>30 mm Hg) or diastolic (>15 mm Hg) values over the baseline blood pressure obtained before 20 weeks of gestation, and proteinuria. PIH has a similar cardiovascular manifestation, but without proteinuria. Despite the prevalence of these diseases, the mechanisms that underlie them have yet to be fully vetted. Because (1) there is a concerted upregulation of ADM family peptides and CLR/RAMP receptors during pregnancy, and (2) ADM is essential for vaso-tone regulation—it has been hypothesized that aberrant regulation of ADM-CLR/RAMP signaling gives rise to high blood pressure in compromised pregnancies, including PIH and preeclampsia [29, 33, 36, 119]. In support of this hypothesis, studies of an animal model of preeclampsia, in which pregnant rats are pre-treated with a nitric oxide synthase inhibitor L-NAME [120–122], showed that infusion of ADM reverses typical preeclampsia-like symptoms, including hypertension and intrauterine growth restriction, and decreases pup mortality.

It has been reported that plasma ADM levels are elevated in women with preeclampsia at term compared to those of normotensive controls [123]. Likewise, a subset of studies have shown that concentrations of ADM in amniotic fluid and umbilical vein plasma in preeclamptic patients are higher than those of normotensive pregnant women [36], and immunoreactive ADM protein in choriodecidua and amnion of women with preeclampsia was significantly increased compared to that of normal pregnancy [124]. However, the relationship between ADM and preeclampsia appears to be complicated. In other reports, ADM concentrations in preeclamptic patients were found to be lower than those of third-trimester pregnant women, or were not significantly different from those of normal pregnant women even though the plasma atrial natriuretic peptide concentrations were significantly elevated in preeclampsia (as compared with those of normal third trimester pregnancies) [32–34, 36, 125, 126].

Similar to analyses of plasma ADM levels, studies of ADM transcripts in placental tissues of preeclamptic patients have reported inconsistent results. In one study, it was reported that ADM mRNA was localized to the syncytiotrophoblasts and the extravillous cytotrophoblasts in the basal plate and choriodecidua regions, and the expression was increased in the choriodecidua, and cytotrophoblasts in peri-infarct regions in preeclampsia [127]. In other studies, ADM mRNA has been shown to be significantly lower in placental villi and villous syncytiotrophoblasts in women with preeclampsia or HELLP syndrome than in controls [128, 129]. A similar reduction of CGRP mRNA levels was also observed in chorionic plates of preeclamptic patients [128].

Functional studies using patient tissues have shown that CGRP dose-dependently relaxes serotonin-induced contraction of umbilical and chorionic arteries from normal pregnancies, and the response to CGRP was significantly reduced in vessels from preeclamptic patients [37]. In addition, both vascular tissues and trophoblasts in preeclamptic placentas showed decreased expression of CLR and RAMP1 proteins and declined CGRP binding sites [37]. However, studies of stem villous arteries from normotensive and preeclamptic pregnancies indicated that there was no significant difference in ADM-induced relaxation of arteries, which were precontracted with a thromboxane A₂ mimetic U46619, from normal and preeclampsia placentas [125].

Furthermore, studies using purified cytotrophoblasts from normal and preeclampsia placentas showed that, after a 3-day culture with epidermal growth factor (EGF), basal ADM production was significantly lower in preeclampsia placental cells than in normal placental cells; contrastingly, pretreatment with TGF- β 1, TNF- α , or interferon- γ had no effect on ADM secretion in either normal or preeclampsia placentas [130]. In addition, EGF significantly stimulated ADM production in normal trophoblast cells but not in preeclampsia placentas, and spontaneous placental syncytialization was impaired in preeclampsia [130], suggesting that the reduced ADM production in trophoblast cells of preeclamptic patients could be due to an impaired EGF response.

On the other hand, investigation of CLR and RAMP2 mRNA in placenta, uterine muscle, umbilical artery, and fetal membranes of PIH patients showed that these receptor components are significantly decreased in the umbilical artery and uterus of the PIH patients, and blood pressure is reversely correlated with RAMP2 mRNA levels in the umbilical artery and uterine muscle [131]. By contrast, the RAMP2 mRNA was significantly increased in the fetal membrane of PIH patients, which suggested that the manifestation of PIH is associated with aberrant regulation of CLR and/or RAMP2 in select tissues at the maternal-fetal interface [131].

Obviously, the relationship between CLR/RAMP receptor signaling and the occurrence of preeclampsia or PIH is complex and require further explication. The reported discrepancies could be related to variations in experimental settings, heterogeneity of patients, or differences in the compensatory vascular responses among tissues. Importantly, most investigations indicated that defective CLR/RAMP signaling in select vascular beds may underlie pathological changes in PIH and preeclampsia. Future investigations that extend these studies could reveal exactly which components of the ADM-CLR/RAMP signaling system and which vascular tissues are affected in PIH and preeclampsia [132].

4. ROLE OF CLR/RAMP RECEPTOR SIGNALING IN THE OVARY

4.1. ADM has Pleiotropic Effects in the Ovary

In mammals, ovarian folliculogenesis is regulated by gonadotropins from the anterior pituitary and a myriad of ovarian factors that include bone morphogenic protein (BMP) family factors, EGF family proteins, IGF family proteins, interleukins, and TNF family proteins among others [133–140]. Studies of ADM and CGRP in rat ovaries have shown that although CGRP is confined to nerve endings [141, 142], ADM expression in ovarian

follicles increases from the small antral stage to the large antral stage and corpora lutea. In addition, levels of ADM in ovarian follicles and the corpora lutea vary during the estrous cycle [17–19, 21], and the ADM level in the corpus luteum is higher in late pregnancy compared to early pregnancy [24]. Although CGRP appeared to have no effect on the basal or follicle-stimulating hormone (FSH)-stimulated release of estradiol or progesterone from cultured rat granulosa cells [141], ADM and IMD stimulated cAMP and estradiol production in cultured granulosa cells [143] (Fig. 3). However, treatment of preantral follicles cultured *in vitro* with ADM did not affect follicular growth or ovulation rate [144]. In addition, FSH and 8-Br-cAMP have been shown to significantly suppress ADM mRNA expression while substantially induce LH receptor mRNA expression in cultured rat granulosa cells.

Whereas CLR, RAMP2, and RAMP3 mRNA levels in ovaries remained unchanged throughout the estrous cycle, RAMP1 mRNA levels were significantly lower at estrus than at diestrus [21]. In the corpus luteum, CLR, RAMP2 and RAMP3 transcripts appeared to increase in late pregnancy compared with early pregnancy [24]. Together, these studies indicated that ADM functions as a paracrine factor during ovarian folliculogenesis and may play a role in the regulation of the hypothalamus-pituitary-ovary feedback.

The expression of ADM has also been detected in growing follicles and corpora lutea in humans. In healthy women with regular menstrual cycles, the plasma ADM profile during the ovarian cycle was similar to that of LH; plasma ADM increased from the 7th to the 14th day, and decreased in the subsequent menses [17–19, 21]. Although no appreciable staining for ADM was observed in granulosa cells of primordial and preantral follicles in humans, immunoreactive ADM was noted in granulosa cells of dominant follicles and in granulosa lutein cells at the midluteal phase [19]. Interestingly, the secretion of ADM by human ovarian follicular cells appeared to lead to the accumulation of ADM to a high concentration in follicular fluid [145–147]. Analysis of follicular fluid and blood samples from women undergoing *in vitro* fertilization (IVF) treatment because of male infertility showed that ADM levels in follicular fluid collected just before ovulation were significantly higher than those in the plasma, and ADM in the follicular fluid could be derived from luteinized granulosa cells and macrophages in the follicle [19, 148, 149]. Based on these observations, a number of investigations have analyzed whether ADM production in follicles is associated with reproductive outcomes of IVF clinics. In patients undergoing ovarian stimulation, the follicular fluid ADM level was found to correlate with serum concentrations of 17 β -estradiol and day 3 FSH [150]. However, ADM levels were inversely correlated with the total number of oocytes retrieved and the number of mature oocytes [145]. Also, ADM concentrations were found to be lower in follicles that resulted in pregnancy than in those that failed. Therefore, a high level of ADM in the follicular fluid could be associated with a decreased ovarian response and negative outcomes in IVF treatments [147]. Because follicular IMD could accumulate in follicular fluid as well, future investigation of the concentration of both ADM and IMD in follicular fluid may provide useful biomarkers for predicting and improving IVF outcomes.

4.2. IMD is Important for Maintaining Cell–Cell Contacts in Cumulus Oocyte Complexes

Based on an analysis of the EST database and the Gene Expression Omnibus data repository [151], we found that IMD transcript has a high representation in female gamete cDNA libraries. These studies suggested that IMD could be an oocyte-derived ligand that acts on neighboring cumulus cells [143]. Interestingly, studies of cumulus oocyte complexes (COCs) cultured *in vitro* showed that IMD is important for the regulation of cumulus-cell survival and cell–cell contacts in COCs (Fig. 3). Suppression of IMD signaling in growing rat ovaries *in vivo* led to oocyte atresia and significantly reduced cyclin D2 expression in follicular cells, suggesting that IMD facilitates follicle development, in part, by suppressing cumulus-cell apoptosis and stimulating cell cycle progression [143]. Although how IMD expression in follicular cells is regulated has not yet been fully explored, we speculated that oxygen tension may play a role in fine-tuning the expression of IMD in COCs given that the follicular oxygen concentration is reversely correlated with follicle diameter, and that the COC microenvironment becomes hypoxic when the follicle grows in size [152]. Because oocyte secreting factors, including growth differentiation factor 9 (GDF9) and bone morphogenetic factor 15 (BMP15), are known to promote granulosa cell proliferation and cumulus expansion, IMD may represent a niche signal from oocytes that is important for the maintenance of cell–cell contacts between the oocyte and cumulus cells in the presence of GDF9 and BMP15 during ovarian folliculogenesis (Fig. 3).

5. THE ROLE OF CLR/RAMP RECEPTOR SIGNALING IN THE OVIDUCT

5.1. CLR/RAMP Receptor Signaling Promotes Gamete Movement in the Oviduct

In mammals, gamete maturation, fertilization, and early embryonic development normally take place inside the oviduct. In the oviduct, immunoreactive ADM has been localized to the ciliated oviductal epithelial cells, especially the cilia on the apical surface [5, 153–155]. Based on the observation that ADM can stimulate sperm progressive motility [153, 156], ADM in the oviduct was hypothesized to facilitate sperm movement by acting directly on sperm. Interestingly, recent reports showed that ADM is also capable of increasing the ciliary beat frequency of human oviductal epithelial tissue in a dose-dependent manner; this suggests that ADM may function as an autocrine factor to indirectly facilitate the movement of male and female gametes in the oviduct by stimulating ciliary beatings of oviduct epithelium cells [153–155]. In addition, because semen contains high levels of ADM and because ADM expression in the human oviduct is up-regulated by sperm contact [23, 154], ADM may represent an uncharacterized factor that mediates semen-induced oocyte movement in the oviduct. Although how sperms stimulate ADM production in the oviduct requires further study, these recent findings suggested that the regulation of ADM expression in oviducts is likely distinct from that found in other tissues.

5.2. A Reduced Plasma ADM Level is Correlated with the Occurrence of Tubal Ectopic Pregnancy

Because defective ciliary motion in the oviduct has been associated with many pathological conditions such as infertility and ectopic pregnancy [155], aberrant regulation of ADM signaling could be related to ectopic pregnancies. Indeed, recent studies showed that plasma and oviductal tissue ADM levels in patients with tubal ectopic pregnancy are lower than

those from patients with normal pregnancy. Importantly, both ciliary beat frequency and frequency of muscle contraction of oviducts from patients with tubal ectopic pregnancy were found to be significantly lower than those from normal pregnancy [157]. Therefore, a low ADM level in the oviduct may lead to the retention and implantation of embryos in the oviduct in tubal ectopic pregnancies as a result of decreased ciliary beating and muscle contraction. Thus, the plasma ADM level may be a useful biomarker for the prediction of tubal ectopic pregnancy [157].

CONCLUSIONS

Using a variety of experimental models, recent investigations have revealed that CLR/RAMP receptors participate in the regulation of vascular development in maternal and fetal compartments during pregnancy as well as gamete development and movement. These new findings suggest that, during the evolution of internal reproduction in placental mammals, ligands and receptors of the CLR/RAMP signaling system were recruited to mediate a variety of newly evolved processes. Among the reproductive processes discussed here, the role of CLR/RAMP receptor signaling in maternal–fetal vascular communication was particularly well characterized. Findings in this area have offered insight into the way maternal vascular cells and fetal cells process the information that makes the coordinated growth of fetus and placenta possible. Also noteworthy, because most studies of CLR/RAMP receptor signaling in reproductive tissues have focused on ADM, the discussion has inadvertently leaned heavily on such studies; however, the relative importance of CGRP and IMD in such processes should not be discounted due to their overlapping receptor specificity with ADM. Finally, because CLR/RAMP receptor signaling is essential for normal transportation of oxygen and nutrients from the mother to the fetus, and because fetal growth retardation from restricted blood flow affects millions of pregnancy each year, these insights may eventually lead to new ways of treating PIH and preeclampsia using therapeutics that target the CLR/RAMP receptor signaling pathway.

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LIST OF ABBREVIATIONS

ADM	Adrenomedullin
ADM2	Adrenomedullin 2
BMP	Bone morphogenetic protein
CGRP	Calcitonin gene-related peptide
CLR	Calcitonin receptor-like receptor
COC	Cumulus oocyte complex
EGF	Epidermal growth factor

FSH	Follicle-stimulating hormone
GDF	Growth differentiation factor
IGF	Insulin-like growth factor
IMD	Intermedin
KCa channel	Calcium-activated K ⁺ channel
PIH	Pregnancy-induced hypertension
RAMP	Receptor activity-modifying protein
TGF-β1	Transforming growth factor- β 1
TNF	Tumor necrosis factor
VEGF	Vascular endothelial growth factor

References

1. Roh J, Chang CL, Bhalla A, Klein C, Hsu SY. Intermedin is a calcitonin/CGRP family peptide acting through the CRLR/RAMP receptor complexes. *J Biol Chem*. 2004; 279:7264–74. [PubMed: 14615490]
2. Takei Y, Inoue K, Ogoshi M, Kawahara T, Bannai H, Miyano S. Identification of novel adrenomedullin in mammals: a potent cardiovascular and renal regulator. *FEBS Lett*. 2004; 556:53–8. [PubMed: 14706825]
3. McLatchie LM, Fraser NJ, Main MJ, Wise A, Brown J, Thompson N, Solari R, Lee MG, Foord SM. RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. *Nature*. 1998; 393:333–9. [PubMed: 9620797]
4. Bell D, McDermott BJ. Intermedin (adrenomedullin-2): a novel counter-regulatory peptide in the cardiovascular and renal systems. *Br J Pharmacol*. 2008; 153(Suppl 1):S247–62. [PubMed: 17965749]
5. Hinson JP, Kapas S, Smith DM. Adrenomedullin, a multifunctional regulatory peptide. *Endocr Rev*. 2000; 21:138–67. [PubMed: 10782362]
6. Muff R, Leuthauser K, Buhlmann N, Foord SM, Fischer JA, Born W. Receptor activity modifying proteins regulate the activity of a calcitonin gene-related peptide receptor in rabbit aortic endothelial cells. *FEBS Lett*. 1998; 441:366–8. [PubMed: 9891972]
7. Moore EL, Salvatore CA. Targeting a family B GPCR/RAMP receptor complex: CGRP receptor antagonists and migraine. *Br J Pharmacol*. 2012; 166:66–78. [PubMed: 21871019]
8. Hong Y, Hay DL, Quirion R, Poyner DR. The pharmacology of adrenomedullin 2/intermedin. *Br J Pharmacol*. 2012; 166:110–20. [PubMed: 21658025]
9. Smillie SJ, Brain SD. Calcitonin gene-related peptide (CGRP) and its role in hypertension. *Neuropeptides*. 2011; 45:93–104. [PubMed: 21269690]
10. Reicher A, Russo AF. Calcitonin gene-related peptide: an update on the biology. *Curr Opin Neurol*. 2009; 22:241–6. [PubMed: 19434786]
11. Michibata H, Mukoyama M, Tanaka I, Suga S, Nakagawa M, Ishibashi R, Goto M, Akaji K, Fujiwara Y, Kiso Y, Nakao K. Autocrine/paracrine role of adrenomedullin in cultured endothelial and mesangial cells. *Kidney Int*. 1998; 53:979–85. [PubMed: 9551407]
12. Kitamura K, Ichiki Y, Tanaka M, Kawamoto M, Emura J, Sakakibara S, Kangawa K, Matsuo H, Eto T. Immunoreactive adrenomedullin in human plasma. *FEBS Lett*. 1994; 341:288–90. [PubMed: 8137955]

13. Chang CL, Roh J, Hsu SY. Intermedin, a novel calcitonin family peptide that exists in teleosts as well as in mammals: a comparison with other calcitonin/intermedin family peptides in vertebrates. *Peptides*. 2004; 25:1633–42. [PubMed: 15476930]
14. Ogoshi M, Inoue K, Naruse K, Takei Y. Evolutionary history of the calcitonin gene-related peptide family in vertebrates revealed by comparative genomic analyses. *Peptides*. 2006; 27:3154–64. [PubMed: 17092606]
15. Nag K, Kato A, Nakada T, Hoshijima K, Mistry AC, Takei Y, Hirose S. Molecular and functional characterization of adrenomedullin receptors in pufferfish. *Am J Physiol Regul Integr Comp Physiol*. 2006; 290:R467–78. [PubMed: 16195494]
16. Cameron VA, Fleming AM. Novel sites of adrenomedullin gene expression in mouse and rat tissues. *Endocrinology*. 1998; 139:2253–64. [PubMed: 9564831]
17. Abe K, Minegishi T, Tano M, Hirakawa T, Tsuchiya M, Kangawa K, Kojima M, Ibuki Y. Expression and effect of adrenomedullin on rat granulosa cell. *Endocrinology*. 1998; 139:5263–6. [PubMed: 9832468]
18. Abe K, Minegishi T, Ibuki Y, Kojima M, Kangawa K. Expression of adrenomedullin in the human corpus luteum. *Fertil Steril*. 2000; 74:141–5. [PubMed: 10899511]
19. Moriyama T, Otani T, Maruo T. Expression of adrenomedullin by human granulosa lutein cells and its effect on progesterone production. *Eur J Endocrinol*. 2000; 142:671–6. [PubMed: 10822232]
20. Upton PD, Austin C, Taylor GM, Nandha KA, Clark AJ, Ghatei MA, Bloom SR, Smith DM. Expression of adrenomedullin (ADM) and its binding sites in the rat uterus: increased number of binding sites and ADM messenger ribonucleic acid in 20-day pregnant rats compared with nonpregnant rats. *Endocrinology*. 1997; 138:2508–14. [PubMed: 9165042]
21. Li YY, Li L, Hwang IS, Tang F, OWS. Coexpression of adrenomedullin and its receptors in the reproductive system of the rat: effects on steroid secretion in rat ovary. *Biol Reprod*. 2008; 79:200–8. [PubMed: 18401014]
22. Laoag-Fernandez JB, Otani T, Maruo T. Adrenomedullin expression in the human endometrium. *Endocrine*. 2000; 12:15–9. [PubMed: 10855685]
23. Fazeli A, Affara NA, Hubank M, Holt WV. Sperm-induced modification of the oviductal gene expression profile after natural insemination in mice. *Biol Reprod*. 2004; 71:60–5. [PubMed: 14973272]
24. Li L, Tang F, OWS. Coexpression of adrenomedullin and its receptor component proteins in the reproductive system of the rat during gestation. *Reprod Biol Endocrinol*. 2010; 8:130. [PubMed: 21034462]
25. Lenhart PM, Caron KM. Adrenomedullin and pregnancy: perspectives from animal models to humans. *Trends Endocrinol Metab*. 2012; 10:524–32. [PubMed: 22425034]
26. Marinoni E, Di Iorio R, Letizia C, Villaccio B, Scucchi L, Cosmi EV. Immunoreactive adrenomedullin in human fetoplacental tissues. *Am J Obstet Gynecol*. 1998; 179:784–7. [PubMed: 9757990]
27. Macri CJ, Martinez A, Moody TW, Gray KD, Miller MJ, Gallagher M, Cuttitta F. Detection of adrenomedullin, a hypotensive peptide, in amniotic fluid and fetal membranes. *Am J Obstet Gynecol*. 1996; 175:906–11. [PubMed: 8885746]
28. Wilson C, Nikitenko LL, Sargent IL, Rees MC. Adrenomedullin: multiple functions in human pregnancy. *Angiogenesis*. 2004; 7:203–12. [PubMed: 15609075]
29. Di Iorio R, Marinoni E, Cosmi EV. New peptides, hormones and parturition. *Gynecol Endocrinol*. 1998; 12:429–34. [PubMed: 10065169]
30. Nikitenko LL, MacKenzie IZ, Rees MC, Bicknell R. Adrenomedullin is an autocrine regulator of endothelial growth in human endometrium. *Mol Hum Reprod*. 2000; 6:811–9. [PubMed: 10956553]
31. Jerat S, Kaufman S. Effect of pregnancy and steroid hormones on plasma adrenomedullin levels in the rat. *Can J Physiol Pharmacol*. 1998; 76:463–6. [PubMed: 9795757]
32. Di Iorio R, Marinoni E, Cosmi EV. Adrenomedullin in pre-eclampsia. *Lancet*. 1998; 351:676–7. [PubMed: 9500359]
33. Hata T, Miyazaki K, Matsui K. Decreased circulating adrenomedullin in pre-eclampsia. *Lancet*. 1997; 350:1600. [PubMed: 9393346]

34. Minegishi T, Nakamura M, Abe K, Tano M, Andoh A, Yoshida M, Takagi T, Nishikimi T, Kojima M, Kangawa K. Adrenomedullin and atrial natriuretic peptide concentrations in normal pregnancy and pre-eclampsia. *Mol Hum Reprod*. 1999; 5:767–70. [PubMed: 10421805]
35. Shinozaki H, Aoki H, Kasahara Y, Kangawa K, Minegishi T. Plasma adrenomedullin levels during multiple pregnancy. *Gynecol Obstet Invest*. 2010; 69:169–73. [PubMed: 20016189]
36. Di Iorio R, Marinoni E, Letizia C, Alo P, Villaccio B, Cosmi EV. Adrenomedullin, a new vasoactive peptide, is increased in preeclampsia. *Hypertension*. 1998; 32:758–63. [PubMed: 9774376]
37. Dong YL, Green KE, Vegiragu S, Hankins GD, Martin E, Chauhan M, Thota C, Yallampalli C. Evidence for decreased calcitonin gene-related peptide (CGRP) receptors and compromised responsiveness to CGRP of fetoplacental vessels in preeclamptic pregnancies. *J Clin Endocrinol Metab*. 2005; 90:2336–43. [PubMed: 15623815]
38. Chauhan M, Ross GR, Yallampalli U, Yallampalli C. Adrenomedullin-2, a novel calcitonin/calcitonin-gene-related peptide family peptide, relaxes rat mesenteric artery: influence of pregnancy. *Endocrinology*. 2007; 148:1727–35. [PubMed: 17218418]
39. Peeters LL, Grutters G, Martin CB Jr. Distribution of cardiac output in the unstressed pregnant guinea pig. *Am J Obstet Gynecol*. 1980; 138:1177–84. [PubMed: 7446627]
40. Leiberman JR, Wiznitzer A, Glezerman M, Feldman B, Levy J, Sharoni Y. Estrogen and progesterone receptors in the uterine artery of rats during and after pregnancy. *Eur J Obstet Gynecol Reprod Biol*. 1993; 51:35–40. [PubMed: 8282141]
41. van der Heijden OW, Essers YP, van Eyndhoven HW, Spaanderman ME, Aardenburg R, van Eys GJ, Peeters LL. Vascular expression of adrenomedullin is increased in Wistar rats during early pregnancy. *Eur J Obstet Gynecol Reprod Biol*. 2005; 123:35–40. [PubMed: 16260338]
42. Hayashi Y, Ueyama H, Mashimo T, Kangawa K, Minamino N. Circulating mature adrenomedullin is related to blood volume in full-term pregnancy. *Anesth Analg*. 2005; 101:1816–20. [PubMed: 16301265]
43. Minamino N, Kikumoto K, Isumi Y. Regulation of adrenomedullin expression and release. *Microsc Res Tech*. 2002; 57:28–39. [PubMed: 11921354]
44. Cormier-Regard S, Nguyen SV, Claycomb WC. Adrenomedullin gene expression is developmentally regulated and induced by hypoxia in rat ventricular cardiac myocytes. *J Biol Chem*. 1998; 273:17787–92. [PubMed: 9651380]
45. Garayoa M, Martinez A, Lee S, Pio R, An WG, Neckers L, Trepel J, Montuenga LM, Ryan H, Johnson R, Gassmann M, Cuttitta F. Hypoxia-inducible factor-1 (HIF-1) up-regulates adrenomedullin expression in human tumor cell lines during oxygen deprivation: a possible promotion mechanism of carcinogenesis. *Mol Endocrinol*. 2000; 14:848–62. [PubMed: 10847587]
46. Nikitenko LL, Smith DM, Bicknell R, Rees MC. Transcriptional regulation of the CRLR gene in human microvascular endothelial cells by hypoxia. *FASEB J*. 2003; 17:1499–501. [PubMed: 12824306]
47. Pfeil U, Aslam M, Paddenberg R, Quanz K, Chang CL, Park JI, Gries B, Rafiq A, Faulhammer P, Goldenberg A, Papadakis T, Noll T, Hsu SY, Weissmann N, Kummer W. Intermedin/adrenomedullin-2 is a hypoxia-induced endothelial peptide that stabilizes pulmonary microvascular permeability. *Am J Physiol Lung Cell Mol Physiol*. 2009; 297:L837–45. [PubMed: 19684198]
48. Ross GR, Yallampalli C. Vascular hyperresponsiveness to adrenomedullin during pregnancy is associated with increased generation of cyclic nucleotides in rat mesenteric artery. *Biol Reprod*. 2007; 76:118–23. [PubMed: 17050860]
49. Gangula PR, Zhao H, Supowit S, Wimalawansa S, DiPette D, Yallampalli C. Pregnancy and steroid hormones enhance the vasodilation responses to CGRP in rats. *Am J Physiol*. 1999; 276:H284–8. [PubMed: 9887042]
50. Ross GR, Yallampalli U, Gangula PR, Reed L, Sathishkumar K, Gao H, Chauhan M, Yallampalli C. Adrenomedullin relaxes rat uterine artery: mechanisms and influence of pregnancy and estradiol. *Endocrinology*. 2010; 151:4485–93. [PubMed: 20631002]

51. Ross GR, Yallampalli C. Endothelium-independent relaxation by adrenomedullin in pregnant rat mesenteric artery: role of cAMP-dependent protein kinase A and calcium-activated potassium channels. *J Pharmacol Exp Ther*. 2006; 317:1269–75. [PubMed: 16551834]
52. Gangula PR, Thota C, Wimalawansa SJ, Bukoski RD, Yallampalli C. Mechanisms involved in calcitonin gene-related Peptide-induced relaxation in pregnant rat uterine artery. *Biol Reprod*. 2003; 69:1635–41. [PubMed: 12855600]
53. Jovanovic A, Jovanovic S, Grbovic L. Endothelium-dependent relaxation in response to acetylcholine in pregnant guinea-pig uterine artery. *Hum Reprod*. 1997; 12:1805–9. [PubMed: 9308816]
54. Yanagita T, Yamamoto R, Sugano T, Kobayashi H, Uezono Y, Yokoo H, Shiraishi S, Minami SI, Wada A. Adrenomedullin inhibits spontaneous and bradykinin-induced but not oxytocin- or prostaglandin F(2alpha)-induced periodic contraction of rat uterus. *Br J Pharmacol*. 2000; 130:1727–30. [PubMed: 10952659]
55. Naghashpour M, Rosenblatt MI, Dickerson IM, Dahl GP. Inhibitory effect of calcitonin gene-related peptide on myometrial contractility is diminished at parturition. *Endocrinology*. 1997; 138:4207–14. [PubMed: 9322931]
56. Samuelson UE, Dalsgaard CJ, Lundberg JM, Hokfelt T. Calcitonin gene-related peptide inhibits spontaneous contractions in human uterus and fallopian tube. *Neurosci Lett*. 1985; 62:225–30. [PubMed: 3911119]
57. Thota C, Gangula PR, Dong YL, Yallampalli C. Changes in the expression of calcitonin receptor-like receptor, receptor activity-modifying protein (RAMP) 1, RAMP2, and RAMP3 in rat uterus during pregnancy, labor, and by steroid hormone treatments. *Biol Reprod*. 2003; 69:1432–7. [PubMed: 12801991]
58. Michishita M, Minegishi T, Abe K, Kangawa K, Kojima M, Ibuki Y. Expression of adrenomedullin in the endometrium of the human uterus. *Obstet Gynecol*. 1999; 93:66–70. [PubMed: 9916958]
59. Yallampalli C, Kondapaka SB, Lanlua P, Wimalawansa SJ, Gangula PR. Female sex steroid hormones and pregnancy regulate receptors for calcitonin gene-related peptide in rat mesenteric arteries, but not in aorta. *Biol Reprod*. 2004; 70:1055–62. [PubMed: 14668209]
60. Nikitenko LL, Brown NS, Smith DM, MacKenzie IZ, Bicknell R, Rees MC. Differential and cell-specific expression of calcitonin receptor-like receptor and receptor activity modifying proteins in the human uterus. *Mol Hum Reprod*. 2001; 7:655–64. [PubMed: 11420389]
61. Dong YL, Vegiraju S, Chauhan M, Gangula PR, Hankins GD, Goodrum L, Yallampalli C. Involvement of calcitonin gene-related peptide in control of human fetoplacental vascular tone. *Am J Physiol Heart Circ Physiol*. 2004; 286:H230–9. [PubMed: 14684361]
62. Dong YL, Vegiraju S, Chauhan M, Yallampalli C. Expression of calcitonin gene-related peptide receptor components, calcitonin receptor-like receptor and receptor activity modifying protein 1, in the rat placenta during pregnancy and their cellular localization. *Mol Hum Reprod*. 2003; 9:481–90. [PubMed: 12837925]
63. Sugo S, Minamino N, Shoji H, Kangawa K, Kitamura K, Eto T, Matsuo H. Interleukin-1, tumor necrosis factor and lipopolysaccharide additively stimulate production of adrenomedullin in vascular smooth muscle cells. *Biochem Biophys Res Commun*. 1995; 207:25–32. [PubMed: 7857273]
64. Sugo S, Minamino N, Shoji H, Kangawa K, Kitamura K, Eto T, Matsuo H. Production and secretion of adrenomedullin from vascular smooth muscle cells: augmented production by tumor necrosis factor-alpha. *Biochem Biophys Res Commun*. 1994; 203:719–26. [PubMed: 8074727]
65. Imai T, Hirata Y, Iwashina M, Marumo F. Hormonal regulation of rat adrenomedullin gene in vasculature. *Endocrinology*. 1995; 136:1544–8. [PubMed: 7895664]
66. Minamino N, Shoji H, Sugo S, Kangawa K, Matsuo H. Adrenocortical steroids, thyroid hormones and retinoic acid augment the production of adrenomedullin in vascular smooth muscle cells. *Biochem Biophys Res Commun*. 1995; 211:686–93. [PubMed: 7794283]
67. Cameron VA, Autelitano DJ, Evans JJ, Ellmers LJ, Espiner EA, Nicholls MG, Richards AM. Adrenomedullin expression in rat uterus is correlated with plasma estradiol. *Am J Physiol Endocrinol Metab*. 2002; 282:E139–46. [PubMed: 11739094]

68. Li M, Wu Y, Caron KM. Haploinsufficiency for adrenomedullin reduces pinopodes and diminishes uterine receptivity in mice. *Biol Reprod.* 2008; 79:1169–75. [PubMed: 18716289]
69. Watanabe H, Takahashi E, Kobayashi M, Goto M, Krust A, Chambon P, Iguchi T. The estrogen-responsive adrenomedullin and receptor-modifying protein 3 gene identified by DNA microarray analysis are directly regulated by estrogen receptor. *J Mol Endocrinol.* 2006; 36:81–9. [PubMed: 16461929]
70. Lin Chang C, Roh J, Park JI, Klein C, Cushman N, Haberberger RV, Hsu SY. Intermedin functions as a pituitary paracrine factor regulating prolactin release. *Mol Endocrinol.* 2005; 19:2824–38. [PubMed: 16002435]
71. Ikeda K, Arao Y, Otsuka H, Kikuchi A, Kayama F. Estrogen and phytoestrogen regulate the mRNA expression of adrenomedullin and adrenomedullin receptor components in the rat uterus. *Mol Cell Endocrinol.* 2004; 223:27–34. [PubMed: 15279908]
72. Ross GR, Chauhan M, Gangula PR, Reed L, Thota C, Yallampalli C. Female sex steroids increase adrenomedullin-induced vasodilation by increasing the expression of adrenomedullin2 receptor components in rat mesenteric artery. *Endocrinology.* 2006; 147:389–96. [PubMed: 16210373]
73. Gangula PRR, Dong YL, Wimalawansa SJ, Yallampalli C. Infusion of Pregnant Rats with Calcitonin Gene-Related Peptide (CGRP)8–37, a CGRP Receptor Antagonist, Increases Blood Pressure and Fetal Mortality and Decreases Fetal Growth. *Biol Reprod.* 2002; 67:624–629. [PubMed: 12135906]
74. Penchalaneni J, Wimalawansa SJ, Yallampalli C. Adrenomedullin antagonist treatment during early gestation in rats causes fetoplacental growth restriction through apoptosis. *Biol Reprod.* 2004; 71:1475–83. [PubMed: 15229133]
75. Chauhan M, Yallampalli U, Reed L, Yallampalli C. Adrenomedullin 2 antagonist infusion to rats during midgestation causes fetoplacental growth restriction through apoptosis. *Biol Reprod.* 2006; 75:940–7. [PubMed: 16971558]
76. Chauhan M, Elkins R, Balakrishnan M, Yallampalli C. Potential role of intermedin/adrenomedullin 2 in early embryonic development in rats. *Regul Pept.* 2011; 170:65–71. [PubMed: 21640761]
77. Robinson SD, Aitken JF, Bailey RJ, Poyner DR, Hay DL. Novel peptide antagonists of adrenomedullin and calcitonin gene-related peptide receptors: identification, pharmacological characterization, and interactions with position 74 in receptor activity-modifying protein 1/3. *J Pharmacol Exp Ther.* 2009; 331:513–21. [PubMed: 19644039]
78. Giudice LC, Dsupin BA, Gargosky SE, Rosenfeld RG, Irwin JC. The insulin-like growth factor system in human peritoneal fluid: its effects on endometrial stromal cells and its potential relevance to endometriosis. *J Clin Endocrinol Metab.* 1994; 79:1284–93. [PubMed: 7525631]
79. Johnson GA, Burghardt RC, Bazer FW, Spencer TE. Osteopontin: roles in implantation and placentation. *Biol Reprod.* 2003; 69:1458–71. [PubMed: 12890718]
80. Smith RS Jr, Gao L, Bledsoe G, Chao L, Chao J. Intermedin is a new angiogenic growth factor. *Am J Physiol Heart Circ Physiol.* 2009; 297:H1040–7. [PubMed: 19592612]
81. Zheng S, Li W, Xu M, Bai X, Zhou Z, Han J, Shyy JY, Wang X. Calcitonin gene-related peptide promotes angiogenesis via AMP-activated protein kinase. *Am J Physiol Cell Physiol.* 2010; 299:C1485–92. [PubMed: 20881236]
82. Imuro S, Shindo T, Moriyama N, Amaki T, Niu P, Takeda N, Iwata H, Zhang Y, Ebihara A, Nagai R. Angiogenic effects of adrenomedullin in ischemia and tumor growth. *Circ Res.* 2004; 95:415–23. [PubMed: 15242974]
83. Marinoni E, Casciani V, Marianetti V, Di Rocco A, Moscarini M, Di Iorio R. Localization and distribution of adrenomedullin receptor in the human placenta: changes with gestational age. *J Reprod Med.* 2007; 52:831–8. [PubMed: 17939601]
84. Liao SB, Kong LH, Tang F, Sum OW. Possible functions of adrenomedullin from the seminal fluid in the female reproductive tract of the rat. *Syst Biol Reprod Med.* 2012
85. Chauhan M, Yallampalli U, Dong YL, Hankins GD, Yallampalli C. Expression of adrenomedullin 2 (ADM2)/intermedin (IMD) in human placenta: role in trophoblast invasion and migration. *Biol Reprod.* 2009; 81:777–83. [PubMed: 19535789]

86. Tsatsaris V, Tarrade A, Merviel P, Garel JM, Segond N, Jullienne A, Evain-Brion D. Calcitonin gene-related peptide (CGRP) and CGRP receptor expression at the human implantation site. *J Clin Endocrinol Metab.* 2002; 87:4383–90. [PubMed: 12213903]
87. Li M, Yee D, Magnuson TR, Smithies O, Caron KM. Reduced maternal expression of adrenomedullin disrupts fertility, placentation, and fetal growth in mice. *J Clin Invest.* 2006; 116:2653–62. [PubMed: 16981008]
88. Chauhan M, Balakrishnan M, Yallampalli U, Endsley J, Hankins GD, Theiler R, Yallampalli C. Adrenomedullin 2/intermedin regulates HLA-G in human trophoblasts. *Biol Reprod.* 2011; 85:1232–9. [PubMed: 21816853]
89. Dong YL, Reddy DM, Green KE, Chauhan MS, Wang HQ, Nagamani M, Hankins GD, Yallampalli C. Calcitonin gene-related peptide (CALCA) is a proangiogenic growth factor in the human placental development. *Biol Reprod.* 2007; 76:892–9. [PubMed: 17267696]
90. Zhang X, Green KE, Yallampalli C, Dong YL. Adrenomedullin enhances invasion by trophoblast cell lines. *Biol Reprod.* 2005; 73:619–26. [PubMed: 15917349]
91. Marinoni E, Pacioni K, Sambuchini A, Moscarini M, Letizia C, RDII. Regulation by hypoxia of adrenomedullin output and expression in human trophoblast cells. *Eur J Obstet Gynecol Reprod Biol.* 2011; 154:146–50. [PubMed: 21094578]
92. Marinoni E, Sambuchini A, Pacioni K, Moscarini M, Letizia C, Di Iorio R. Effect of corticosteroids and progesterone on adrenomedullin output and expression in human fetal membranes and placenta trophoblasts. *Am J Obstet Gynecol.* 2010; 202:196, e1–6. [PubMed: 20035916]
93. Marinoni E, Zacharopoulou C, Di Rocco A, Letizia C, Moscarini M, Di Iorio R. Effect of betamethasone *in vivo* on placental adrenomedullin in human pregnancy. *J Soc Gynecol Investig.* 2006; 13:418–24.
94. Shindo T, Kurihara Y, Nishimatsu H, Moriyama N, Kakoki M, Wang Y, Imai Y, Ebihara A, Kuwaki T, Ju KH, Minamino N, Kangawa K, Ishikawa T, Fukuda M, Akimoto Y, Kawakami H, Imai T, Morita H, Yazaki Y, Nagai R, Hirata Y, Kurihara H. Vascular abnormalities and elevated blood pressure in mice lacking adrenomedullin gene. *Circulation.* 2001; 104:1964–71. [PubMed: 11602502]
95. Caron KM, Smithies O. Extreme hydrops fetalis and cardiovascular abnormalities in mice lacking a functional Adrenomedullin gene. *Proc Natl Acad Sci U S A.* 2001; 98:615–9. [PubMed: 11149956]
96. Fritz-Six KL, Dunworth WP, Li M, Caron KM. Adrenomedullin signaling is necessary for murine lymphatic vascular development. *J Clin Invest.* 2008; 118:40–50. [PubMed: 18097475]
97. Lu JT, Son YJ, Lee J, Jetton TL, Shiota M, Moscoso L, Niswender KD, Loewy AD, Magnuson MA, Sanes JR, Emeson RB. Mice lacking alpha-calcitonin gene-related peptide exhibit normal cardiovascular regulation and neuromuscular development. *Mol Cell Neurosci.* 1999; 14:99–120. [PubMed: 10532808]
98. Dackor R, Fritz-Six K, Smithies O, Caron K. Receptor activity-modifying proteins 2 and 3 have distinct physiological functions from embryogenesis to old age. *J Biol Chem.* 2007; 282:18094–9. [PubMed: 17470425]
99. Gangula PR, Zhao H, Supowit SC, Wimalawansa SJ, Dipette DJ, Westlund KN, Gagel RF, Yallampalli C. Increased blood pressure in alpha-calcitonin gene-related peptide/calcitonin gene knockout mice. *Hypertension.* 2000; 35:470–5. [PubMed: 10642343]
100. Tsujikawa K, Yayama K, Hayashi T, Matsushita H, Yamaguchi T, Shigeno T, Ogitani Y, Hirayama M, Kato T, Fukada S, Takatori S, Kawasaki H, Okamoto H, Ikawa M, Okabe M, Yamamoto H. Hypertension and dysregulated proinflammatory cytokine production in receptor activity-modifying protein 1-deficient mice. *Proc Natl Acad Sci U S A.* 2007; 104:16702–7. [PubMed: 17923674]
101. Dackor RT, Fritz-Six K, Dunworth WP, Gibbons CL, Smithies O, Caron KM. Hydrops fetalis, cardiovascular defects, and embryonic lethality in mice lacking the calcitonin receptor-like receptor gene. *Mol Cell Biol.* 2006; 26:2511–8. [PubMed: 16537897]
102. Nicoli S, Tobia C, Gualandi L, De Sena G, Presta M. Calcitonin receptor-like receptor guides arterial differentiation in zebrafish. *Blood.* 2008; 111:4965–72. [PubMed: 18326814]

103. Guidolin D, Albertin G, Spinazzi R, Sorato E, Mascarin A, Cavallo D, Antonello M, Ribatti D. Adrenomedullin stimulates angiogenic response in cultured human vascular endothelial cells: involvement of the vascular endothelial growth factor receptor 2. *Peptides*. 2008; 29:2013–23. [PubMed: 18692535]
104. Maki T, Ihara M, Fujita Y, Nambu T, Harada H, Ito H, Nakao K, Tomimoto H, Takahashi R. Angiogenic roles of adrenomedullin through vascular endothelial growth factor induction. *Neuroreport*. 2011; 22:442–7. [PubMed: 21558971]
105. Ichikawa-Shindo Y, Sakurai T, Kamiyoshi A, Kawate H, Iinuma N, Yoshizawa T, Koyama T, Fukuchi J, Iimuro S, Moriyama N, Kawakami H, Murata T, Kangawa K, Nagai R, Shindo T. The GPCR modulator protein RAMP2 is essential for angiogenesis and vascular integrity. *J Clin Invest*. 2008; 118:29–39. [PubMed: 18097473]
106. Kadmiel M, Fritz-Six K, Pacharne S, Richards GO, Li M, Skerry TM, Caron KM. Research resource: Haploinsufficiency of receptor activity-modifying protein-2 (RAMP2) causes reduced fertility, hyperprolactinemia, skeletal abnormalities, and endocrine dysfunction in mice. *Mol Endocrinol*. 2011; 25:1244–53. [PubMed: 21566080]
107. Hoopes SL, Willcockson HH, Caron KM. Characteristics of multi-organ lymphangiectasia resulting from temporal deletion of calcitonin receptor-like receptor in adult mice. *PLoS One*. 2012; 7:e45261. [PubMed: 23028890]
108. Jin D, Harada K, Ohnishi S, Yamahara K, Kangawa K, Nagaya N. Adrenomedullin induces lymphangiogenesis and ameliorates secondary lymphoedema. *Cardiovasc Res*. 2008; 80:339–45. [PubMed: 18708640]
109. Wootten D, Lindmark H, Kadmiel M, Willcockson H, Caron KM, Barwell J, Drmota T, Poyner DR. Receptor Activity Modifying Proteins (RAMPs) Interact with the VPAC(2) Receptor and CRF(1) Receptors and modulate their function. *Br J Pharmacol*. 2012
110. Cueille C, Garel JM. RAMPs and G protein coupled receptors. *Med Sci (Paris)*. 2004; 20:773–8. [PubMed: 15361343]
111. Christopoulos A, Christopoulos G, Morfis M, Udawela M, Laburthe M, Couvineau A, Kuwasako K, Tilakaratne N, Sexton PM. Novel receptor partners and function of receptor activity-modifying proteins. *J Biol Chem*. 2003; 278:3293–7. [PubMed: 12446722]
112. Christopoulos G, Perry KJ, Morfis M, Tilakaratne N, Gao Y, Fraser NJ, Main MJ, Foord SM, Sexton PM. Multiple amylin receptors arise from receptor activity-modifying protein interaction with the calcitonin receptor gene product. *Mol Pharmacol*. 1999; 56:235–42. [PubMed: 10385705]
113. Fujii T, Nagaya N, Iwase T, Murakami S, Miyahara Y, Nishigami K, Ishibashi-Ueda H, Shirai M, Itoh T, Ishino K, Sano S, Kangawa K, Mori H. Adrenomedullin enhances therapeutic potency of bone marrow transplantation for myocardial infarction in rats. *Am J Physiol Heart Circ Physiol*. 2005; 288:H1444–50. [PubMed: 15539427]
114. Iwase T, Nagaya N, Fujii T, Itoh T, Ishibashi-Ueda H, Yamagishi M, Miyatake K, Matsumoto T, Kitamura S, Kangawa K. Adrenomedullin enhances angiogenic potency of bone marrow transplantation in a rat model of hindlimb ischemia. *Circulation*. 2005; 111:356–62. [PubMed: 15655128]
115. Albertin G, Sorato E, Oselladore B, Mascarin A, Tortorella C, Guidolin D. Involvement of vascular endothelial growth factor signaling in CLR/RAMP1 and CLR/RAMP2-mediated pro-angiogenic effect of intermedin on human vascular endothelial cells. *Int J Mol Med*. 2010; 26:289–94. [PubMed: 20596610]
116. Aslam M, Pfeil U, Gunduz D, Rafiq A, Kummer W, Piper HM, Noll T. Intermedin (adrenomedullin2) stabilizes the endothelial barrier and antagonizes thrombin-induced barrier failure in endothelial cell monolayers. *Br J Pharmacol*. 2012; 165:208–22. [PubMed: 21671901]
117. Hippenstiel S, Witzenrath M, Schmeck B, Hocke A, Krisp M, Krull M, Seybold J, Seeger W, Rascher W, Schutte H, Suttorp N. Adrenomedullin reduces endothelial hyperpermeability. *Circ Res*. 2002; 91:618–25. [PubMed: 12364390]
118. Dosiou C, Giudice LC. Natural killer cells in pregnancy and recurrent pregnancy loss: endocrine and immunologic perspectives. *Endocr Rev*. 2005; 26:44–62. [PubMed: 15689572]

119. Gruenwald P. Chronic Fetal Distress and Placental Insufficiency. *Biol Neonat.* 1963; 5:215–65. [PubMed: 14081642]
120. Yallampalli C, Dong YL, Wimalawansa SJ. Calcitonin gene-related peptide reverses the hypertension and significantly decreases the fetal mortality in pre-eclampsia rats induced by N(G)-nitro-L-arginine methyl ester. *Hum Reprod.* 1996; 11:895–9.
121. Makino I, Shibata K, Makino Y, Kangawa K, Kawarabayashi T. Adrenomedullin attenuates the hypertension in hypertensive pregnant rats induced by N(G)-nitro-L-arginine methyl ester. *Eur J Pharmacol.* 1999; 371:159–67. [PubMed: 10357253]
122. Yallampalli C, Garfield RE. Inhibition of nitric oxide synthesis in rats during pregnancy produces signs similar to those of preeclampsia. *Am J Obstet Gynecol.* 1993; 169:1316–20. [PubMed: 8238200]
123. Lauria MR, Standley CA, Sorokin Y, Yelian FD, Cotton DB. Adrenomedullin levels in normal and preeclamptic pregnancy at term. *J Soc Gynecol Investig.* 1999; 6:318–21.
124. Al-Ghafra A, Gude NM, Brennecke SP, King RG. Increased adrenomedullin protein content and mRNA expression in human fetal membranes but not placental tissue in pre-eclampsia. *Mol Hum Reprod.* 2006; 12:181–6. [PubMed: 16517613]
125. Jerat S, Morrish DW, Davidge ST, Kaufman S. Effect of adrenomedullin on placental arteries in normal and preeclamptic pregnancies. *Hypertension.* 2001; 37:227–31. [PubMed: 11230276]
126. Dikensoy E, Balat O, Pence S, Balat A, Cekmen M, Yurekli M. The changes of plasma malondialdehyde, nitric oxide, and adrenomedullin levels in patients with preeclampsia. *Hypertens Pregnancy.* 2009; 28:383–9. [PubMed: 19843001]
127. Gratton RJ, Gluszynski M, Mazzuca DM, Nygard K, Han VK. Adrenomedullin messenger ribonucleic acid expression in the placenta of normal and preeclamptic pregnancies. *J Clin Endocrinol Metab.* 2003; 88:6048–55. [PubMed: 14671210]
128. Knerr I, Dachert C, Beinder E, Metzler M, Dotsch J, Repp R, Rascher W. Adrenomedullin, calcitonin gene-related peptide and their receptors: evidence for a decreased placental mRNA content in preeclampsia and HELLP syndrome. *Eur J Obstet Gynecol Reprod Biol.* 2002; 101:47–53. [PubMed: 11905404]
129. Kanenishi K, Kuwabara H, Ueno M, Sakamoto H, Hata T. Immunohistochemical adrenomedullin expression is decreased in the placenta from pregnancies with pre-eclampsia. *Pathol Int.* 2000; 50:536–40. [PubMed: 10886735]
130. Li H, Dakour J, Kaufman S, Guilbert LJ, Winkler-Lowen B, Morrish DW. Adrenomedullin is decreased in preeclampsia because of failed response to epidermal growth factor and impaired syncytialization. *Hypertension.* 2003; 42:895–900. [PubMed: 14517225]
131. Makino Y, Shibata K, Makino I, Kangawa K, Kawarabayashi T. Alteration of the adrenomedullin receptor components gene expression associated with the blood pressure in pregnancy-induced hypertension. *J Clin Endocrinol Metab.* 2001; 86:5079–82. [PubMed: 11600589]
132. Di Iorio R, Marinoni E, Letizia C, Cosmi EV. Adrenomedullin in perinatal medicine. *Regul Pept.* 2003; 112:103–13. [PubMed: 12667631]
133. Sugimoto H, Hamano Y, Charytan D, Cosgrove D, Kieran M, Sudhakar A, Kalluri R. Neutralization of circulating vascular endothelial growth factor (VEGF) by anti-VEGF antibodies and soluble VEGF receptor 1 (sFlt-1) induces proteinuria. *J Biol Chem.* 2003; 278:12605–8. [PubMed: 12538598]
134. De A, Park JI, Kawamura K, Chen R, Klein C, Rauch R, Mulders SM, Sollewijn Gelpke MD, Hsueh AJ. Intraovarian tumor necrosis factor-related weak inducer of apoptosis/fibroblast growth factor-inducible-14 ligand-receptor system limits ovarian preovulatory follicles from excessive luteinization. *Mol Endocrinol.* 2006; 20:2528–38. [PubMed: 16762976]
135. Park JY, Su YQ, Ariga M, Law E, Jin SL, Conti M. EGF-like growth factors as mediators of LH action in the ovulatory follicle. *Science.* 2004; 303:682–4. [PubMed: 14726596]
136. George EM, Arany M, Cockrell K, Storm MV, Stec DE, Granger JP. Induction of heme oxygenase-1 attenuates sFlt-1-induced hypertension in pregnant rats. *Am J Physiol Regul Integr Comp Physiol.* 2011; 301:R1495–500. [PubMed: 21865547]

137. Murphy SR, LaMarca BB, Cockrell K, Granger JP. Role of endothelin in mediating soluble fms-like tyrosine kinase 1-induced hypertension in pregnant rats. *Hypertension*. 2010; 55:394–8. [PubMed: 20026766]
138. Bridges JP, Gilbert JS, Colson D, Gilbert SA, Dukes MP, Ryan MJ, Granger JP. Oxidative stress contributes to soluble fms-like tyrosine kinase-1 induced vascular dysfunction in pregnant rats. *Am J Hypertens*. 2009; 22:564–8. [PubMed: 19265787]
139. Falcao S, Stoyanova E, Cloutier G, Maurice RL, Gutkowska J, Lavoie JL. Mice overexpressing both human angiotensinogen and human renin as a model of superimposed preeclampsia on chronic hypertension. *Hypertension*. 2009; 54:1401–7. [PubMed: 19822800]
140. Richards JS, Russell DL, Ochsner S, Espey LL. OVULATION: New Dimensions and New Regulators of the Inflammatory-Like Response. *Annual Review of Physiology*. 2002; 64:69–92.
141. Calka J, McDonald JK, Ojeda SR. The innervation of the immature rat ovary by calcitonin gene-related peptide. *Biol Reprod*. 1988; 39:1215–23. [PubMed: 3265338]
142. Schultea TD, Dees WL, Ojeda SR. Postnatal development of sympathetic and sensory innervation of the rhesus monkey ovary. *Biol Reprod*. 1992; 47:760–7. [PubMed: 1282372]
143. Chang CL, Wang HS, Soong YK, Huang SY, Pai SY, Hsu SY. Regulation of oocyte and cumulus cell interactions by intermedin/adrenomedullin 2. *J Biol Chem*. 2011; 286:43193–203. [PubMed: 22009752]
144. Li L, OWS, Tang F. Adrenomedullin in rat follicles and corpora lutea: expression, functions and interaction with endothelin-1. *Reprod Biol Endocrinol*. 2011; 9:111. [PubMed: 21824440]
145. Manau D, Balasch J, Jimenez W, Fabregues F, Civico S, Casamitjana R, Creus M, Vanrell JA. Follicular fluid concentrations of adrenomedullin, vascular endothelial growth factor and nitric oxide in IVF cycles: relationship to ovarian response. *Hum Reprod*. 2000; 15:1295–9. [PubMed: 10831558]
146. Manau D, Balasch J, Jimenez W, Fabregues F, Casamitjana R, Creus M, Arroyo V, Vanrell JA. Adrenomedullin and nitric oxide in menstrual and *in vitro* fertilization cycles. Relationship to estradiol. *Acta Obstet Gynecol Scand*. 1999; 78:626–31. [PubMed: 10422910]
147. Marinoni E, Feliciani E, Muzzonigro F, Letizia C, Tranquilli A, Tranquilli D, Aragona C, Moscarini M, Di Iorio R. Intrafollicular concentration of adrenomedullin is associated with IVF outcome. *Gynecol Endocrinol*. 2010; 26:435–9. [PubMed: 20170350]
148. Balasch J, Guimera M, Martinez-Pasarell O, Ros J, Vanrell JA, Jimenez W. Adrenomedullin and vascular endothelial growth factor production by follicular fluid macrophages and granulosa cells. *Hum Reprod*. 2004; 19:808–14. [PubMed: 15016771]
149. Guimera M, Morales-Ruiz M, Jimenez W, Balasch J. LH/HCG stimulation of VEGF and adrenomedullin production by follicular fluid macrophages and luteinized granulosa cells. *Reprod Biomed Online*. 2009; 18:743–9. [PubMed: 19490776]
150. Marinoni E, Di Iorio R, Villaccio B, Letizia C, Aragona C, Schimberni M, Cosmi EV. Follicular fluid adrenomedullin concentrations in spontaneous and stimulated cycles: relationship to ovarian function and endothelin-1 and nitric oxide. *Regul Pept*. 2002; 107:125–8. [PubMed: 12137974]
151. Clark HF, Gurney AL, Abaya E, Baker K, Baldwin D, Brush J, Chen J, Chow B, Chui C, Crowley C, Currell B, Deuel B, Dowd P, Eaton D, Foster J, Grimaldi C, Gu Q, Hass PE, Heldens S, Huang A, Kim HS, Klimowski L, Jin Y, Johnson S, Lee J, Lewis L, Liao D, Mark M, Robbie E, Sanchez C, Schoenfeld J, Seshagiri S, Simmons L, Singh J, Smith V, Stinson J, Vagts A, Vandlen R, Watanabe C, Wieand D, Woods K, Xie MH, Yansura D, Yi S, Yu G, Yuan J, Zhang M, Zhang Z, Goddard A, Wood WI, Godowski P, Gray A. The secreted protein discovery initiative (SPDI), a large-scale effort to identify novel human secreted and transmembrane proteins: a bioinformatics assessment. *Genome Res*. 2003; 13:2265–70. [PubMed: 12975309]
152. Fischer B, Kunzel W, Kleinstein J, Gips H. Oxygen tension in follicular fluid falls with follicle maturation. *Eur J Obstet Gynecol Reprod Biol*. 1992; 43:39–43. [PubMed: 1737607]
153. Chiu PC, Liao S, Lam KK, Tang F, Ho JC, Ho PC, OWS, Yao YQ, Yeung WS. Adrenomedullin regulates sperm motility and oviductal ciliary beat via cyclic adenosine 5'-monophosphate/protein kinase A and nitric oxide. *Endocrinology*. 2010; 151:3336–47. [PubMed: 20444935]
154. Li HW, Liao SB, Chiu PC, Tam WW, Ho JC, Ng EH, Ho PC, Yeung WS, Tang F, OWS. Expression of adrenomedullin in human oviduct, its regulation by the hormonal cycle and contact

- with spermatozoa, and its effect on ciliary beat frequency of the oviductal epithelium. *J Clin Endocrinol Metab.* 2010; 95:E18–25. [PubMed: 20534761]
155. Lyons RA, Saridogan E, Djahanbakhch O. The reproductive significance of human Fallopian tube cilia. *Hum Reprod Update.* 2006; 12:363–72. [PubMed: 16565155]
156. Chiu PC, Lam KK, Lee CL, Huang VW, Wong BS, Zhao W, Li K, Ho PC, Yeung WS. Adrenomedullin enhances the progressive motility and zona pellucida-binding capacity of spermatozoa from normozoospermic but not asthenozoospermic men. *Mol Reprod Dev.* 2012; 79:76. [PubMed: 21990183]
157. Liao SB, Li HW, Ho JC, Yeung WS, Ng EH, Cheung AN, Tang F, OWS. Possible role of adrenomedullin in the pathogenesis of tubal ectopic pregnancy. *J Clin Endocrinol Metab.* 2012; 97:2105–12. [PubMed: 22456622]
158. Hay DL, Christopoulos G, Christopoulos A, Poyner DR, Sexton PM. Pharmacological discrimination of calcitonin receptor: receptor activity-modifying protein complexes. *Mol Pharmacol.* 2005; 67:1655–65. [PubMed: 15692146]
159. Kuwasako K, Kitamura K, Nagata S, Hikosaka T, Takei Y, Kato J. Shared and separate functions of the RAMP-based adrenomedullin receptors. *Peptides.* 2011; 32:1540–50. [PubMed: 21645567]
160. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP, Karumanchi SA. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest.* 2003; 111:649–58. [PubMed: 12618519]
161. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM, Sukhatme VP, Karumanchi SA. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med.* 2004; 350:672–83. [PubMed: 14764923]
162. Robinson CJ, Johnson DD. Soluble endoglin as a second-trimester marker for preeclampsia. *Am J Obstet Gynecol.* 2007; 197:174, e1–5. [PubMed: 17689640]
163. Al-Ghaffra A, Brennecke SP, King RG, Gude NM. Expression of adrenomedullin mRNA is altered with gestation and labour in human placenta and fetal membranes. *Clin Sci (Lond).* 2006; 110:337–42. [PubMed: 16316317]
164. Hay DL, Smith DM. Knockouts and transgenics confirm the importance of adrenomedullin in the vasculature. *Trends Pharmacol Sci.* 2001; 22:57–9. [PubMed: 11421200]

Fig. (1).

The role of CLR/RAMP receptor signaling in **a)** main arteries that supply reproductive organs, **b)** uteroplacental tissues, **c)** ovaries, and **d)** oviducts.

Fig. (2).

The role of CLR/RAMP receptor signaling at the utero-placental interface. ADM family peptides play critical roles in trophoblast invasion and migration. Peptides derived from maternal resources appear to act on select processes in both the maternal and fetal compartments.

Fig. (3).

The role of ADM and IMD in intercellular communication in tertiary ovarian follicles. Both ADM and IMD have been shown to promote estrogen production of cultured granulosa cells. IMD signaling has also been shown to promote cyclin D2 expression in granulosa cells *in vivo*. In addition, the promotion of cell–cell contacts within cumulus oocyte complexes (COCs) by IMD may be important for maintaining normal COC tertiary structure in the presence of oocyte-derived cumulus expansion-promoting factors, GDF9 and BMP15, during ovarian follicle development.

Table 1

The Differences in the Response to CGRP, ADM, and IMD for the Three CLR/RAMP Receptors.

	Responses to ADM family peptides	References
CLR/RAMP1	CGRP > IMD > ADM	[1–3, 6, 158, 159]
CLR/RAMP2	ADM > IMD > CGRP	[1–3, 6, 158–160]
CLR/RAMP3	ADM = IMD > CGRP	[1–3, 6, 158, 159]

Table 2

Evidences of Expression of mRNA and/or Protein of CGRP, ADM, IMD, and the Three CLR/RAMP Receptors in Maternal Vasculatures and Feto-Placental Tissues.

	Mesenteric and uterine arteries	Uterine tissues	Feto-placental tissues	References
ADM	++	+++	++	[24, 41, 67, 68, 87, 92–94, 124, 127–130]
CGRPs	+	+	+	[86, 160–162] [128]
IMD	unknown	++	+	[76, 85, 88]
CLR	+	+	+	[20, 24, 37, 50, 57, 59–62, 68, 72, 86, 89, 90, 131, 160]
RAMP1	+	++	++	[20, 24, 37, 50, 57, 59–62, 86, 160]
RAMP2	+	++	+	[20, 24, 50, 57, 60, 68–71, 131]
RAMP3	+	++	++	[20, 24, 50, 60, 68–72]

Table 3

The Role of CLR/RAMP Receptor Signaling in Reproductive Tissues.

Gene	Phenotypes of knockout mice	Major functions in reproductive tissues			Associated reproductive diseases	References
		Maternal –fetal communication	Ovarian functions	Oviduct functions		
<i>ADM</i>	embryonic lethality, systemic hemorrhage, hydrops fetalis	vasodilation, trophoblast proliferation, differentiation	estrogen production	sperm and oocyte movement	PIH, preeclampsia, tubal ectopic pregnancy	[17, 33, 36, 87, 94, 95, 123, 124, 127, 145, 153, 156, 163, 164]
<i>CGRPβ</i>	negligible	vasodilation	neurotransmission, estrogen production, cyclin	—	preeclampsia	[37, 61, 99, 128, 141]
<i>IMD</i>	unknown	vasodilation, trophoblast migration	D2 expression, cell-cell contacts in COCs	—	unknown	[13, 38, 47, 75, 76, 85, 115, 143]
<i>CLR</i>	embryonic lethality, lack of blood circulation, hydrops fetalis	vasodilation, trophoblast differentiation and migration	—	—	preeclampsia	[57, 62, 101]
<i>RAMP1</i>	negligible	vasodilation	—	—	unknown	[24, 60, 100]
<i>RAMP2</i>	embryonic lethality, paracellular leakage	vasodilation, trophoblast proliferation and differentiation	—	—	unknown	[24, 98, 105, 106]
<i>RAMP3</i>	negligible	vasodilation	—	—	unknown	[24, 98]