Calcitonin Gene Related Family Peptides: Importance in Normal Placental and Fetal Development

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Abstract

Synchronized molecular and cellular events occur between the uterus and the implanting embryo to facilitate successful pregnancy outcome. Nevertheless, the molecular signaling network that coordinates strategies for successful decidualization, placentation and fetal growth are not well understood. The discovery of calcitonin/calcitonin gene-related peptides (CT/CGRP) highlighted new signaling mediators in various physiological processes, including reproduction. It is known that CGRP family peptides including CGRP, adrenomedulin and intermedin play regulatory functions during implantation, trophoblast proliferation and invasion, and fetal organogenesis. In addition, all the CGRP family peptides and their receptor components are found to be expressed in decidual, placental and fetal tissues. Additionally, plasma levels of peptides of the CGRP family were found to fluctuate during normal gestation and to induce placental cellular differentiation, proliferation, and critical hormone signaling. Moreover, aberrant signaling of these CGRP family peptides during gestation has been associated with pregnancy disorders. It indicates the existence of a possible regulatory role for these molecules during decidualization and placentation processes, which are known to be particularly vulnerable. In this review, the influence of the CGRP family peptides in these critical processes is explored and discussed.

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1 Introduction

Mammalian reproduction is a complex but well-coordinated process designed to produce successful pregnancy by safeguarding critical steps of the regulatory systems. Normal preimplantation embryo development, timely journey of embryos from the oviduct into the uterine lumen, differentiation of the uterus to the receptive state, trophoblast cell fusion, trophoblast invasion and hormone production are all prerequisites for successful pregnancy; dysregulation of these processes leads to pregnancy complications, including fetal growth restriction, preeclampsia, preterm birth, gestational diabetes, etc. In recent years, calcitonin/calcitonin gene-related peptide (CT/CGRP) family peptides, a group of endogenously produced peptides, have emerged as major players in reproduction. Supported by mechanistic studies, the importance of CT/CGRP family peptides during pregnancy is growing through their involvement in vascular adaptations, utero placental functions and fetal growth.

The CT/CGRP family consists of five peptides: calcitonin (CT), amylin (AMY), CGRP, adrenomedullin (AM), and AM2/intermedin (IMD). These peptides share similar molecular structure and the majority of their functions overlap. Although these peptides have little sequence homology, they share similar secondary structure consisting of an amino acid ring structure formed by a single disulfide bond and an amidated carboxyl terminus [1, 2]. Moreover, the receptors for these peptides consist of components that are common to the majority of these peptides further adding to the overlapping functionality. Recent studies implicate CT/CGRP family peptides in multiple essential roles in a variety of functions including vascular adaptations, uteroplacental functions and fetal growth for a successful healthy pregnancy. For detailed up to date information on the role of CGRP family peptides in vascular adaption please refer to our recent review [3]. In this review, we now provide a comprehensive account of functions, shared receptors and mechanisms of action for CGRP, AM and IMD with respect to their role in regulating various functions during pregnancy especially placental development fetal growth and their implications for pregnancy complications.

2 CGRP Family Peptides and Their Receptor Components

2.1 Peptides

The CT, AMY, CGRP, AM, and IMD originated phylogenetically from CALC1 gene (CALCA) [4] and share structural similarities. C-cells in the thyroid gland were found to be the source of CT which was presented as an endocrine hormone based on its potent hypocalcemic activity [5].

The *CALCA* gene produces CT mRNA after alternative splicing when exons 1, 2, 3, and 4 are spliced together, and αCGRP mRNA when exons 5 and 6 are included instead of exon 4 [6]. A second form of CGRP, βCGRP, which differs from αCGRP by 1 amino acid in the rat and 3 amino acids in the human, is produced by a separate gene. AMY was isolated from amyloid deposits in pancreatic islets of patients with type II diabetes, is produced mainly by β-islet cells, and is co-secreted with insulin [7, 8]. AMY shares only 40 % amino acid identity with CGRP. AM was originally isolated from human pheochromocytoma but is also expressed in various other tissues [9].

The preproAM is processed to form a 164-amino acid peptide proAM, which is then cleaved to give rise to biologically active AM [9]. Rat AM is 50 amino acids long and differs from human AM in only 6 positions [10]. There is a 27 % homology between the peptide sequence of AM and CGRP. The human IMD gene encodes a prepro-protein of 148 amino acids and a predicted 47-amino-acid mature peptide. IMD has ~28 % sequence identity with AM and <20 % with CGRP, and is expressed primarily in the intermediate lobe of the pituitary and gastrointestinal tract, and appears to have distinct physiological effects [2].

All these peptides share several common features of secondary structure which includes a ring structure formed by an intramolecular disulfide linkage and a C-terminal amide structure that are essential structural features of the CT/CGRP family. The N-terminus of these peptides has di-sulfide bonded residues important for their biological activity and approximately 30 amino acids required for binding [1]. Distinct biological activity is exhibited by these peptides despite their sequence homology due to the interaction with their receptors.

2.2 Receptors

Seven-transmembrane (TM) domain G-protein-coupled receptors, CT receptor (CTR) and calcitonin receptor-like receptor (CRLR) are the two G-protein coupled receptors assigned to this peptide family that interact with their respective ligands. The CTR is for CT and AMY, whereas CGRP, AM and IMD exhibit their function through CRLR. The ligand binding specificity of these receptors is conferred by 1 of the 3 receptor activity modifying proteins (RAMPs). CTR and CRLR share >50 % amino acid identity, and CTR by itself acts as a specific receptor for CT, whereas transport of CRLR to the plasma membrane and its ability to bind ligands are dependent on its heterodimerization with one of the RAMPs [11–13]. The RAMPs are 150 amino acids long single TM proteins. RAMPs share a similar basic structure—a large extracellular domain of 120 amino acids containing 4 conserved cysteine residues, a single TM domain of 20 amino acids, and a short intracellular domain of 10 amino acids. $RAMP_1$ was discovered while screening an expression cDNA library from the human neuroblastoma cell line SK-N-MC in *Xenopus* oocytes while aiming to clone a specific CGRP receptor [13]. A sequence database search for expressed sequences similar to $RAMP_1$ identified $RAMP_2$ and $RAMP_3$. The 3 RAMPs show only about 30 % amino acid sequence identity. The type of RAMP protein associated with CTR or CRLR would dictate the specificity of binding to each of the CT/CGRP family peptides.

$RAMP_1$ co-expressed with CRLR constitutes CGRP receptor, which is antagonized by truncated $CGRP_{8–37}$ [14]. Co-expression of $RAMP_2$ or $RAMP_3$ with CRLR constitutes specific AM receptors. CRLR forms a heterodimer with either $RAMP_2$ to form AM_1 receptor or with $RAMP_3$ to form AM_2 receptors. Studies of the reconstituted CGRP and AM receptors in yeast suggest that $CGRP_{8–37}$ and $AM_{22–52}$ are selective for the CRLR receptor/$RAMP_1$ and CRLR receptor/RAMP2 combinations, respectively [15]. IMD actions are mediated via formation of a heterodimeric complex of CRLR with any 1 of the 3 RAMPs. However, as demonstrated by Roh et al. actions of IMD are more potent in the presence of CRLR/$RAMP_1$ or CRLR/$RAMP_3$ compared to CRLR/$RAMP_2$ [2]. A truncated form of IMD, $IMD_{17–47}$, is the suggested antagonist of IMD [2]. Since IMD functions through CGRP and AM receptors, some of the effects of IMD are antagonized by both CGRP and AM antagonists. It appears that CT/CGRP peptides can bind to CRLR, but the presence of RAMPs is critical for signaling.

3 Expression and Localization of CGRP Family Peptides and Their Receptor Components

CGRP, AM and IMD and their receptors have been shown to be expressed in placenta and implantation sites [16–19]. CRLR and RAMPs are localized in numerous reproductive tissues

including the uterine endometrium [20], fetal membranes [21, 22], placenta [23], and trophoblast cells [16, 21, 24–27], suggesting an important role for CGRP, AM and IMD in feto-placental development. mRNA for receptor components CRLR, $RAMP_1$, $RAMP_2$ and $RAMP_3$ are expressed in implantation sites, inter-implantation sites, fetus and placenta in rat gestation. These receptor components are differentially regulated during pregnancy in a spatio-temporal manner [17]. CRLR and $RAMP_1$ immunoreactivity was specifically shown to be concentrated in the cytotrophoblast and syncytium in labyrinth, trophoblastic giant cells and basophilic cells in trophospongial cell layer, and endothelium and smooth muscle cells in fetal vessels [28]. Transcripts of CRLR were detected by RT-PCR in both decidual and extravillous trophoblast cells, whereas transcripts of $RAMP_1$ were detected in decidual cells only [16]. No $RAMP_1$ immunostaining was observed in either EVCTs or immune cells [16]. Thus, a functional CGRP receptor appears to be present in decidual cells but not in extravillous trophoblast cells.

AM localizes to the human epithelium and endothelium of the endometrium [20] and in stromal macrophages [29]. In the rat AM was localized in the endometrial stroma with increased immunoreactivity from nonpregnancy to pregnancy [17]. Robust levels of *AM* gene expression were found in the mural trophectoderm cells of a preimplanting blastocyst at E3.5. The relative level of fluorescence in the trophectoderm lineage was slightly higher than the levels observed in the inner cell mass [30]. AM along with CRLR and $RAMP_2$ mRNAs was reported in fetal membranes and umbilical vein endothelial cells (HUVECs) [31–33]. $RAMP_3$ mRNA is also expressed in HUVECs.

Expression of IMD is reported in placenta throughout gestation [19, 34]. Immunoreactive IMD was reported in cytotrophoblast cells, syncytio-trophoblast, placental vascular endothelial cells, decidual trophoblast cells and natural killer (NK) cells that are infiltrated into deciduas [35]. Expression of CGRP, AM and IMD and their receptor components are also demonstrated in the immortalized first trimester trophoblast derived normal extravillous cytotrophoblast cell and trophoblast cells derived from choriocarcinoma such as JEG-3, HTR, JAr and TEV-1 cells [34, 36]. Availability of these cell lines has greatly facilitated many in vitro studies to explore functional roles of these peptides in pregnancy [36].

Expression of these peptides and their receptor components appear to be regulated by sex steroid hormones. During pregnancy, 17beta-estradiol inhibits, while progesterone stimulates, placental mRNA and proteins for CRLR and $RAMP_1$. Antiestrogen, ICI 182780, increased, whereas antiprogesterone, RU 486, inhibited expression of CRLR and $RAMP_1$ [28, 37].

4 Physiological Roles of CGRP Family Peptides During Pregnancy

4.1 Implantation

After fertilization, the morula becomes a blastocyst as fluid accumulates and polarization of cells occurs. The blastocyst has an outer layer of cells (trophoblast) that will form the placenta and fetal membranes, an inner cell mass at one pole that will form the embryo, and a fluid filled cavity. The inner and outer cell masses multiply and the fluid cavity enlarges until the expanded blastocyst hatches out of the zona shell. Initially it is bathed in uterine secretions that provide oxygen and metabolic substrates; however, these secretions soon become inadequate for support of further development. Therefore, within 24 h of hatching (about day 6 after fertilization), the blastocyst implants in the uterine lining, which provides access to substrates (glycogen filled stromal cells) necessary for continued growth. Implantation involves movement of the blastocyst to an optimal location (typically the mid to upper anterior or posterior wall of the human uterus), adhesion, and invasion.

CGRP is found to be produced by decidual cells (but not by extravillous trophoblast cells) at the implantation site, where it is suggested to be involved in important events, such as the complex immunomodulation that abrogates rejection

of trophoblast cells by decidual cells and immunocompetent cells present in the decidua [16]. CGRP stimulates cAMP production in cultured decidual cells while it also acts on the nearby extravillous trophoblasts to increases NO release [16]. Thus, CGRP has paracrine and autocrine effect on decidual and extravillous trophoblast cells, two major players in implantation [16].

Fetal trophoblast cells and the maternal uterine wall have coordinated and localized increases in *AM* gene expression at the time of implantation. AM peptide is abundantly expressed by both the maternal uterine luminal epithelium and the fetal trophectoderm at the time and site of implantation [30]. Northern blot and in situ hybridization analyses showed that the AM mRNA is detected just after implantation and its level peaks at 9.5 days of post conception and decreases coincidentally with the completion of the mature chorioallantoic placenta. Decidual *AM* expression is strictly localized and concentrated around the implanting and developing embryo. Specifically, the luminal epithelium and several surrounding subepithelial cell layers of the stroma express high levels of AM, which rapidly dissipate away laterally from the implanted embryo. The robust expression of *AM* in the maternal decidua, compared with weak expression in the fetal placenta, persists throughout development. In contrast, an AM receptor was not detected in either embryo or trophoblast giant cells at 7 days, suggesting that the AM produced and secreted from the embryo's trophoblast giant cells acts on the maternal tissues rather than on the embryonic tissues [27]. Vasodilation being the hallmark function of AM [38] suggests that the most likely role for AM in the maternal uterine tissue is to maintain uterine quiescence in pregnancy and promote blood flow to the implantation site. In addition, AM is also an angiogenic factor [32, 39, 40] that has been shown to regulate vascular permeability [41] and trophoblast invasion [42, 43] that may support maternal vascular remodeling and permeability that occur during implantation. This notion is supported by maternal expression of AM in both the epithelium and endothelium of receptive uterine tissue [20] as well as stromal macrophages [29]. Inhibition of endogenous AM action via the AM receptor for just 3 days from post-copulation to preimplantation caused deleterious effects, including irregular implantation spacing at mid-pregnancy, and this effect is shown to be mediated by the AM receptor and not by the CGRP receptor [44]. $AM^{+/-}$females are shown to display abnormal spacing of and overcrowded conceptuses within the uterine horns [30]. These results demonstrate that maternal *AM* expression is tightly coordinated, localized to implantation sites, and persistently robust throughout development [30, 35].

Expression of IMD is reported in implantation sites at decidual trophoblast cells and infiltrated decidual NK cells. Recent report shows secretion of IMD by human blastocysts on day 5 [35] and its mRNA expression in day 9 rat implantation sites. Further, infusion of IMD antagonist from day 3 causes a significant decline in weights of implantation sites on day 9 [19]. Therefore, IMD may have a potential role in arterial remodeling and thus contribute to efficient implantation to ensure a healthy pregnancy.

4.2 Syncytialization and Hormone Production

After implantation, cytotrophoblasts differentiate into the villous cytotrophoblast and the extravillous cytotrophoblast (EVCT). The former fuse to form the multinucleated syncytiotrophoblasts responsible for fetomaternal exchange and production of hormones. The latter form migratory cell columns that invade the endometrium [45]. Placental hormones such as human chorion gonadotrophin (hCG) are crucial for maintenance of gestation and successful pregnancy outcome. In placental tissue, the major source of hCG is the multinucleated syncytiotrophoblast layer. Transcription of hCG subunit, mRNA expression and secretion strongly increase during in vitro cell fusion of primary trophoblasts [46–48]. Since production of hCG could be stimulated by placental as well as decidual growth factors, several investigators attempted to study the involvement of CGRP family peptides in syncytialization and placental hormone production.

The first evidence for a critical role of CGRP peptides in hormone production was obtained from studies in choriocarcinoma cell lines. Studies have shown that CGRP stimulates human villous cytotrophoblasts to aggregate and fuse to form multinucleated syncytiotrophoblasts [49]. CGRP also increases hCG, 17β-estradiol and progesterone secretion from human term trophoblasts. This CGRP-induced increase in trophoblast hormone secretion is time- and dose-dependent, and is blocked by CGRP antagonist, $CGRP_{8–37}$ [49]. Although AM and IMD immunoreactivity has been reported in syncytiotrophoblast cells of placental villi, their role in syncytialization and hormone production is yet to be established [25, 34].

4.3 Trophoblast Proliferation and Invasion

The progenitor cytotrophoblast cells are the stem cells of the placenta. These cells proliferate throughout gestation, differentiating along two pathways to form either villous cytotrophoblast, which ultimately can become syncytiotrophoblasts (outer cellular layer) or EVCTs (inner cellular layer). Syncytiotrophoblast is a specialized epithelium that has several functions, including transport of gases, nutrients, and waste products and synthesis of peptide and steroid hormones that regulate placental, fetal, and maternal systems. Extravillous trophoblasts (EVT) have a proliferative component and an invasive component. There is also a migratory EVT, which is neither invasive nor proliferative. These cells populate the cell islands, septum, chorionic plate and chorion laeve.

Invasion by trophoblast cells involves cellular proliferation, attachment of cells to and degradation of extracellular matrix, and migration through connective tissue [50]. It is well established that signaling through the RAMPs in general promotes cellular division and differentiation. Specifically, in human or rodent cells, reducing CGRP family peptides signaling by infusion with antagonists directly correlates with increased apoptosis or programmed cell death both in vitro and even more so in vivo [51, 52]. In preimplantation rats, antagonist infusion induces apoptosis, which manifests as increased resorption rates. In vivo, AM and IMD contribute for protection against apoptosis especially in trophoblast cells in the labyrinth zone of placenta and uterine decidua in rats [51, 52]. AM increases the invasive capacity and migration of trophoblast cells [34–36, 53].

Fetal *AM* gene expression is upregulated in invasive trophoblast giant cells [30]. AM is shown to enhance the invasive capabilities of JAr cells and HTR-8/SV neo cells through increasing the gelatinolytic activity of MMP-2 [43], increased expression/activity of uPA [36] and reduction in plasminogen activator inhibitor-1 expression [43]. These actions of AM were completely blocked by administration of human $ADM_{22–52}$ [36]. It is likely that AM secreted either from maternal or fetal tissues act as a migratory factor for these cells during trophoblast invasion. In support of this possibility, AM is shown to be an effective chemoattractant and migratory factor for a variety of cell types [54, 55], including cultured choriocarcinoma JAr cells and first-trimester cytotrophoblast cells [43].

Similar to AM, IMD also stimulates an increase in trophoblast invasion and migration [34, 35]. Recent report shows that IMD regulates the invasive capacity of first trimester EVCTs via suppressing decidual expression of tumor/metastasis suppressor KAI-1(Kangai-1) in human pregnancy [35]. In addition, based on the in-vivo studies IMD may involve NO and MMP/uPA system to facilitate trophoblast invasion [19].

4.4 Fetal Growth and Developmental Consequences of Direct Perturbations

Infusion of CGRP antagonist, $CGRP_{8–37}$, caused significant fetal growth restriction and pup mortality [56]. Rats infused with AM antagonist, AM_{22-52} during pre- (i.e., from gestational day 1–4) or post-implantation period (i.e., from gestational day 8–15 or 14–22) is shown to induce dose-dependent decrease in both placental and

fetal weights along with increase in fetal resorption sites [44, 52]. The AM antagonist induced placental and fetal growth restrictions appeared more pronounced when infused during late gestation (day 14–22). Fetal as well as placental growth restriction with impaired placental vasculature was also reported in pregnant rats infused with IMD antagonist in mid gestation [51]. Reduced weights of implantation sites were observed when the infusion of IMD antagonist was done during peri-implantation period [19]. Thus, IMD has a potential role in mediating early placentation and fetal-placental growth.

Genetically modified mouse models have been developed for most of the CGRP family peptides and their receptor components. CRLR, $RAMP_2$, and AM—but not CGRP, $RAMP_1$, and $RAMP_3$—null mice are embryonically lethal [3]. A modest 50 % reduction in uterine AM in $AM^{+/-}$ female mice caused significant reductions in fertility and fetal growth restriction even in wild-type and $AM^{+/-}$embryos, demonstrating a critical role for maternal AM on fetal growth. Genotype analysis from $AM^{+/-}$ intercrosses and results from reciprocal crosses using wild-type females mated to $AM^{+/-}$males did not reveal a significant dose effect of heterozygous loss of fetal AM on fertility or fetal growth. However, the incidence of fetal growth restriction was significantly exacerbated when the implanting blastocyst was null for AM. These data suggest that fetal expression of AM also contributes significantly to the early stages of embryonic development. Taken together, these data implicates that both maternal and, to a lesser extent, fetal sources of AM peptide are involved in early fetal growth [30].

5 CGRP Family Peptides and Pregnancy Diseases

5.1 Immunological Effects

Uterine NK cells constitute the largest proportion of immune cells in the decidua. The uterine NK cells have an important role in spiral artery remodeling and cooperation between decidual NK cells and the EVT are considered primary events in this vascular remodeling event [57, 58]. Recently, a direct link between AM and uterine NK immune cell function was identified [59]. Dynamic differences were reported in uterine NK cell recruitment between AM null and AM wild type placentas in a mouse model of gestation, reflecting concomitant changes in the expression of numerous chemokines and cytokines [59]. Uterine NK cells expressed high levels of CRLR, and treatment with AM responded with increases in MMP9 but not MMP2 consistent with the previously described functions of uterine NK-derived MMP9 in SA remodeling [59]. Thus, fetal AM may greatly influence the immune milieu of the placenta by recruiting and activating uterine NK cells to secrete chemokines, cytokines, and MMPs to facilitate SA remodeling. Understanding regulation of uterine NK cell effector molecule expression is important, as in addition to MMPs and cytokines such as IFN-γ, uterine NK cells also secrete angiogenic factors vascular endothelial growth factor and angiopoietin II [60]. In support of Li and colleagues [59], we observed that NK cells in human decidua express abundant AM receptor components (CRLR, RAMP2, RAMP3) in early gestation (Yallampalli and coworkers, unpublished observations). In ongoing studies in a rat pregnancy model [52], in vivo antagonism of AM results in decreased uterine NK numbers, and reduced IFN-γ expression by uterine NK cells, at implantation sites at day 8 of gestation (Yallampalli and coworkers, unpublished observations). Uterine NK cells are the predominant source of IFN-γ in the decidua microenvironment while exogenously administered IFN-γ is sufficient for SA remodeling in NK cell deficient mice [61]. Inhibition of trophoblast invasion during SA remodeling, however, is linked to induction of EVT apoptosis by uterine NK cell-derived IFN-γ [62, 63]. These opposing effects suggest the IFN-γ levels may be tightly regulated to optimize vasculogenesis while controlling EVT invasion. Understanding how neuroendocrine peptide mediators, such as CGRP and AM, regulate recruitment and activation of uterine NK cells at the fetal/maternal interface is

an important avenue of investigation relevant to diseases associated with vascular dysfunction (e.g., preeclampsia).

5.2 Spontaneous Abortion

The causes of recurrent pregnancy loss are classified as genetic, anatomic, hormonal, metabolic, immunologic, microbiologic, and environmental [64, 65]. Plasma AM concentrations were similar in women who are spontaneously aborting and their controls. However another recent study reported that the plasma AM levels in women with recurrent pregnancy loss (5.6 ± 1.9, mean ± standard deviation) were significantly higher ($P > 0.001$) than that in control women (3.6 ± 1.7) [65]. In the placenta, AM was localized at the fetomaternal interface, and the prevalence of positive cells, particularly of trophoblast cells, stained for AM was significantly lower in spontaneous abortions than in controls [66]. Our preliminary studies [67] and that of Urban et al. [68] showed that ir-AM is reduced at the fetomaternal interface in women with spontaneous abortion compared to controls. In spontaneous abortion before 10 gestational weeks, AM immunopositive cells are reduced by more than 50 % in the decidua and up to 30 % in the extravillous trophoblast cells [59]. Recent study shows that lower serum as well as placental IMD levels are associated with spontaneous abortion compared to the age matched controls [35]. IMD mRNA expression in the first trimester villous tissue from spontaneously aborted placenta were 100-fold lower at all weeks of gestation compared with elective abortion [35]. This suggests that either pathological pregnancy decreases IMD levels or the effect of lower IMD levels in these spontaneous abortions could possibly be a cause for spontaneous abortion. However, the limitations of the studies involving tissues from spontaneous abortions in human pregnancy cannot be ignored. Future studies, perhaps in a nonhuman primate model, may help to address the relationship of lower IMD levels in spontaneous abortion with the occurrence of pathology.

5.3 Preeclampsia and Intrauterine Growth Restriction

Transcripts of CRLR and $RAMP_1$ are substantially reduced in fetoplacental vessels from preeclamptic women [42]. In addition, trophoblast cells also showed decreased expressions for CRLR and $RAMP_1$ proteins and CGRP binding sites were lower in preeclamptic placentas. In addition, relaxation of umbilical and chorionic arteries to CGRP in preeclamptic women is significantly attenuated compared to their age-matched controls. Therefore, it is likely that the fetoplacental vascular resistance in normal pregnancies is regulated by CGRP, which appears to be compromised in preeclamptic pregnancies [42, 69].

Maternal circulating AM has been reported as either increased [70], decreased [71] or unchanged [23, 59, 72–74], whereas in umbilical plasma and amniotic fluid, its concentrations are higher than in normotensive pregnancies [72]. Conflicting results have been reported also in the expression of AM in fetoplacental tissues in preeclampsia. Ir-AM in placentas of preeclamptic women was found to be decreased [75] or unchanged [72], and AM mRNA expression has been shown to be either decreased [18] or unchanged in the placenta and uterine muscle [76], decreased in fetal membranes and increased in umbilical artery [76]. Similarly, receptor component for AM ($RAMP_2$) has been shown to be unchanged in the placenta [77], decreased in cord and uterus and increased in fetal membranes [77] although no correlation was found between mRNA level and blood pressure. Li et al. [78] reported a ninefold decrease in AM output from cultured preeclamptic placentae. Differences in the criteria for diagnosis of preeclampsia or in the uteroplacental or fetal hemodynamic condition between studies may account for the controversial results found. However, recent study in AM knockout mice showed characteristics of preeclamptic placenta such as failed SA remodeling and reendothelialization with retained smooth muscle actin layer as they approached the maternal fetal interface and reduced number of uNK cells in AM-null placentas [59].

Di Iorio and colleagues found that fetoplacental levels of AM peptide were increased in human patients with intrauterine growth restriction [79], while another group found no significant differences in fetal or maternal AM levels between normal pregnancies and pregnancies with fetal growth restriction [80].

Since expression of receptor components for CGRP peptide family are altered in preeclampsia, involvement of IMD in the pathophysiology of this pregnancy complication cannot be ruled out. Our unpublished data shows that expression of IMD transcript are significantly lower in preeclamptic villi compared to the age matched controls and this effect appeared to be more pronounced in pregnancies with male fetus compared to the female. Clearly, more studies are needed to understand the mechanisms of IMD action and the cause and consequences of altered IMD levels in pathological pregnancies such as miscarriages and preeclampsia.

6 Summary

It is evident from in-vivo and ex vivo studies that, the CGRP family peptides involving CGRP, AM, and IMD in endometrium, decidua and placental tissue could be an important system for various adaptive changes that occur during pregnancy. CGRP family peptides are found to have pleiotrophic effects on placenta and endometrium. In the uterus, these peptides may facilitate decidualization, favor implantation, angiogenesis and proliferation of endometriotic tissue and amplify the uNK cell responses. In the placenta, CGRP family peptides may facilitate trophoblast cell proliferation and fusion and estradiol and progesterone production.

Despite our increasing knowledge on the diverse functions CGRP family peptides in normal and pathological reproduction, much remains to be learned about CGRP family peptides-dependent signaling cascades in the diverse gestational tissues and its interactions with other molecules. Although CGRP, AM and IMD appears to play an important role in regulating feto-placental growth and development, most of the functions of these peptides appear redundant, yet knock out or inhibition of one peptide or its receptor component cause deleterious effects on fetal growth and development. Whether function of each of these peptides are complimentary, additive or synergistic; and if the signaling of these peptides integrates at some point downstream is not known. Conspicuous similarities in the structure and function and yet distinct physiological roles of these peptides provoke future studies to identify their relative roles in placental functions and if these peptides compete for their shared receptor components to create a favorable physiological milieu in pregnancy. Future explicit mechanistic studies may identify these peptides and or their receptor components as a new class of clinically useful tools in pregnancy related disorders such as recurrent miscarriages, preeclampsia and intrauterine growth restriction. Therefore, continuous research and improvement of model systems are required to gain more insights into the complex functions of CGRP family peptides in physiological and pathological placenta and endometrium.

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