Adrenomedullin: A Novel Peptide Requires Coordination Of Genetic, Physiologic And Environmental Factors

K. R. Padma, Dr. P. Josthna

ABSTRACT: A healthy pregnancy requires strict coordination of genetic, physiologic, and environmental factors. The relatively common incidence of infertility and pregnancy complications has resulted in increased interest in understanding the mechanisms that underlie normal versus abnormal pregnancy. The peptide hormone adrenomedullin has recently been the focus of some exciting breakthroughs in the pregnancy field. Adrenomedullin (ADM) is a 52-amino acid peptide with structural homology to calcitonin gene-related peptide (CGRP) initially isolated from human pheochromocytoma. ADM is synthesized by many mammalian tissues including the adrenal medulla, endothelial and vascular smooth muscle cells, myocardium and central nervous system. ADM binds to plasma membrane receptors composed of calcitonin receptor-like receptor (CRLR), a member of serpentine receptor superfamily, and receptor activity modifying protein (RAMP) type 2 or 3. ADM has also some affinity for CGRP receptor composed of CRLR and RAMP1. Supported by mechanistic studies in genetic animal models, there continues to be a growing body of evidence demonstrating the importance of adrenomedullin protein levels in a variety of human pregnancy complications. With measurement of foetal resorption sites, we can examine the importance of adrenomedullin, a peptide hormone in pregnancy which alters due to genetic, physiologic and environmental factors. A growing body of evidence illustrates AM as a pivotal component in normal physiology and disease with marked beneficial effects in the host defense mechanism.

Key words: Adrenomedullin, Calcitonin, Amylin, Preeclampsia, Phaeochromocytoma, RAMP, pregnancy, foetal resorption.

Introduction of Adrenomedullin:

Adrenomedullin is a novel vasodilatory peptide isolated first in 1993. It was originally isolated from human phaeochromocytoma from adrenal glands of Zona glomerulosa region.[1]This plueripotent hormone increases during implantation. Any change in environment may result in pregnancy complications.[2] The area of study is the calcitonin gene-related peptide (CGRP) family and the critical roles these peptides play in female reproductive biology. The CGRP family is composed of six known peptides, CGRP (α- and β receptors), adrenomedullin (AM), calcitonin (CT), amylin(AMY), and intermedin/ adrenomedullin2 (IMD), that share a similar molecular structure and overlapping biological functions .[3]

Biochemistry of Adrenomedullin:

Human AM is a 52-aa peptide with a ring structure formed by a disulfide bond and amidated tyrosine at the C terminus (Figure1), both essential for binding to receptors and biological activity. Based on sequence homology, AM is thought to belong to the calcitonin gene–related peptide (CGRP) superfamily.[4] ADM and CGRP belong to a family of structurally related peptides, which also includes calcitonin (CT) and amylin. Cloning of the cDNA encoding AM revealed the AM precursor peptide preproAM to comprise 185 amino acids, with the C terminus followed by a pair of basic amino acids, Arg-Arg, a typical processing signal.

ADM acts as a circulating or paracrine hormone and exerts its effects via the G-protein coupled calcitonin-like receptor (CL), a member of the serpentine receptor super family complexed with a receptor accessory modifying protein known as $RAMP_2$ (ADM_1 receptor) or $RAMP_3$ (ADM_2 receptor).[5] The preproadrenomedullin precursor is 185 aminoacids long and the precursors give rise to cleavage products, they are Adenotensin, PAMP-20, PAMP-12, and prodepin (Figure2). Adrenomedullin is synthesized and secreted in many cell types. Amylin is also a 37Amino acid peptide present on chromosome 12 instead of chromosome11.[6]

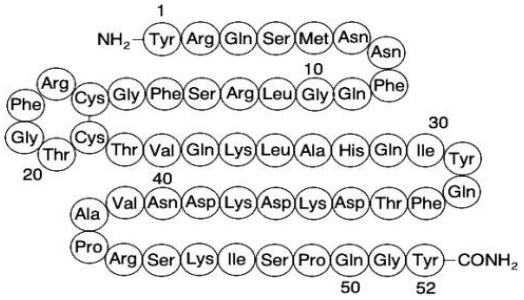

Figure 1. *Amino-acid sequence of human AM.*

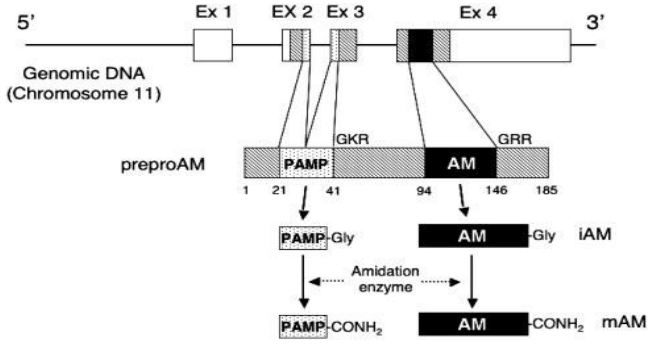

Figure 2. *Schematic representations of the AM gene and of the processing of AM and PAMP from preproAM. Ex indicates exon; PAMP, proadrenomedullin N-terminal 20 peptide; iAM and iPAMP, intermediate forms of AM and*

- *Dr. P. Josthna, Assistant Professor, Department of Biotechnology, Sri Padmavati Mahila Visvavidyalayam (Women's University), Tirupati, 517 502 Andhra Pradesh, India. E-mail: penchalanenijosthna@gmail.com*

PAMP, respectively; mAM and mPAMP, mature forms of AM and PAMP, respectively.

Mechanism of AM dilation:

The mechanisms by which AM dilates blood vessels are not completely understood; however, based on the numerous articles published to date, it is clear that AM directly dilates blood vessels of the systemic and pulmonary circulation in an endothelium-dependent or independent manner.[7] The biological feature of AM initially characterized after subcutaneous infusion in a relatively short period of time. We evaluated the role of AM in the regulation of uteroimplantation growth during pregnancy.[8] There is a growing body of evidence indicating that AM possess many important physiologic and pathophysiologic properties.[9] In addition, ADM has protective effects against vascular injury, including oxidative stress.[10] Although little is known about the biology of AM in cells of the immune system, a few reports have recently began to define its role in immune cell function. Neutrophils are usually the first cells arriving to inflammatory sites and represent cardinal cellular effectors of the innate host response.[11]

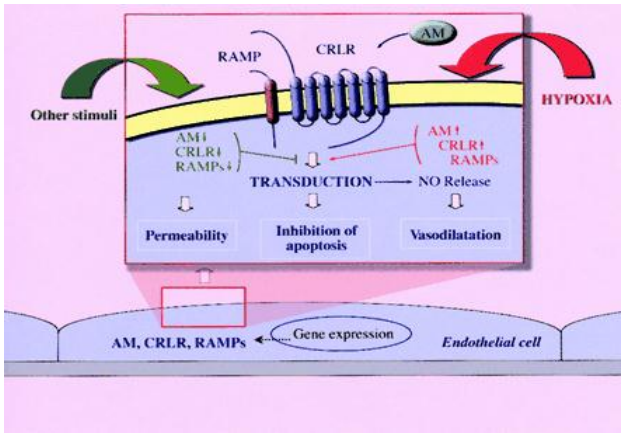

Figure 3: *AM in Vasodilation*

Environmental factors affects AM in foetus growth:

In the present study, we evaluated the role of AM in the regulation of uteroimplantation growth during pregnancy. There are a multitude of genetic, physiologic, and environmental factors that must all work in perfect harmony throughout pregnancy to produce the so-called “miracle” that is a healthy, full-term baby.[12] Any aberration in this process may result in pregnancy complications, which can include implantation failure, miscarriage, fetal growth restriction, gestational diabetes, preeclampsia (PE), and preterm birth. Thus there is currently major interest and effort in the field, to expand our understanding of the factors that contribute to healthy versus unhealthy pregnancies. To demonstrate how an environmental changes affects during early pregnancy, an antagonist of AM, $AM_{22–52}$, was continuously infused through osmotic minipumps beginning on gestational day 2 in rats. These animals received either 125 or 250 µg rat/day of $AM_{22–52}$ or vehicle only and were killed on day 9 of gestation to assess uterus and implantation weights. Table-1, shows that both uterus and implantation weights in rats receiving two doses of AM antagonist were significantly lower than in controls ($P < 0.05$). These reductions in uterus and implantation weights were more substantial with $AM_{22–52}$ at 250 µg rat/day compared with 125 µg rat/day. Thus AM is the potent vasodilatory peptide which has strong influence in pregnancy and any disturbance in environment indirectly effects pregnant women and leads to reduction in size of foetus.[13]

Table: 1 *Uterus and implantation sites weight for rats killed on gestational day 9*

Treatment group	pregnant rats per group (n)	uterus weight (g)	implantation wt (mg)
Control	04	42.6±0.9	8.92±0.1
125µgAM_{22-52}	04	39.4±0.3	7.95±0.1
250µgAM_{22-52}	04	22.1±0.7	5.55±0.3

Figure : *A & B*

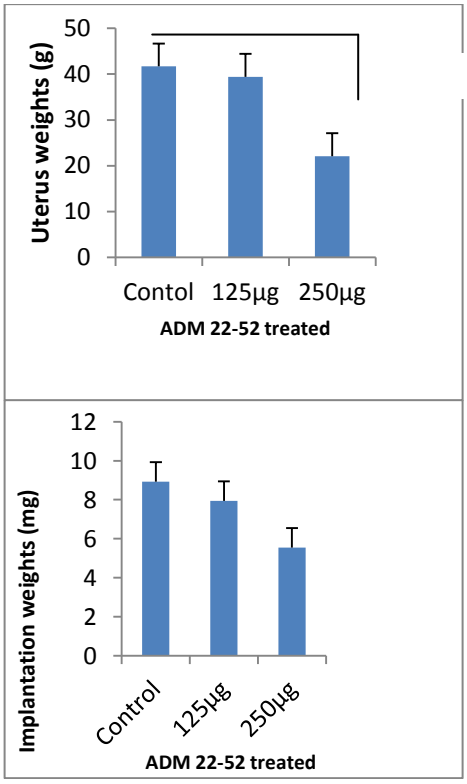

Figure:*4*

Effects of AM_{22-52} on rat uteroimplantation growth. Rats received continuous infusion of different doses of AM_{22-52} (125µg vs. 250µg) or vehicle (control) from Day 2-8 of pregnancy. Uterus (fig: A) and implantation (fig: B) weights of rats were recorded on Day 9 of gestation. Bars are mean± SEM values for four replicative animals in each group. *P < 0.05 indicates significantly different when compared with the control, and **P < 0.05 indicates the significance between the two doses.

Expression of Adrenomedullin:

AM and its receptor components are highly expressed in reproductive tissues, including the uterine endometrium fetal membranes , placenta , stromal macrophages and trophoblast cells.[13] AM expression is regulated by multiple factors involved in the physiology of reproduction. The earliest stages of placental development in humans and rodents occur during implantation, when trophectoderm cells from the blastocyst attach and invade into the wall of the receptive uterus.[14] These trophectoderm cells differentiate into multinucleate trophoblast cells termed extravillous cytotrophoblasts in humans and giant trophoblast cells in rats and mice, which invade the uterine lining and establish the vascular connection between fetal placental tissue and the maternal blood supply.[15]

Adrenomedullin in fertility and implantation:

An important role of AM in fertility and implantation has come from well-characterized animal models. Recent findings have implicated AM in even the earliest stages of pregnancy. Lei et. al. showed that in a rat model, ovarian AM expression increases from small antral follicles to large antral follicles to the formation of the corpus luteum, and AM appears to be involved in the regulation of progesterone production from the corpus luteum.[16] In the present work carried by us we observed in (figure-5) foetal resorption sites, when physiologic and environmental factors varied which resulted in resorption sites in early pregnancy.

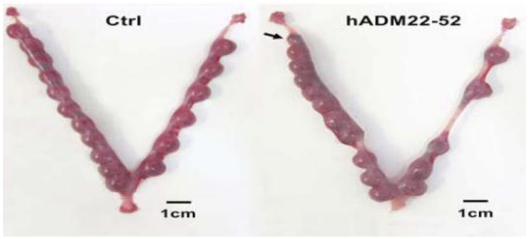

Fig. 5. *The effect of preimplantation of ADM antagonism on implantation rate*

CONCLUSION AND PERSPECTIVE:

Since the discovery of the novel vasodilator peptide AM, much research, basic and clinical, has been done to clarify the vascular actions of AM and its role in host defence mechanism. Over the past decade, a growing body of evidence has implicated AM, and PAMP as important pleiotropic effectors of the host defense mechanism. The importance of CGRP family peptide AM in the establishment and maintenance of the healthy pregnancy is clearly supported by many studies spanning the past 15 years. Our understanding of the functions of AM in normal and in complicated pregnancies has advanced significantly in the past five years in large part to studies using genetic animal models. However, unanswered questions remain. Altogether, the aforementioned studies point to the complexity of the control of immune cells specific to the transient environment of the pregnant uterus. Here, we have emphasized that both maternal and fetal-derived AM is important for establishing and maintaining a successful pregnancy.[17]Molecular identification of PAMP receptors and functional interactions between different proADM-derived peptides with parallel and opposite activities are an important challenge for future research.[18]

REFERENCES:

[1] Kitamura K, Kangawa K, Kawamoto M, Ichiki Y, Nakamura S, Matsuo H, Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. 1993. Eto T.Biochem Biophys Res Commun. 2012 Aug 31; 425 (3):548-55. Do I: 10.1016/j.bbrc.2012.08.022.

[2] Patricia M. Lenharta and Kathleen M. Carona,* Adrenomedullin and Pregnancy: Perspectives from Animal Models to Humans.Trends Endocrinol Metab. 2012 October; 23(10): 524–532. doi:10.1016/j.tem.2012.02.007. .

[3] K.R.Padma, Dr.P.Josthna, K.R.Don. Adrenomedullin receptors and functions of this novel peptide in implantation.Vol: I Issue: I Aug.-2013, ISSN 2347-2723

[4] Johji Kato, Toshihiro Tsuruda, Toshihiro Kita, Kazuo Kitamura, Tanenao Eto Adrenomedullin A Protective Factor for Blood Vessels (Arterioscler Thromb Vasc Biol. 2005; 25:2480-2487.)

[5] McLatchie LM, Fraser NJ, Main MJ, Wise A, Brown J, Thompson N, Solari R, Lee MG, and Foord SM. 1998. RAMPs regulate the transport and ligand binding specificity of the calcitonin-receptor-like receptor. Nature 393:333-339. doi: 10.1038/30666

[6] Penchalaneni J, Wimalawansa SJ, Yallampalli C: Adrenomedullin antagonist treatment during early gestation in rats causes fetoplacental growth restriction through apoptosis. Biol Reprod 2004, 71:1475-1483.

[7] Hayakawa H, Hirata Y, Kakoki M, Suzuki Y, Nishimatsu H, Nagata D, Suzuki E, Kikuchi K, Nagano T, Kangawa K, Matsuo H, Sugimoto T, Omata M. Role of nitric oxide-cGMP pathway in adrenomedullin induced vasodilation in the rat. Hypertension. 1999; 33:689–693.

[8] Ishiyama Y, Kitamura K, Ichiki Y, Nakamura S, Kida O, Kangawa K, Eto T. Hemodynamic effects of a novel hypotensive peptide, human adrenomedullin, in rats. Eur J Pharmacol. 1993;241:271–273.

[9] Kano H,Kohno M, Yasunari K, Yokokawa K,Horio T,Ikeda M, Minami M,Hanehira T, Takeda T, Yoshikawa J (1996) Adrenomedullin as a novel antiproliferative factor of vascular smooth muscle cells. J. Hypertens 14:209-213. 10.

[10] Kawai J, Ando K, Tojo A, Shimosawa T, Takahashi K, Onozato ML, Yamasaki M, Ogita T, Nakaoka T, and Fujita T. 2004. Endogenous adrenomedullin protects against vascular response to injury in mice.

Circulation109:11471153.doi:10.1161/01.CIR.0000117231.40057.6D

[11] Marshall, J. C. (2005) Neutrophils in the pathogenesis of sepsis. Crit.Care Med. **33,** S502–S505.

[12] Ogoshi M, et al. Evolutionary history of the calcitonin gene-related peptide family in vertebrates revealed by comparative genomic analyses. Peptides. 2006; 27:3154–3164. [PubMed: 17092606]

[13] Minegishi T, et al. Adrenomedullin and atrial natriuretic peptide concentrations in normal pregnancy and pre-eclampsia. Mol Hum Reprod. 1999; 5:767–770. [PubMed: 10421805]

[14] Zhao Y, et al. PCR display identifies tamoxifen induction of the novel angiogenic factor adrenomedullin by a non estrogenic mechanism in the human endometrium. Oncogene. 1998;16:409–415. (PubMed: 9467966)

[15] Lee KY, DeMayo FJ. Animal models of implantation. Reproduction. 2004; 128:679–695.[PubMed: 15579585]

[16] Li L, et al. Adrenomedullin in rat follicles and corpora lutea: expression, functions and interaction with endothelin-1. Reprod Biol Endocrinol. 2011; 9:111. [PubMed: 21824440]

[17] Kraus DM, Feng L, Heine RP, Brown HL, Caron KM, Murtha AP et al.Cigarette smoke-induced placental adrenomedullin expression and trophoblast cell invasion. Reprod Sci 2014; 21: 63–71.

[18] Li J, Ren Y, Dong X, Zhong G, Wu S, Tang C: Roles of different peptide fragments derived from proadrenomedullin in the regulation of vascular tone in isolated rat aorta. Peptides, 2003, 24, 563–568.