International Journal Of

Recent Scientific Research

ISSN: 0976-3031

Volume: 7(6) June -2016

ADRENOMEDULLIN: A UBIQUITOUS HORMONE REQUIRED FOR HEALTHY PREGNANCY

Padma K.R and Josthna P

THE OFFICIAL PUBLICATION OF
INTERNATIONAL JOURNAL OF RECENT SCIENTIFIC RESEARCH (IJRSR)
http://www.recentscientific.com/ recentscientific@gmail.com

ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 7, Issue, 6, pp. 11710-11713, June, 2016

International Journal of Recent Scientific Research

Research Article

ADRENOMEDULLIN: A UBIQUITOUS HORMONE REQUIRED FOR HEALTHY PREGNANCY

Padma K.R and Josthna P*

Department of Biotechnology Sri Padmavati Mahila Visvavidyalayam (Women's University)
Tirupati, 517 502 Andhra Pradesh, India

ARTICLE INFO

Article History:

Received 11th March, 2016
Received in revised form 14th April, 2016
Accepted 18th May, 2016
Published online 28th June, 2016

Key Words:

Adrenomedullin, Phaeochromocytoma, CGRP, pregnancy, mini pumps.

ABSTRACT

Concentrations of adrenomedullin (ADM) in circulation enhances during pregnancy. The pattern of ADM level and gene expression of ADM, its receptor components from early pregnancy has been studied in our present work. Calcitonin gene-related peptide (CGRP) and its related peptide, adrenomedullin (ADM), have vasorelaxant activity in a variety of tissues. The CGRP plays an important role in maintaining uterine relaxation during pregnancy. 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. Hence, in the present study, the AM-antagonist AM_{22-52} is continuously infused through osmotic mimipumps during 2–8 days of gestation. On the 9th day, we assessed the weights of uteroimplantation site to know the growth of uterus and implantation tissues. Later we examined apoptotic changes in uteroimplantation site by Haematoxylin and eosin staining.

INTRODUCTION

Adrenomedullin is a multiregulatory peptide isolated first in 1993 by kitamura *et al*. It was predominantly isolated from human phaeochromocytoma from adrenal gland regions.[1] This ubiquitous hormone enhances during implantation. Any change in genetic, physiologic and environmental deterioration may result in pregnancy complications.[2] The CGRP is a seven transmembrane family proteins and is known to be proposed of six related peptides, CGRP (α- and β receptors), adrenomedullin (AM), calcitonin (CT), amylin(AMY), and intermedin/ adrenomedullin2 (IMD), that shares a similar molecular structure and binds to receptor modifying protein which is composed of three isoforms, RAMP1,2 &3.[3]ADM shares structural homology with rat as it consists of 52 amino acids in the human and 50 amino acids in the rat. [4,5] In the present study, the AM-antagonist $AM_{22–52}$ was profusely infused during Days 2–8 of gestation, and on Day 9, we have sacrificed the animals to observe uteroimplantation site which showed fetal resorption[6] and in modest the uterus and implanted weights have been reduced considerably. By histopathological examination of this peptide hormone, it has been proved that AM is a multifunctional regulatory and vasodilatory peptide hormone which is essential not only during late gestation period but also plays a critical role in early gestation in rodents as well as humans.

MATERIALS AND METHODS

Animal maintenance

Animal studies were performed as per institute animal ethics committee regulations and approved by the committee (Reg. No. 1677/PO/a/12/CPCSEA/SPMVV-IEC/2014/01). Healthy rats of Wistar strain were purchased from authorized vendor (M/S Raghavendra Enterprises, Bangalore, India). All rats were housed in polypropylene cages (20" 8"x 9") lined with sterilized paddy husk, and provided filtered tap water and rat food in an air-conditioned environment (26±2°C) with a 12-h light and 12-in dark cycle.

Experimental Treatment Protocol

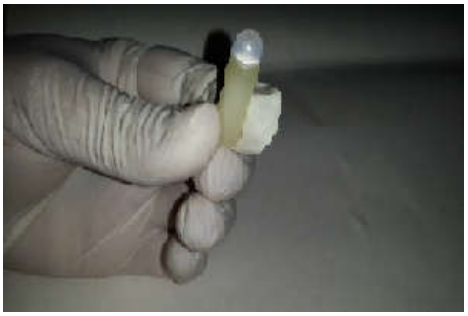
Female wistar rats (Rattus norvergicus), weighing 220g ± 280 g were used for the experiment. Three to four pregnant rats were used for each of the experimental study. Female rats were mated with male proven breeders. The next day morning, collecting of vaginal secretion with a plastic pipette filled with 10 µL of normal saline (NaCl 0.9%) by inserting the tip into the rat vagina. One drop of vaginal fluid was placed on glass slides the unstained material was observed under a light

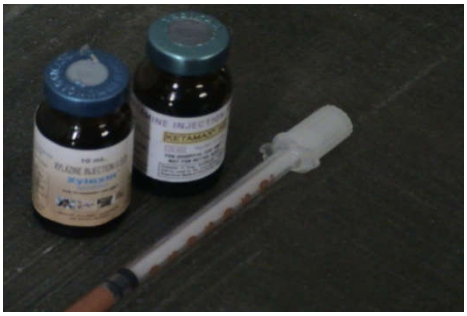
*Corresponding author: **Josthna P***
Department of Biotechnology Sri Padmavati Mahila Visvavidyalayam (Women's University) Tirupati, 517 502 Andhra Pradesh, India

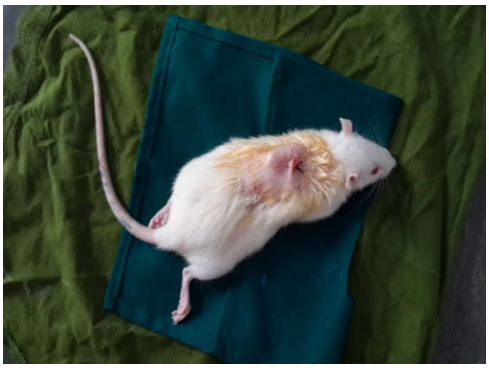
microscope [7-8]. Two females of pro-estrous stage were paired with a male overnight and the next morning, males were removed and females were assessed for the presence of sperms in the vaginal flush. Animals with positive sperm in the flushes are designated as day 1 of gestation.

Osmotic (ALZET) pumps

The mean ± SEM body weight was 228 ± 260 g in the rats on day 2 of gestation. The osmotic minipumps (model 2001 Alzet pump; 1.0 µl/h were inserted subcutaneously into the dorsum of pregnant rats while the animals were under anaesthesia (anaesthesia consisted of a combination of ketamine (45 mg/kg) and xylazine (5 mg/kg). The minipumps were filled with saline alone or with saline containing different concentrations of $AM_{22–52}$. These concentrations were chosen based on the earlier findings of Witlin, *et al*.[9] and Penchalaneni, *et al*.[6] to deliver $AM_{22–52}$ at 125 and 250µg/rat/day.







Histopathological examination

For this part of the study, uteroimplantation sites were collected from rats infused with 125 and 250 µg/day of ADM $_{22–52}$ along with vehicle control. Immediately the collected uteroimplantation sites were placed in fixative solution (10% formaldehyde) later the uteroimplanted tissue block was dehydrated with different series of isopropyl alcohol concentrations then the tissues are embedded in paraffin and sectioned the tissues into 6- µm thickness finally tissue sections are transferred on to microscopic slide and were stained with haematoxylin and eosin and then studied by light microscope. Related photographs were taken to observe for any hADM $_{22-52}$ associated morphological changes.

Statistical analysis

Uterus and implantation weights were performed by taking the mean of the uterus and implantation weights per rat first followed by the mean of all the rats. Weights are expressed as the mean ± SEM and were analyzed for differences using one-way ANOVA. Values were found to be significant when *P< 0.05.

RESULTS

Evaluation of $AM_{22–52}$ on uteroimplantation Weights

In the present study, we examined the role of AM in the regulation of uteroimplantation growth during pregnancy. The decadent effects of ADM antagonist has shown that there was a significant decrease in pregnancy which was clearly observed in uterus and implantation weights ($P < 0.05$; Table-1 and Figure-1 A and B) when they were continuously infused through osmotic minipumps beginning on gestational day 2. 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.

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 and vehicle control.

Table 1 Uterus and implantation sites weight of rats killed on gestational day 9

Treatment group	pregnant rats per group (n)	uterus weight (g)	implantation wt (g)
Control	04	15.1±0.3	1.6±0.1
125µgAM$_{22-52}$	04	12.5±0.1	1.2±0.06
250µgAM$_{22-52}$	04	8.15±0.2	0.8±0.03

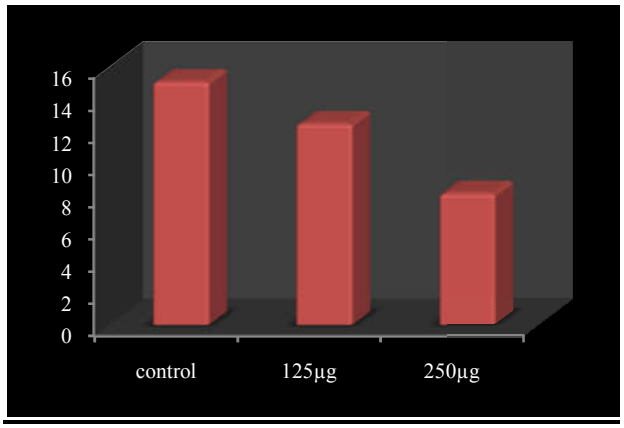


Figure A Uterus weight in (g)

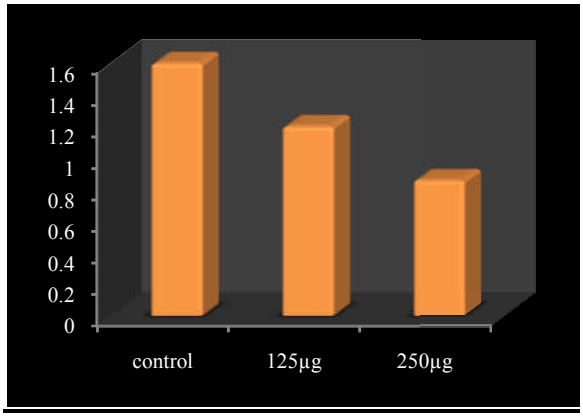
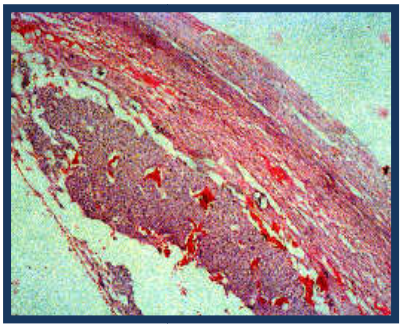
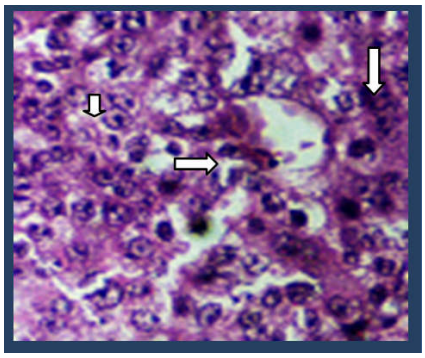


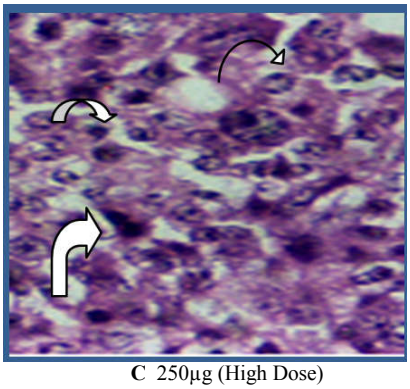
Figure B Implantation weight in (g)



A Control



B 125µg (Low Dose)



C 250µg (High Dose)

Histopathological examination for morphological changes

In our current study, we examined H&E stained microphotograph of rat uteroimplantation sections obtained from ADM22-52 treated and vehicle control on gestational day9. In the ADM treated rats histopathological changes were more prominent in the stromal endometrium and in uterine glands or endometrial glands lining the endometrium of uterus. In the stromal endometrium regions we observed apoptotic changes in high dose group rats and mild pyknosis in low dose treated rats in (Fig-b). Pyknosis, or karyopyknosis, is the irreversible condensation of chromatin in the nucleus of a cell undergoing necrosis or apoptosis. It is followed by karyorrhexis, or fragmentation of the nucleus which is well depicted in (Fig-c)

Representative Photomicrograph of H&E staining in sections of uteroimplantation from control (A) and AM$_{22-52}$ treated (B&C) rats on day 9 of gestation. AM$_{22-52}$ infusion was started on day 2-8. Arrows indicate pyknosis and stromal breakdown showed haphazard nuclei and pale, sparsely vacuolated cytoplasm, are markedly distorted in AM$_{22-52}$ treated animals compared with controls. Original magnification X 100**.**

DISCUSSION

The present study perceived that, since the discovery of ADM, it has been found to be expressed by various reproductive tissues including the uterus, ovary, the oviduct, the placenta and the fetus membrane reviewed by (Garayoa *et.al*.2002) This exalt in the ADM levels occurred immediately after implantation i.e once trophoblast started to differentiate by (Di Iorio *et al*.1999). ADM has been counseled to be involved in implantation, placentation, embryogenesis, and uterine relaxation during pregnancy as an angiogenic factor, growth enhancing factor, immune modulator and vasodilator reviewed by (Wilson *et al*.2004) Previously, we have reported significant detraction in fetal and placental growth in pregnant rats when AM$_{22–52}$ was continuously infused from gestational 14th day (Penchalaneni *et al*.2004) In our present study we demonstrated that ADM, a novel member of the calcitonin related family of peptides which has a crucial role in pregnancy and is important for uterimplantation growth and fetal development. Inconsistent results from human studies attempting to characterize changes in AM levels in pregnancy-related diseases have left us with few conclusions. In our present study on ADM on animal models resulted in a meekly view

that ADM is responsible for healthy pregnancy. Any aberration due to environment detoriation may detract in implantation. Furthermore, additional in vitro and in vivo methods are employed to gain a greater understanding of the mechanisms by which AM mediates its effects in reproductive biology, with the intention of linking the biological functions of the peptide to physiologically relevant paradigms such as host defense, vasodilation, angiogenesis or regulation of innate immunity. Hence a legal action has to be taken by the government to stop the environmental pollution. A law has to be made effective against the pollution, to protect females during pregnancy.

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. Kitamura K, Sakata J, Kangawa K, Kojima M, Matsuo H, Eto T. Cloning and characterization of cDNA encoding a precursor for human adrenomedullin. Biochem Biophys Res Commun 1993; 194:720 –5.
5. Sakata J, Shimokubo T, Kitamura K, Nakamura S, Kangawa K, Matsuo H, *et al*. Molecular cloning and biological activities of rat adrenomedullin, a hypotensive peptide. Biochem Biophys Res Commun 1993; 195:921–7.
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. Marcondes, F.K., Bianchi, F.J., Tannon, A.P. Determination of estrous cycle phase of rats: Some helpful considerations. *Brazilian Journal of Biology* 2002, 62(4a): 609.
8. Spornitz, U.M., Socin, C.D., David, A.A. Estrous stage determination in rats by means of scanning electron microscopic images of uterine surface epithelium. The Anatomical Research 1999, 254: 116-126.
9. Witlin AG, Li ZY, Wimalawansa SJ, Grady JJ, Grafe MR, Yallampalli C. Placental and fetal growth and development in late rat gestation is dependent on adrenomedullin. Biol Reprod2002, 67:1025–31.
10. Garayoa M, Bodegas E, Cuttitta F, Montuenga LM. Adrenomedullin in mammalian embryogenesis. Microsc Res Tech 2002; 57:40–54.
11. Di Iorio R, Marinoni E, Letizia C, Villaccio B, Alberini A, Cosmi EV. Adrenomedullin production is increased in normal human pregnancy. *Eur J Endocrinol* 1999; 140:201–206.
12. Wilson, C., L. L. Nikitenko, I. L. Sargent & M. C. Rees (2004) Adrenomedullin:1 multiple functions in human pregnancy. *Angiogenesis,* 7**,** 203-12.

****

How to cite this article:

Padma K.R and Josthna P.2016, Adrenomedullin: A Ubiquitous Hormone Required For Healthy Pregnancy. *Int J Recent Sci Res.* 7(6), pp. 11710-11713.

TSSN 0976-3031