http://www.medscidiscovery.com

OPEN ACCESS JOURNAL ISSN: 2148-6832

MSD Medical Science and Discovery
2019; 6(7):114-8

Research Article Doi: 10.17546/msd.578488

Adrenomedullin has effects on hypoxia-inducible factor 1-alpha in hypoxia in several organs of female rats

Ayse Asiye Culum[1]*, Muhittin Yürekli[1]

Abstract

Objective: Vascular dysfunction induces impaired O_2 delivery and hypoxia, and adrenomedullin (AdM) have a role in hypoxia and angiogenesis. The reaction of the organism exposed to hypoxia and the effect of AdM over HIF-1α has been investigated in this study.

Material and Methods: Female rats were divided into 4 groups (control, hypoxia, control + AdM and hypoxia + AdM). Hypoxia groups were provided hypoxia containing 10% oxygen and 90% nitrogen for 1 week. AdM treatment groups were injected 1.25 nmol/kg AdM for 4 days intraperitoneally. AdM and HIF-1α levels were measured in heart, lung, and kidney tissues with ELISA.

Results: AdM levels increased in hypoxia + AdM group than hypoxia group, however, was not increased in control + AdM group when compared with the control group in heart tissue. Contrarily, AdM decreased in control + AdM group in the kidney, while increased in control + AdM, and hypoxia + AdM groups in the lung when compared to the control group. The HIF-1α level was higher in control + AdM group in the kidney, and hypoxia + AdM group in the lung. HIF-1α levels in heart tissue were decreased in hypoxia group when compared to the control group.

Conclusion: Tissues respond to hypoxic conditions at different times, and at distinct levels. AdM may be used to induce HIF-1α therapeutically before ischemic conditions.

Key words: Adrenomedullin, hypoxia, hypoxia-inducible factor 1α, angiogenesis

Introduction

Angiogenesis is regulated by hypoxia for supplying adequate oxygen to the cells. The lungs, heart, vascular, and red blood cell system involve in angiogenesis for providing balance against inadequate and excessive oxygenation (1). De novo vessels form in embryogenesis by vasculogenesis, in addition, new blood vessels form in pathological and physiological conditions including tumor neovascularization, ischemia, rheumatoid arthritis, regeneration, wound healing, menstrual cycle in adulthood (2). A hypoxic microenvironment is generated by physical and capillary injury and tumor development. This condition triggers angiogenesis by stimulating angiogenic factors (1).

Hypoxia-inducible factor (HIF) is the key transcriptional regulator of angiogenic pathways including the triggering vascular endothelial growth factor (VEGF) by hypoxia (1). HIF-1 is a DNA binding factor consisting of α and β subunits (3) and binds to 3' enhancer of erythropoietin which is a hormone that regulates vascular oxygen content (3,4).

HIF-1 also regulates other responses to hypoxia including angiogenesis and the activation of several genes for surviving on low oxygen conditions (5).

HIF-1α is inducible by hypoxia and it is rapidly degraded in normoxic conditions in the oxygen-dependent degradation domain by the ubiquitin-proteasome pathway (6). The overexpression and dysregulation of HIF-1α by either genetic alternations or hypoxia have been massively involved in cancer biology and several pathophysiologies, including tumor invasion, energy metabolism, angiogenesis, cell survival, and vascularization (7).

Adrenomedullin (AdM) is a vasodilator peptide and was discovered in a neuroendocrine tumor pheochromocytoma (8). AdM is synthesized in many tissues, however, it is chiefly synthesized in the medulla (9). AdM has a wide range of effects involving inhibition of cardiovascular alteration, the regulation of vascular endothelial function, vasodilatation, decreasing insulin resistance, and adjusting adipogenesis (10). The AdM binding sites were found in the membranes of kidney, lung, and heart (11).

Received 16-06-2019 **Accepted** 22-07-2019 **Available Online** 23-07-2019 **Published** 30-07-2019
1 Inonu University, The Faculty of Science and Literature, Dept of Biology, Malatya, TR
*** Corresponding Author:** Ayse Asiye Culum **E-mail:** D3613110221@ogr.inonu.edu.tr **Phone:** +90 422 377 37 01

http://dx.doi.org/10.17546/msd.578488

AdM provides enough oxygen to tissues by expanding the blood vessels as a vasodilator peptide in hypoxia (12). AdM protects against ischemia-reperfusion injury after stroke (9). Furthermore, there are hypoxia response elements in human AdM promoter (9).

The reaction of the organism exposed to hypoxia and the effect of AdM over HIF-1α has been investigated in this study.

Material and Methods

The rats were obtained from Inonu University Laboratory Animal Reproduction and Research Center. Rats were housed in cages at a 12/12-hour light/dark cycle, and the temperature of 22 ± 2°C. All processes with rats were managed in guidelines established by the Institutional Animal Care and Use Committee of the university, Public Health Services Policy, and the Animal Welfare Act.

Twenty-eight 12 months old female Sprague dawley rats were portioned into 4 groups randomly: control (n = 8), hypoxia (n = 7), control + AdM (n = 6) and hypoxia + AdM (n = 7). Hypoxia groups were provided with 10% oxygen (13,14) and 90% nitrogen for 1 week, and the control group was provided normoxia. During hypoxia, AdM groups were treated with 1.25 nmol/kg AdM (Rat Adrenomedullin 24-50, Phoenix Pharmaceuticals, Inc., CA, USA) for last 4 days intraperitoneally.

Rats were anesthesized with 1500 µL/kg ketamine, 500 µL/kg xylazine, then, heart, lung, and kidney tissues were collected. Tissues were homogenized in 2 mM PBS buffer (pH 7.3), and AdM and HIF-1α concentrations were measured by ELISA (Rat Adrenomedullin, ADM ELISA Kit CK-E30105; Rat hypoxia-inducible factor 1α, HIF-1α ELISA Kit CK-E30271, Hangzhou Eastbiopharm Co., Ltd., Zhejiang, China) according to manufacturer's instructions.

The results were expressed as means ± SD. The differences between groups were calculated with One-way ANOVA and LSD posthoc test (IBM SPSS Statistics Version 24), and values smaller than 0.05 were accepted as statistically significant.

Results

AdM level was decreased in the hypoxia group than the control group and hypoxia + AdM group in heart tissue ($p < 0.05$). Furthermore, AdM was higher in control group than hypoxia and control + AdM groups in kidney tissue ($p < 0.05$). Additionally, AdM was increased in all groups when compared with the control group in lung tissue (Table 1, Figure 1, $p < 0.05$).

HIF-1α was lower in hypoxia and hypoxia + AdM groups than the control group in heart tissue ($p < 0.05$). HIF-1α levels of control + AdM group was significantly increased than other groups in kidney tissue ($p < 0.05$). Control and hypoxia group HIF-1α levels were different from hypoxia + AdM and control + AdM groups in lung tissue ($p < 0.05$). Control + AdM and hypoxia + AdM group levels were increased when compared with control and hypoxia groups ($p < 0.05$, Table 2, Figure 2).

Table 1. AdM levels. (Means ± SD)

AdM (ng/L)	Heart	Kidney	Lung
Control	14.4 ± 2.7	16.7 ± 4.2	11.2 ± 1.3
Hypoxia	11.9 ± 1.3	12.9 ± 2.1	13.6 ± 2.1
Hypoxia + AdM	15.5 ± 1.4	15.4 ± 3.3	14.6 ± 2.5
Control + AdM	13.5 ± 2.9	12.8 ± 2.7	15.3 ± 1.6

Table 2. HIF-1α levels. (Means ± SD)

HIF-1α (ng/mL)	Heart	Kidney	Lung
Control	0.84 ± 0.33	0.70 ± 0.10	0.47 ± 0.1
Hypoxia	0.50 ± 0.13	0.78 ± 0.09	0.47 ± 0.05
Hypoxia + AdM	0.61 ± 0.11	0.86 ± 0.20	0.89 ± 0.20
Control + AdM	0.68 ± 0.14	1.03 ± 0.15	0.81 ± 0.28

Discussion

HIF-1α is induced in specific cell types including kidney, brain, liver, heart, skeletal muscle, lung, pancreas, intestine, and additionally, different levels of responses are observed in these specific cells (15–17). The oxygen concentration varies between tissues, for instance, the pO_2 of the kidney is 9.5 ± 2.6%, lung 5.6% (18), and myocardium 10% (19). It is possible that the hypoxia conditions of this study could remain normoxic to heart and kidney tissues. HIF-1α levels of heart, kidney, and lung were different from each other and the responses of these tissues to hypoxia were varied. HIF-1α was increased in hypoxia and hypoxia + AdM groups than the control group in the heart, suggesting that the amplitude of induction of heart is lower than other tissues. In the lung, alveolar cells are oxygenated from inspired air, rather than oxygenation with blood (17). It is possible that the response to hypoxia in the lung may earlier and distinct from other tissues. Although AdM expression is activated by HIF-1α (9), AdM treatment induced HIF-1α in hypoxia + AdM and control + AdM groups in lung. These results show that AdM may induce HIF-1α in the lung.

HIF-1α is found in the nucleus of the brain, heart, kidney, and liver cells in normoxia. The maximal expression of HIF-1α in hypoxia is 5 h, then expression decreases to basal levels in 12 h (16). It is observed in vivo studies that prolonged hypoxia increases HIF-1α mRNA (20). Rats provided hypoxia for 1 week in this study, thus, we could unable to observe peaks in HIF-1α levels. The induction of HIF-1α in kidney and liver is permanent only for 3 hours, then induction is disappeared. Additionally, HIF-1α is induced in more serious hypoxia in the liver and kidney (17). The HIF-1α level in kidney was higher in control + AdM group in this study. It is striking that AdM increased HIF-1α in control group independently from hypoxia.

Studies showed that HIF-1α induces AdM in hypoxic conditions to provide vascularization. Furthermore, AdM is overexpressed in human malignant tumors (21). AdM is elevated in several cardiovascular diseases according to the severity of the disease. Nitric oxide (NO) synthesis is activated by AdM resulting regulation of blood fluid and protective effect in ischemia/reperfusion injury and myocardial ischemia-induced arrhythmias (9).

http://dx.doi.org/10.17546/msd.578488

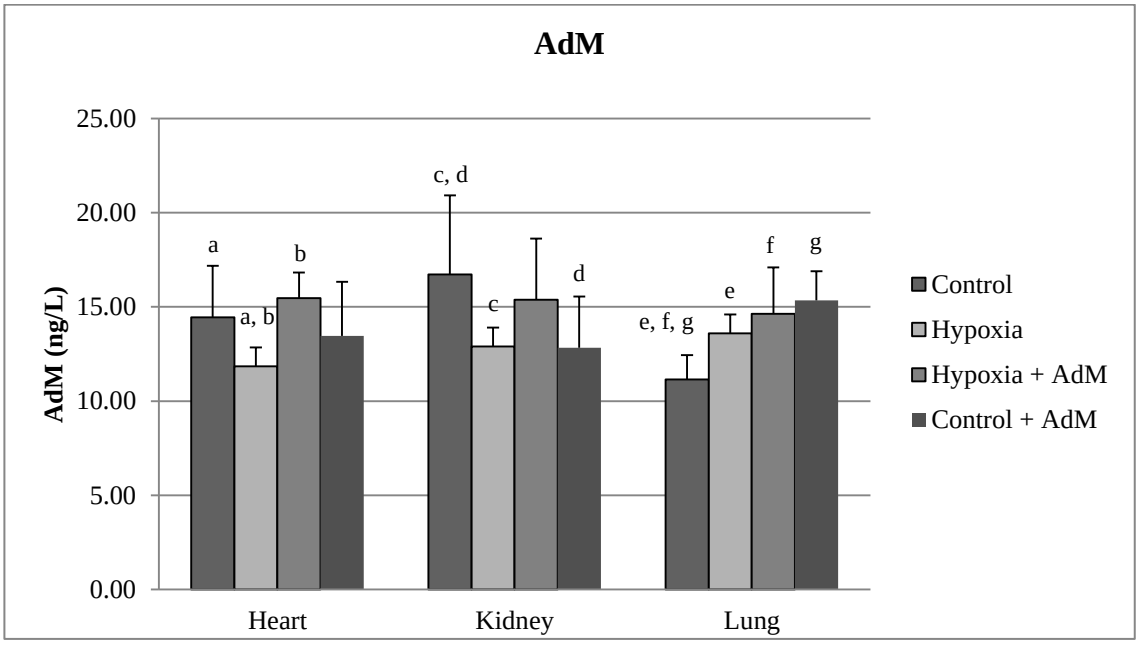

Figure 1. The differences between groups in AdM levels. Levels with same letters indicate significant differences ($p < 0.05$)

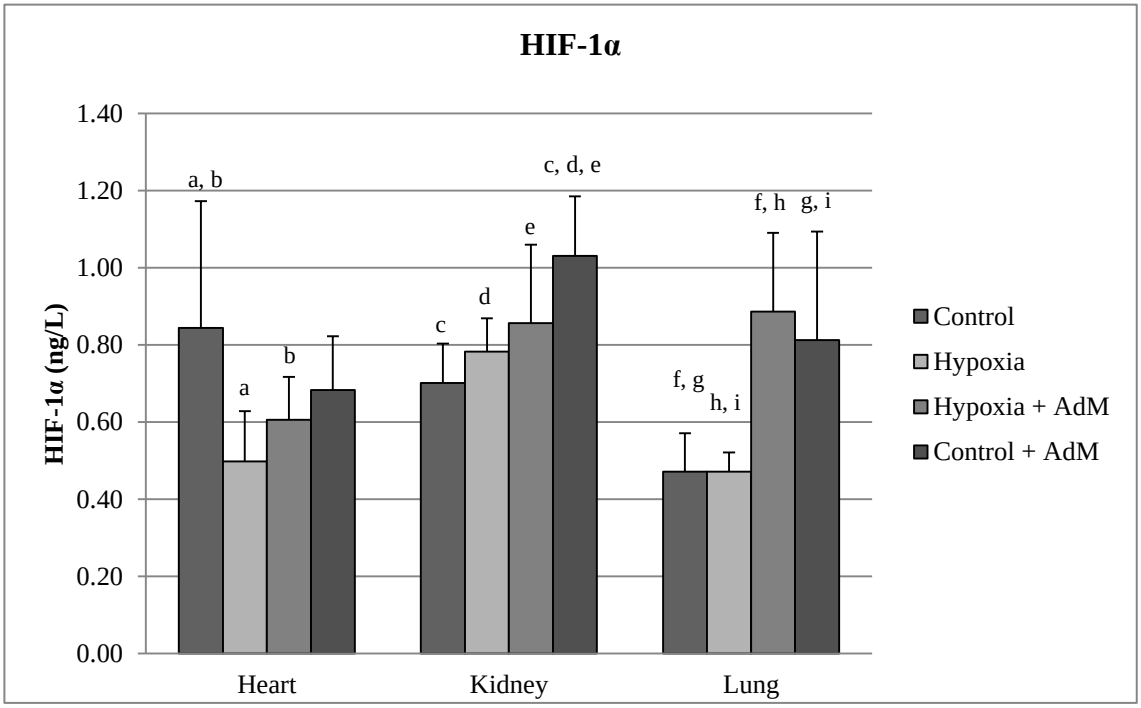

Figure 2. The differences between groups in HIF-1α levels. Levels with same letters indicate significant differences ($p < 0.05$)

In this study, hypoxia decreased AdM levels in hypoxia group in heart tissue; however, AdM treatment increased the level in hypoxia + AdM group. This increase might trigger angiogenesis for supplying enough oxygen to the cells. AdM is a controller of renal function and also have a protective effect in ischemia/reperfusion injury in the kidney (9).

AdM treatment in hypoxia + AdM group kept AdM to control levels in kidney suggesting that AdM decreased in heart and kidney after its contribution to angiogenesis or protection against hypoxia. AdM preserves pulmonary hypertension caused by high blood flow (9). AdM was increased in all groups when compared to control group in the lung in this study.

This may be the result of the sensibility of lung tissue to hypoxia, and AdM treatment. HIF-1α levels induced by hypoxia are the cell type-specific (1).

Sexual dimorphism draws attention, and the National Institutes of Health (NIH) emphasizes the necessity of researching diseases in both sexes (22). Besides, angiogenesis is crucial for females due to the female reproductive system. According to the former studies, hypoxia induces HIF-1α in kidney transiently in male (15), female rats (16), as in our study. Additionally, HIF-1α is upregulated in myocardial endothelial cells, cardiomyocytes (17), and heart in female rats (16), however, we could not detect an increase. Regarding AdM, AdM is a protective molecule against myocardial ischaemia in male rats (23). AdM is expressed averagely strong in the cortex of kidney; lower in lining epithelial cells of lung and myocardium in the male and female rats (24).

Conclusion

Angiogenesis is an important process recently as abnormal, inadequate and excessive vessels are responsible for many pathophysiological conditions including carcinogenesis. Tumor cells generate hypoxic microenvironment and then, angiogenesis is stimulated by hypoxia. Additionally, AdM is synthesized by tumor cells and its expression is stimulated by HIF-1α (9). Occlusive vascular diseases trigger a wide proportion of morbidity and death recently. The HIF pathway regulates initial responses to hypoxia (19); hence HIF may be activated therapeutically before ischemic situations for accelerating response to hypoxia (1), ameliorating ischemic vascular diseases such as atherosclerosis (19) and regulating angiogenesis to prevent vascular dysfunctions.

Promoting angiogenesis causes tumor growth; however, it would be significant in ischemic conditions. According to the results, AdM treatment may provide a rapid and continuous adjustment to hypoxia, and, AdM may be a part of therapeutic vasculogenesis. Our study showed that AdM induces HIF-1α, thereby; AdM may be used to induce HIF-1α therapeutically before ischemic conditions. We suggest that AdM could regulate HIF pathway that is a potential therapeutic target for vascular dysfunctions including neovascular eye disease, peripheral artery disease, and cancer. Further studies about the role of AdM in Notch signalling pathway would be useful before therapeutic adjustments. Additionally, the determination of VEGF that induced by AdM and HIF-1α will be a guide for the dose of AdM.

Acknowledgements: This research was funded by Inonu University, Department of Scientific Research Projects (Project No: 2012/176).

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's Contributions: **AAC, MY**; Research concept and design, Animal studies, Research the literature, preparation of the article. Chemical Analysis**. AAC;** Revision of the article.

References

1. Pugh CW, Ratcliffe PJ. Regulation of angiogenesis by hypoxia HIF system. Nat Med 2003;9(6):677–84.
2. Costa C, Soares R, Schmitt F. Angiogenesis: now and then. APMIS 2004;112(7–8):402–12.
3. Semenza GL, Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Mol Cell Biol 1992;12(12):5447–54.
4. Jelkmann W. Erythropoietin: structure, control of production, and function. Physiol Rev 1992;72(2):449–89.
5. Wenger RH. Cellular adaptation to hypoxia: O2 -sensing protein hydroxylases, hypoxia-inducible transcription factors, and O2 -regulated gene expression. FASEB J 2002;16(10):1151–62.
6. Wiesener MS, Turley H, Allen WE, Willam C, Eckardt KU, Talks KL, et al. Induction of endothelial PAS domain protein-1 by hypoxia: characterization and comparison with hypoxia-inducible factor-1alpha. Blood. 1998;92(7):2260–8.
7. Kim S, Kim J, Lee S, Park J. Adrenomedullin protects against hypoxia / reoxygenation-induced cell death by suppression of reactive oxygen species via thiol redox systems. FEBS Lett 2010;584(1):213–8.
8. Kitamura K, Kangawa K, Ichiki Y, Nakamura S, Matsuo H, Eto T. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. Biochem Biophys Res Commun 1993;192:553–60.
9. Larráyoz IM, Martínez-herrero S, García-sanmartín J, Ochoa-callejero L, Martínez A. Adrenomedullin and tumour microenvironment. J Transl Med 2014;12(339):1–15.
10. Park SC, Yoon J-H, Lee J-H, Yu SJ, Myung SJ, Kim W, et al. Hypoxia-inducible adrenomedullin accelerates hepatocellular carcinoma cell growth. Cancer Lett 2008;271(2):314–22.
11. Hinson JP, Kapas S, Smith DM. Adrenomedullin, a Multifunctional Regulatory Peptide. Endocr Rev. 2000;21(2):138–67.
12. Karpinich NO, Hoopes SL, Kechele DO, Lenhart PM, Caron KM. Adrenomedullin Function in Vascular Endothelial Cells: Insights from Genetic Mouse Models. Curr Hypertens Rev 2011;7(4):228–39.
13. Meyrick B, Miller J, Reid L. Pulmonary oedema induced by antu, or by high or low oxygen concentrations in rat - an electron microscopic study. Bri J exp Path 1972;53(4):347–58.
14. Chunyu Z, Junbao D, Dingfang B, Hui Y, Xiuying T, Chaoshu T. The regulatory effect of hydrogen sulfide on hypoxic pulmonary hypertension in rats. Biochem Biophys Res Commun 2003;302(4):810–6.
15. Rosenberger C, Mandriota S, Jürgensen JS, Wiesener MS, Hörstrup JH, Frei U, et al. Expression of hypoxia-inducible factor-1 alpha and -2 alpha in hypoxic and ischemic rat kidneys. J Am Soc Nephrol 2002;13(7):1721–32.
16. Stroka DM, Burkhardt T, Desbaillets I, Wenger RH, Neil DAH, Bauer C, et al. HIF-1 is expressed in normoxic tissue and displays an organ-specific regulation under systemic hypoxia. FASEB J 2001;15:2445–53.
17. Wiesener MS, Jürgensen JS, Rosenberger C, Scholze C, Hörstrup JH, Warnecke C, et al. Widespread hypoxia-inducible expression of HIF-2α in distinct cell populations of different organs. FASEB J 2003;17(1):271–3.

18. Carreau A, Hafny-rahbi B El, Matejuk A, Grillon C, Kieda C. Why is the partial oxygen pressure of human tissues a crucial parameter ? Small molecules and hypoxia Imaging of hypoxic areas. J Cell Mol Med 2011;15(6):1239–53.

19. Krock BL, Skuli N, Simon MC. Hypoxia-Induced Angiogenesis : Good and Evil. Genes Cancer 2011;2(12):1117–33.

20. Jin KL, Mao XO, Nagayama T, Goldsmith PC, Greenberg DA. Induction of vascular endothelial growth factor and hypoxia-inducible factor-1alpha by global ischemia in rat brain. Neuroscience 2000;99(3):577–85.

21. Oehler MK, Fischer DC, Orlowska-Volk M, Herrle F, Kieback DG, Rees MCP, et al. Tissue and plasma expression of the angiogenic peptide adrenomedullin in breast cancer. Br J Cancer 2003;89:1927–33.

22. Palmer BF, Clegg DJ. The sexual dimorphism of obesity. Mol Cell Endocrinol 2015;402:113–9.

23. Looi YH, Kane KA, McPhaden AR, Wainwright CL. Adrenomedullin acts via nitric oxide and peroxynitrite to protect against myocardial ischaemia-induced arrhythmias in anaesthetized rats. Br J Pharmacol 2006;148:599–609.

24. Cameron VA, Fleming AM. Novel Sites of Adrenomedullin Gene Expression in Mouse and Rat Tissues. Endocrinology 1998;139(5):2253–64.