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Brief communication (Original)

Adrenomedullin administration alters vascular endothelial growth factor levels in rats in cold stress

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Background: Many endogenous peptides play important regulatory roles in angiogenesis by modulating endothelial cell behavior. Adrenomedullin (AdM) is one of such factors. Angiogenesis and vascular endothelial growth factor (VEGF) are indistinguishable. Exposure to cold environment stimulates capillary angiogenesis.
Objectives: Examine the effect of the bioactive peptide AdM on VEGF levels in rat liver, lung, brain, and heart tissues after cold stress treatment.
Methods: Male wistar rats were divided into four groups as control, AdM treatment, cold stress and AdM+cold stress treated groups. In AdM-treated group, animals received intraperitoneal injection of AdM (2000 ng/kg body weight) once a day during a week. For the cold stress exposure, the rats were kept in separate cages at 10°C for a week.
Results: The administration of AdM increased VEGF levels in all tissues in cold exposed rats.
Conclusion: AdM may be a major regulatory factor in angiogenesis by modulating VEGF levels that is closely associated with cold exposure-related metabolic stimulation.

Keywords: Adrenomedullin, angiogenesis, cold stress, rat, vascular endothelial growth factor (VEGF)

Angiogenesis, or the formation of new blood vessels from pre-existing ones [1], plays a pivotal role during embrional development and later, in adult life, in a variety of physiological and pathological conditions, such as malignancy and chronic inflammation [2]. Diseased or injured cells in response to genetic alterations, hypoxia, hypoglycemia, mechanical stress, and/or inflammatory proteins, release pro-angiogenic growth factors such as vascular endothelial growth factor (VEGF) into the surrounding tissue. The released growth factors bind to endothelial cells nearby blood vessels and stimulate their proliferation [3]. The angiogenic cascade is a complex multistep process [4]. It includes sequential basement membrane degradation, endothelial cell (EC) migration and proliferation, and self-organization of the endothelial cells to form a capillary lumen. It is followed by inhibition of EC proliferation and stabilization of the new vessel by basement membrane reconstitution and recruitment of periendothelial cells. Modulation of any one of these steps would affect the formation of new vessels [5].

EC properties are regulated by many trophic factors [6], such as fibroblast growth factor-2 (FGF-2) and transforming growth factors (TGFs). However, VEGF is the major endogenous regulator [7] of EC proliferation, migration, and differentiation. Angiogenesis and VEGF are indistinguishable. VEGF is the potent and critical vascular regulator required to initiate the formation of immature vessels by angiogenic sprouting. Inappropriate induction of VEGF, in the absence of the entire angiogenic program, leads to formation of immature and leaky vessels that cause disease [8-10]. VEGF acts as a survival factor for VEGF, thereby protecting tumor cells from apoptosis and/or necrosis [11].

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Exposure to cold environment and/or hypoxia induces an increase in wall tension and shear stress in capillary, and stimulates capillary angiogenesis [12]. An increase in the number and density of capillaries may help improve the oxygen supply to muscle tissues during cold exposure [13-17].

In recent years, evidence has accumulated to indicate that many endogenous peptides play important regulatory roles in angiogenesis by modulating endothelial cell behavior. Adrenomedullin (AdM) is one of such factors [18]. It was shown that AdM induced proliferation, migration, and tube formation in ECs in vitro. It was also confirmed that AdM induced sprouting by ECs. AdM promoted endothelial DNA synthesis, migration, and tube formation, which are the essential steps for angiogenesis. Furthermore, AdM promoted sprouting in vitro and neovessel formation in vivo in gel plugs. Thus, it was a demonstrated novel biological function of AdM in angiogenesis [19].

Truly, cold stress may stimulate capillary angiogenesis [12]. However, the mechanism of angiogenesis due to cold stress is still unknown. Furthermore, there is no data available on the effects of prolonged cold stress on the VEGF levels in liver, lung, brain, and heart tissues. In the present study, we examined whether AdM affects the levels of VEGF in cold stress-exposed rats.

Materials and methods

Treatment of animals

Rats were obtained from Experimental Animal Research Facility, Inonu University. Twenty-four male Wistar albino rats (eight months old, 190-240 g) were housed individually under diurnal lighting conditions (12-12 hour) with free access to drinking water and a standard pellet diet. The environment in the animal rooms was maintained at a temperature of 22±2°C, relative humidity of 45±5%. The rats were divided into four experimental groups: control group (n = 6), adrenomedullin group (AdM) (n = 6), cold stress group (n = 6), and cold stress+adrenomedullin group (AdM+cold stress) (n = 6). The AdM-treated groups received an *i.p.* injection of AdM (2000 ng/g body weight) for a week. In cold stress treatment group, animals were exposed +10°C cold during a week. In cold stress+AdM treatment groups, both animals were exposed +10°C cold during a week and were given AdM intraperitoneally at a single dose of 2000 ng/g body weight for a week.

Experimental design

At the end of the experiment, the rats were anaesthetized by intraperitoneal injection of ketamine/xylazine hydrochloride (20/2 mg kg body weight), and the liver was removed immediately. Liver, lung, brain and heart tissues were homogenized (PCV Kinematica Status Homogenizator, Littau-Luzern, Switzerland; Bronson sonifier 450, Danburg, USA) in ice-cold phosphate buffered saline (pH 7.4). The homogenate was sonified with an ultrasonifier (Bronson sonifier 450) using six cycles (20 second sonications and 40 second pause on ice). The homogenate was centrifuged (15000 g, 10 minutes, 4°C), and the supernatant was subjected to enzyme assays immediately.

The animal experiments were performed in accordance with the guidelines for animal Research from the National Institute of Health and were approved by the Committee of Animal Research at Inonu University, Malatya.

ELISA measurement

VEGF levels were assayed by an enzyme-linked immunosorbent assay (ELISA) kit (The Cayman Chemical VEGF Assay kit, Michigan, USA).

Statistical analysis

Statistical analysis was carried out using the SPSS 12.0 statistical program. All data were expressed as mean±standard errors (SE). Duncan test was used to determine the differences between the groups having two parts. One-way analysis of variance was used to determine the differences between the groups having more than two parts.

Results

In the cold stress group, the effects of cold stress on VEGF levels were investigated in each tissue, which are summarized in **Table 1**.

VEGF levels significantly increased in lung and brain tissues ($p > 0.05$) but decreased in liver and heart tissues, compared to the control ($p < 0.05$). Depending on AdM treatment, VEGF levels increased in liver ($p < 0.05$) and lung ($p > 0.05$) tissues but decreased in brain and heart tissue, compared to the control ($p < 0.05$). In AdM+cold stress group, VEGF levels increased in all tissues compared to control ($p < 0.05$). Interestingly, AdM application in addition to cold stress increase VEGF levels in liver, lung, brain, and heart tissues, compared to AdM treatment group ($p < 0.05$).

Table 1. The effect of AdM treatment and cold stress on VEGF levels in rat liver, lung, brain, and heart tissues.

Groups	VEGF Levels (pg/mL)			
	Liver	Lung	Brain	Heart
Control	326±12,81^{bc}	259±17,13^{c}	750±47,3^{a}	429±42,95^{b}
AdM	392±3,65^{b}	325±18,46^{c}	458±37,98^{b}	332±18,13^{c}
Stress	219±20,6^{c}	327±27,08^{c}	888±73,3^{a}	369±11,98^{bc}
Stress+AdM	536±46,42^{a}	649±34,19^{b}	775±17,86^{a}	583±13,77^{a}

*Values shown are mean±SE for six replicate experiments. Different letters (a,b, and c) in each column indicate significant difference between groups according to Duncan's Multiple Range Test, ($p < 0.05$)

Discussion

The increased shear stress associated with elevated flow and blood viscosity in the cold, as indicated by the reduction in capillary blood transit times, has been shown to be a powerful stimulus for angiogenesis [20]. Possibly, arterialization of pre-existing and newly sprouted venular capillaries may be facilitated by chronic cold exposure, as exposure to cold environment induces an increase in wall tension and shear stress in capillary and stimulates capillary angiogenesis [12]. An increase in the number and density of capillaries may improve the oxygen supply to tissues during cold exposure [13-17].

Deveci et al. [21] suggested that cold exposure differentially stimulates angiogenesis in glycolytic and oxidative muscles of rats and hamsters. Kim et al. [19] showed an increase in the expression of VEGF mRNAs after prolonged cold acclimatization. Gao et al. [22] reported that cardiac cells were hypertrophied, the total capillary density was increased, and the diffusion area was reduced along the capillary path from the arteriolar to venular capillary portions in rats when reared at 5 C for 68 generations. All the changes suggest that the hypertrophy in cardiac cells is accompanied by the improvements of oxygen delivery capacity after cold adaptation.

It was hypothesized that AdM plays an important role in the vascular remodeling by affecting the extracellular matrix turnover [18]. Guidolin et al. [23] suggested that the angiogenic actions of AdM appeared independent of VEGF secretion and AdM might act directly through up-regulation of VEGF. AdM treatment did not alter the secretion of VEGF and block antibodies to VEGF. Furthermore, AdM treatment did not significantly inhibit AdM-induced in vitro capillary-like tube formation by human umbilical vein ECs.

It has been reported that specific conditions (such as hypoxia) associated to increased VEGF expression and angiogenesis stimulate AdM synthesis and secretion leading to a strong increase of the peptide concentration in the cell microenvironment [23].

The present results showed that the administriation of AdM increases VEGF levels in liver and lung tissues but decreases VEGF levels in the brain and heart in cold exposed rats.

In conclusion, AdM treatment alters the secretion of VEGF in rat liver, brain, and heart in cold exposed rats, but no changes were found in lung tissue. VEGF may be a major regulatory factor of angiogenesis that is closely associated with cold-exposure-related metabolic stimulation. Further studies are needed to understand the exact role of AdM in response to cold exposure.

The authors have no conflict of interest to report.

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