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Angiogenic Role of Adrenomedullin through Activation of Akt, Mitogen-activated Protein Kinase and Focal Adhesion Kinase in Endothelial Cells

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Purpose: It is known that AM has important roles in tumor angiogenesis, vascular development, and in angiogenesis of the female reproductive system. However, the signaling pathway used by AM in vascular angiogenesis has not been reported. In this report, we investigated the angiogenic effect of AM on endothelial cells and the possible mechanisms of AM-induced angiogenesis in vascular endothelial cells.

Materiala and Methods: The porcine pulmonary artery endothelial cells (PPAECs) and human umbilical vein endothelial cells (HUVECs) were isolated using methods previously described. The DNA synthetic activity of HUVECs was measured by a [^{3}H] thymidine incorporation assay. The migration assay with HUVECs was performed using a modified Boyden chamber. The sprouting assay with PPAECs was performed. *In vitro* tube formation was performed in three-dimensional cultures of HUVECs on gel matrices with rat tail collagen (type I, Roche Molecular Biochemicals) at a final concentration of 1.75 mg/ml. A Matrigel plug assay was performed as previously described. In brief, C57/BL6 mice were injected subcutaneously with 0.5 ml of Matrigel with heparin (50 units/ml) that contained control buffer, AM, or AM plus wortmannin (30 nmol/L) and PD98059 (2 μmol/L). Data are expressed as mean±standard deviation (SD). Statistical significance was tested using 1-way ANOVA followed by the Student-Newman-Keuls test. Statistical significance was set at $p<0.05$.

Results: In this report, we demonstrate that AM induces angiogenesis through intracellular Akt, MAPK, and p125^{FAK} activation in endothelial cells. Our Western blot analyses indicated that AM induced phosphorylation of Akt and ERK in HUVECs, and AM fragment AM_{22-52} partially suppressed AM-induced phosphorylation of Akt and ERK. Therefore, AM-induced activation of Akt and ERK could occur, at least in part, through an AM_{22-52}-sensitive receptor. Our Western blot data also suggest that there is no crosstalk between AM-induced Akt and ERK pathways. Thus, AM mediates phosphorylation of Akt and ERK using distinct signaling pathways. Using thin-layer chromatography, we also found that AM (10^{-8} mol/L) caused maximal activation of PI 3′-kinase activity within 3 to 5 min in HUVECs. Thus, AM-induced Atk activation can be mediated through activation of PI 3′-kinase. Our results showed that AM induced proliferation, migration, and tube formation in endothelial cells *in vitro*. We also confirmed that AM induced sprouting from endothelial cells. Furthermore, we demonstrated that AM-induced sprout formation was mediated through PI 3′-kinase and MEK1/2 pathway. In this study, we found that AM induces tyrosine phosphorylation of p125^{FAK} in a time- and concentration-dependent manner in endothelial cells. Next, we found that the PI 3′-kinase inhibitors completely inhibit AM-stimulated tyrosine phosphorylation of p125^{FAK} in HUVECs. These results suggest that PI 3′-kinase is part of the signal transduction pathway inducing the tyrosine phosphorylation of p125^{FAK}. As p125FAK has been implicated in migration, all of these results suggest that the migratory effect of AM in endothelial cells may be mediated through AM-induced tyrosine-phosphorylated p125^{FAK} by a PI 3′-kinase dependent pathway. We confirmed the angiogenic activity of AM by performing an in vivo mouse Matrigel plug assay. AM promoted neovessel formation in the Matrigel plug, and we noted that these newly synthesized vessels participated actively in the circulation of blood cells. Moreover, our pharmacological inhibition study indicated that the activation of MAPK and Akt by AM is crucial in endothelial tube formation and neovessel formation in the Matrigel plug. These results suggest that the activities and pathways of AM found in our in vitro experiments are likely to be valid *in vivo* as well.

Conclusion: In this study, we demonstrated that AM can be a new angiogenic factor and it's novel signaling is through activation of Akt, MAPK, and focal adhesion kinase in endothelial cells.