Organ-Protective Effects of Adrenomedullin

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Adrenomedullin (AM), a vasodilatory peptide, has recently been shown to have multipotent properties. Among its other pharmacological actions, AM has been hypothesized to protect organs from hypertension, hypoxia, or infection. In vitro studies have shown that AM has an inhibitory effect on vascular smooth muscle cell proliferation and oxidative stress, but that it enhances nitric oxide (NO) production, which in turn is thought to protect against organ damage. Recent advances in genetic engineering have made it possible to investigate the chronic effects of AM in vivo. Applying genetic engineering, it is revealed that adrenomedullin was shown to protect liver, kidney, vasculature, and heart from septic shock, ischemia and hypertension. However, speculation as to the mechanism of its organ-protective effect varies from report to report. Possible mechanisms include preservation of blood flow, interaction with NO and/or oxidative stress. And although there continue to be technical limitations to the use of these genetically modified models, their application in further investigations should help to clarify the potential efficacy of AM as a new therapeutic agent.


Key Words: oxidative stress, nitric oxide, knockout mouse

Introduction

Adrenomedullin (AM), a potent vasodilator, was originally isolated from pheochromocytoma cells, is produced in and secreted by vascular endothelial cells (1). The DNA sequence encoding the precursor of AM, proadrenomedullin, has been identified and the first paired basic amino acid of this precursor (Lys43-Arg44) is a representative site for proteolytic cleavage, yielding proadrenomedullin N-terminal 20 peptide (PAMP) (2), a compound showing physiological effects different from those of AM (3-5). Several studies have revealed that AM is a multipotent peptide (6-9) and a new tool for use in diagnosis of extracellular volume in renal insufficiency (10). However, technical limitations have restricted the study of the physiological relevance of AM and PAMP. The two available antagonists calcitonin gene-related peptide (CGRP) (8-37) and AM 22-52, must be administered systemically at very high doses in order to study the chronic effects of AM and PAMP. Passive immunoneutralization or antisense oligonucleotides (11, 12) have also been tried, but concerns remain as to their specificity and duration of action. To overcome these limitations, gene-delivery, gene-transfer, gene-disruption and ribozyme treatments have been reported (13). In this review, we focus on the organ-protective effects of AM and their mechanisms, as revealed in studies using these new techniques and gene-manipulated mouse models of AM and AM/PAMP.

Generating AM/PAMP Transgenic Mice and Knockout Mice

As mentioned above, AM and PAMP share the same propeptide and signal peptide and thus are encoded on one gene (Fig. 1). This construction makes it difficult to separate the AM transgene from the PAMP transgene. Our preliminary studies showed that direct conjugation of the original signal peptide and AM peptide sequences was not sufficient to induce secretion of AM from any of the cell types studied. Therefore, gene delivery models (14-17) and AM transgenic...
mice (18) induced both AM and PAMP at the same time. The transgenic mice were applied endothelin promoter to express AM/PAMP only in endothelial cells. AM is normally expressed not only in endothelial cells but also in vascular smooth muscle cells, endocrine organs and kidney interstitial cells. In order to clarify the physiological effects of AM, other promoters could be applied and other AM transgenic mice models could be developed.

As for disruption of AM, both gene-knockout and ribozyme methods have been reported. Three independent groups reported the development of AM or AM/PAMP knockout mice (19–21). In two of these groups, AM/PAMP knockout mice were generated by replacing one exon with a neomycin (NEO)-resistant gene (20, 21), whereas in our previous study, AM knockout mice were generated by inserting a stop mutation at the beginning of the AM-coding region (19). Our preliminary studies and later studies with AM knockout mice revealed that our mutation did not affect the expression of PAMP, which was secreted to the same degree as in the wild type.

The major limitation of the use of AM knockout mice is that they do not survive the embryo stage (22). For this reason, Taylor and Samson administered ribozyme to mice in an attempt to block AM synthesis genetically (13). Because

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**Fig. 1.** AM locus and targeting strategies. Restriction map of the mouse AM locus (top), the targeting vector (middle) and the mutation-containing locus following homologous recombination (bottom). Filled boxes indicate exons and filled triangles indicate lox-P sequences. A stop mutation was inserted at the beginning of the AM-coding region at exon 4. The restriction fragments detected by probes A and NEO are indicated by bars. Arrowheads represent the primers used in PCR screening. E, EcoRI; Rv, EcoRV; S, SalI; A, ApaI; H, HindIII; K, KpnI; N, NotI.

**Fig. 2.** AM knockout and wild littermate fetuses. A fetus in a yolk sac (a) and a fetus without a yolk sac (b) are shown. No apparent differences were observed either on yolk sac or in E 14.5 fetus.
AM and PAMP are formed by the processing of a preprohormone, this method is not specific to AM, but rather is equally disruptive to both AM and PAMP. Further, these authors limited their study of AM disruption to its effects on water intake, and did not investigate organ protection.

**Fetus Growth**

AM/PAMP knockout mice and AM knockout mice revealed embryonic lethal. The cause of this early mortality varies from report to report. Caron and Smithies reported that AM/PAMP knockout mice are hydrops fetalis, and they assume that lymphatic circulation plays a role in their early mortality (21). On the other hand, Shindo et al. also generated AM/PAMP knockout mice, and their mice had poor vascular formation in the yolk sac, and the fetuses showed massive hemorrhage with abnormal basement membrane of vessels (20). Our AM knockout mice were also embryonically lethal, but no hydrops fetalis or hemorrhage was found (Fig. 2). In addition, histological examination throughout the body showed no apparent abnormalities in homozygotes. The precise mechanism of this early mortality remains unclear, although it has been demonstrated that exogenous supplementation of AM fails to rescue the fetus, and thus AM is likely to play an essential role in gestation.

**Regulation of Blood Flow and Organ Protection**

Gene delivery to spontaneously hypertensive rats, deoxycorticosterone acetate (DOCA)-salt rats or Dahl salt-sensitive rats has shown that AM decreases blood pressure concomitant with an increased level of cAMP. As a result of the decrement of blood pressure, renal and cardiovascular damage are also attenuated (14–17). These findings suggest that the direct vasodilatory action of AM plays a key role in the hypotensive effect, thereby leading to the protection of organs from hypertension.

In addition to its direct action, AM has been reported to indirectly protect organs from several stressors. A septic shock model of AM transgenic mice has revealed that AM preserves liver function after septic shock, and that this effect is canceled by blockade of nitric oxide (NO) synthesis (20). Also, ischemic damages in the kidney have been shown to be protected in AM transgenic mice and deteriorated in AM/PAMP knockout mice (23). In this model, too, the blockade of NO synthesis canceled the effect of AM. Based on these observations, we speculate that AM protects organs by preserving blood flow via an increase in NO synthesis. This speculation is supported by studies in which inhibition of guanylate cyclase attenuated AM-induced vasodilation in rat aorta (24) or blockade of NO synthesis attenuated AM-induced increment of renal blood flow (25).

**Antioxidant Effect and Organ Protection**

Recent studies have shown that oxidative stress is one cause of organ damage in a variety of pathological conditions. In particular, ischemia-reperfusion, hypertension, or diabetes increases oxidative stress and induces vascular damage, renal damage or other organ damages. AM expression is increased by oxidative stress (6, 26). At the same time, AM inhibits oxidative stress (27). These observations lead us to hypothesize that AM is an intrinsic antioxidant. To clarify this hypothesis, we administered angiotensin II and a salt diet—both of which was known to increase oxidative stress—to AM knockout mice. Following this treatment, we showed that AM knockout mice had higher oxidative stress in three ways: by measuring urinary excretion of oxidative stress markers such as isoprostane and 8-hydroxydeoxyguanosine (8-OHdG), by immunostaining of 3-nitrotyrosine to localize oxidative stress, and by measuring oxidant production in real-time using the electron spin resonance method (19). AM content in heterozygotes was half of that in the wild-type mice, and the increased oxidative stress was reduced by supplementation with AM. The blood pressure were comparable between the wild-type and AM-deficient mice, but the fibrotic changes and occlusion of the coronary artery were more apparent in AM-deficient than in wild-type mice. This change was reversed by 4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPO), a superoxide dismutase mimetic (28). From this study, it was revealed that the AM peptide conferred protection against organ damage via an inhibition of oxidative stress.

Collectively, the above studies suggest that AM protects organs in the presence of various conditions by at least two mechanisms, inhibition of oxidative stress and blood-flow regulation through an increase in NO production.

**Future Directions**

Gene knockout models of AM/PAMP are intrinsically limited by the structure of AM and PAMP themselves. In order to clarify the effect of AM, we can target specific receptors and their modifying protein for AM. As for PAMP, the specific receptors have not yet been identified. On the other hand, we can generate PAMP transgenic mice. A few studies have shown that PAMP modifies sympathetic nerve tone and regulates blood pressure. The physiological effect is totally different from that of AM, and thus PAMP transgenic mice have the potential to reveal new information for both therapy and diagnosis.

**References**


