Amino Acids (2007) 32: 157–164
DOI 10.1007/s00726-005-0279-5
Printed in The Netherlands

Amino Acids

Adrenomedullin: molecular mechanisms and its role in cardiac disease

Review Article

B. Yanagawa and **N. Nagaya**

Department of Regenerative Medicine and Tissue Engineering, National Cardiovascular Center Research Institute, Osaka, Japan

Received August 24, 2005
Accepted December 3, 2005
Published online April 4, 2006;

Summary. Adrenomedullin (AM) is a potent, long-lasting vasoactive peptide originally isolated from human pheochromocytoma. Since its discovery, serum and tissue AM expression have been shown to be increased in experimental models and in patients with cardiac hypertrophy, myocardial infarction and end-stage heart failure with several beneficial effects. Considerable evidence exists for a wide range of autocrine, paracrine and endocrine mechanisms for AM which include vasodilatory, anti-apoptotic, angiogenic, anti-fibrotic, natriuretic, diuretic and positive inotropic. Thus, through regulation of body fluid or direct cardiac mechanisms, AM has additive and beneficial effects in the context of heart disease. Notable molecular mechanisms of AM include cyclic adenosine monophosphate, guanosine-3′,5′-monophosphate, PI3K/Akt and MAPK-ERK-mediated cascades. Given the endogenous and multifunctional nature of AM, we consider this molecule to have great potential in the treatment of cardiovascular diseases. In agreement, early experimental and preliminary clinical studies suggest that AM is a new and promising therapy for cardiovascular diseases.

Keywords: Adrenomedullin – Heart disease – Signaling

Introduction

Adrenomedullin (AM) is a member of the calcitonin gene-related peptide (CGRP) family, which was originally discovered in human adrenal medulla as a hypotensive factor produced by pheochromocytoma cells (Kitamura, 1993). This molecule is synthesized as an immature 53-amino acid precursor and modified by amidation into a mature 52-amino-acid peptide with an intramolecular disulfide bond, sharing slight homology with CGRP and amylin. Since its discovery, this molecule has subsequently been found in plasma and in a variety of tissues including the vasculature, lungs, heart and adipose tissue (Ichiki, 1994; Saito, 1987; Fukai, 2004). In the heart, AM is present in ventricular tissue and is particularly abundant in rat atria (Ichiki, 1994; Sakata, 1994). Although mainly produced by vascular endothelial cells, vascular smooth muscle cells and macrophages, AM can also be produced by fibroblasts, adipocytes and cardiac myocytes (Sugo, 1994, 1994a; Kubo, 1998). Earlier studies have shown that plasma AM levels are increased in patients with heart failure (Nishikimi, 1995). Since then, increased AM has been detected in hearts of patients with acute myocardial infarction, hypertension, chronic heart failure and pulmonary hypertension.

In this review, although we will refer to studies involving AM signaling in various cell types and diseases settings, our focus on the role of AM in cardiovascular illness. Certainly, the critical importance of AM in cardiovascular development is highlighted by the severe tissue edema and cardiac abnormalities seen in AM-null mice (Caron, 2001). Here, we review the current literature regarding the molecular mechanisms of AM, investigate its role in cardiovascular disease and look ahead to the potential of AM in the treatment of heart disease.

Signaling

Since its discovery, the role of AM in heart failure and its molecular mechanisms of action have been an intense area of study. Signaling studies of AM have largely been performed in endothelial and smooth muscle cell types with calcium- and cAMP-mediated cascades having been identified as the predominant mechanisms of AM action, respectively. Here, we will review the AM receptors and AM signaling pathways including cyclic adenosine 3′,5′-monophosphate (cAMP) and calcium, cyclic guanosine 3′,5′-monophosphate (cGMP), phosphatidylinositol

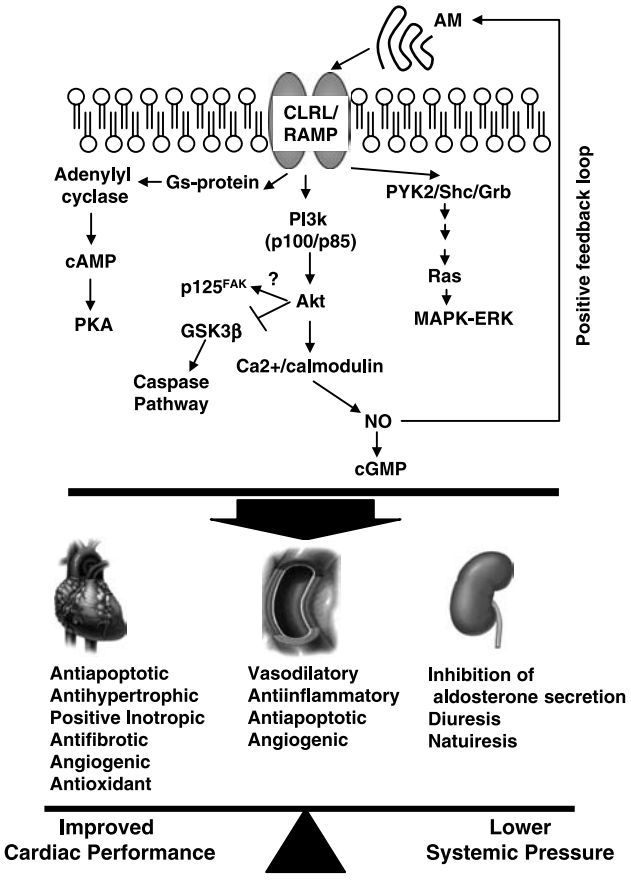

Fig. 1. Adrenomedullin (AM) signaling pathways and downstream consequences. Activation of three main signaling pathways has been identified thus far: cyclic adenosine monophosphate (cAMP), phosphoinositide 3-kinase (PI3K)/Akt and mitogen activated protein kinase (MAPK)-extracellular signal-regulated protein kinase (ERK). The downstream physiological effects are seen primarily in the vasculature, kidneys and heart, which results ultimately in a balance between improved cardiac performance and lower systemic blood pressure

3-kinase (PI3K)/Akt, and MAPK-ERK (Iwasaki, 1998, 2001; Shimekake, 1995) as summarized in Fig. 1.

The actions of AM are mediated by the 7 transmembrane G-protein-coupled calcitonin receptor-like receptor (CRLR), which co-assembles with subtypes 2 and 3 of a family of receptor-activity-modifying proteins (RAMPs) thus forming a receptor-co-receptor system (McLatchie, 1998). In rat hearts, CRLR and RAMP2 expression influences AM intracellular signaling, strongly suggesting that these are the functional receptors (Autelitano, 2001). The co-receptors RAMP2 and RAMP3 modify the effects of AM on CRLR, a G-protien-coupled receptor. Interestingly, AM and CRLR are both up-regulated under hypoxic conditions in microvascular endothelial cells (Kinitenko, 2003). The promoter for CRLR is at least partially regulated by HIF-1, which has also been shown to regulate the expression of AM under hypoxic conditions. Therefore, CRLR shares a common transcriptional mechanism with AM.

The PI3K and downstream serine-threonine kinase Akt (or protein kinase B) pathway is a well-characterized protective signaling program (Matsui, 1999; Fujio, 2000). AM has a potent protective, anti-apoptotic role through the PI3K/Akt pathway (Kim, 2002). Adenovirus-mediated gene delivery of AM has been shown to protect the myocardium from apoptosis in a rat model of myocardial infarction whereas this effect was blocked in dominant-negative Akt mice (Yin, 2004). GSK-3β is a downstream protein kinase of Akt, which when phorphorylated, causes inactivation and reduced caspase signaling. In hypoxic and reoxygenated cardiomyocytes, the AM-mediated antiapoptotic effect is associated with increased GSK-3β signaling. The angiogenic effect of AM is mediated by activation of Akt as well as mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) 1/2 and focal adhesion kinase (FAK) in endothelial cells (Kim, 2003).

The MAPK-ERK is also a well-characterized stress-induced protective signaling program whose expression is triggered in heart injury. In particular, ERK is part of an antiapoptotic and compensatory hypertrophic program (Baines, 2005). AM triggers smooth muscle cell proliferation at least in part via ERK (Shichiri, 2003). Following receptor binding in smooth muscle cells, AM triggers rapid ERK activation which likely limits acute myocardial injury (Iwasaki, 1998).

Binding of AM to CRLR can activate Gs, trigger cAMP accumulation and activate cyclic AMP-dependent protein kinase. cAMP/protein kinase in smooth muscle cells (SMCs) regulates the vasodilatory effects of AM (Ishizaka, 1994). AM strongly increases cAMP and Ca^{2+} concentration in bovine aortic endothelial cells (Shimekake, 1995) and it is 10-times more potent than CGRP in increasing the cAMP level in rat vascular SMC (Eguchi, 1994). In endothelial cells, vasodilation predominantly occurs by an eNOS/NO pathway (Hirata, 1995). Nishimatsu et al. (2001) have shown that AM induces Akt activation in the endothelium via the Ca^{2+}/calmodulin-dependent pathway. This is implicated in the production of nitric oxide, which in turn induced endothelium-dependent vasodilation. The increase in cAMP in SMCs by AM activates protein kinase A, resulting in decreased calcium content in SMCs (Ishizaka, 1994). The unique combination of the above signaling mechanisms results in a number of *in vivo* effects such as vasodilation, anti-apoptotosis, angiogenesis, and positive inotropy. An improved understanding

of the signaling pathways whereby AM mediates its physiologic effect may bring us closer to using AM as a therapeutic agent.

Adrenomedullin and the cardiovascular system

It is clear that AM expression is upregulated in patients with both acute and chronic cardiovascular disease and that such increases are associated with multiple host protective effects. Thus far, AM has been found to be increased in patients with essential hypertension (Ishimitsu, 1994), cardiac hypertrophy (Tsuruda, 2003), heart failure (Kreminski, 2002; Jougasaki, 1995; Nishikimi, 1995), acute myocardial infarction (Kobayashi, 1996; Miyao, 1998; Nagaya, 1999a), and peripheral arterial occlusive disease (Suzuki, 2004). In acute myocardial infarction, levels of AM expressed in patients correlates with the severity of illness (Miyao, 1998; Nagaya, 1999a). Tissue levels of AM peptide and mRNA are also markedly increased in the ischemic (Nagaya, 2000; Hofbauer, 2000) and failing heart (Cueille, 2002; Nishikimi, 2003; Tadokoro, 2003; Totsune, 2000). Thus, expression of AM is up-regulated both in serum and in the target tissue.

Even in a chronic setting, AM is increased in patients with end stage heart failure, a final common pathway for cardiomyopathies and ischemic heart disease, for which the only definitive treatment is heart transplantation. Notably, AM expression, as determined by immunohistochemistry on biopsy specimens, correlates with the severity of disease (Jougasaki, 1995; Nishikimi, 1995). Furthermore, evidence from patients with acute myocardial infarction shows plasma AM to be an independent prognostic indicator for mortality, although not for hemodynamic variables (Nagaya, 1999a; Katayama, 2004). Nagaya et al. (2000a) have shown that intravenous infusion of AM markedly increases cardiac index and improves hemodynamics, renal function and hormonal parameters in patients with left sided heart failure. Therefore, increase in AM above the endogenously mediated increase is of therapeutic benefit.

Although the precise mechanism of AM upregulation is unclear, the expression of proinflammatory cytokines TNF-α and IL-1β as well as IFN-γ and NO can directly trigger increased AM expression in cultured endothelial cells, myocytes and SMCs (Hofbauer, 2002; Sugo, 1994, 1995; Ihara, 2000). Upregulation may occur via the hypoxia-inducible factor-1 (HIF-1) as observed under hypoxic conditions (Garaoya, 2000). Considering the lack of AM stores, transcriptional control is considered rather important for AM regulation. However, the precise mechanism of AM transcriptional upregulation is incompletely understood.

The potential functions of AM include vasodilator, natriuretic, diuretic, antiapoptotic and pro-survival roles (Fig. 1). Also, the angiogenic and inflammatory modulating properties of AM have been recently described. Experimental and clinical evidence for the cardiovascular effects of AM will now be examined in greater detail.

Positive inotropic effects

Considerable evidence exists for a positive inotropic effect of AM. AM increases cardiac cAMP, which is known to mediate the positive inotropic action of β-adrenergic stimulants. Szokodi et al. (1998) have shown that AM produces positive inotropic action through cAMP-independent Ca^{2+} release. A recent binding study demonstrated the presence of abundant binding sites for AM in the ventricular myocardium (Owji, 1995). Furthermore, our laboratory has demonstrated that infusion of AM markedly increases cardiac index and stroke volume index in patients with congestive heart failure (Nagaya, 2000b, 2002). Considering the strong vasodilator effect of AM, a decrease in mean arterial pressure may be partially responsible for increased cardiac index during infusion. Oya et al. (2000) reported that AM administration increased cardiac index and stroke volume index in patients. However, some reports are indicative of negative inotropy or no effect on contractility following AM treatment in cultured cardiomyocytes and *in vivo* (Ikenouchi, 1997; Perret, 1993; Stangl, 2000). In primary cultured cardiocytes, AM induced a biphasic acute increase and decrease of myocyte shortening and Ca^{2+} transients (Mittra, 2004). Thus, it is possible that timing of AM administration *in vivo* is an important determinant of pump function. The particular circumstances for a positive inotropic effect of AM remain to be determined.

Vasodilation

AM was originally characterized as a potent vasodilatory molecule (Kitamura, 1993). The vasodilatory effect of AM is mediated by cAMP (Ishizaka, 1994) and nitric oxide/cGMP-dependent mechanisms (Nakamura, 1997; Hayakawa, 1999). AM increases cAMP thus activating protein kinase A, resulting in decreased calcium content. Vasodilatation and natiuresis is NO- and cGMP-dependent as E-4021, a cGMP-specific phosphodiesterase inhibitor, blocked these effects (Hayakawa, 1999). In patients with congestive heart failure, intravenous infusion

of AM decreases mean arterial pressure but to a lesser extent than in normal patients (Nagaya, 2000). The fall in mean arterial pressure is associated with a significant increase in heart rate (Nagaya, 2000). Infusion of AM causes a greater and more prolonged reduction of mean arterial pressure than that of an equimolar amount of atrial natriuretic peptide (Oya et al., 2000). In patients with pulmonary hypertension, AM administration also decreases pulmonary vascular resistance and is a potential new treatment for this condition (Nagaya, 2004).

Diuresis and natiuresis

In vivo studies in sheep have shown that intravenously administered AM causes diuresis, in an experimental model of heart failure (Rademaker, 1997). Natriuretic activity that is characterized by increased glomerular filtration and decreased sodium reabsorption was also demonstrated in a NO-dependent manner (Elhawary, 1995; Majid, 1996). Even in normal animals, AM causes diuresis and natriuresis as well as vasodilation (Majid, 1996; Parkes, 1997). Systemically administered AM increases urine volume and urinary sodium excretion in patients with congestive heart failure, consistent with results obtained from earlier animal studies (Majid, 1996; Rademaker, 1997; Nagaya, 1999).

Inhibition of aldosterone production

The renin-angiotensin-aldosterone system is excessively activated in patients with heart failure, leading to adverse effects related to hypertension. In this setting, infusion of AM significantly and selectively decreases plasma aldosterone in patients with congestive heart failure, although there is no significant change in plasma renin (Nagaya, 2000a). *In vitro* studies showed that AM inhibits Ang II-induced secretion of aldosterone from dispersed rat adrenal zona glomerulosa cells (Yamaguchi, 1995). Thus, we speculate that AM may play a compensatory role in the pathophysiology of heart failure by inhibiting the augmented production of aldosterone thus preventing sodium retention.

Antihypertrophic, anti-apoptotic and antifibrotic effects

AM has direct cardioprotective effects in reducing ventricular remodeling following MI in rats in the absence of changes in mean arterial pressure (Nakamura, 2002). In this regard, it is a possible endogenous suppressor of myocyte hypertrophy and fibroblast proliferation (Tsuruda, 1998, 1999). AM inhibits collagen synthesis and cardiac fibroblast proliferation, and AM+/− mice have increased fibrosis compared to wildtype littermates (Nishikimi, 2005; Niu, 2004). As well, it confers an anti-apoptotic effect on cardiomyocytes via the PI3K/Akt pathway (Okumura, 2004). Inhibition of vascular endothelial cell apoptosis and induction of angiogenesis by AM also occurs through the PI3K/Akt pathway (Kim, 2002; Tokunaga, 2004). Thus, AM has protective effects in the myocardium and vasculature, which may have beneficial effects in patients with congestive heart failure.

Angiogenesis

An angiogenic effect of AM could partly explain the protective effect of AM seen following myocardial infarction. Recent studies using homozygous AM knockout mice highlight the importance of AM in vascular morphogenesis (Caron, 2001; Imai, 2001; Shindo, 2001). AM signaling is of particular significance in endothelial cell biology since the peptide protects cells from apoptosis (Kato, 1997), promotes angiogenesis (Kim, 2003; Oehler, 2002) and affects vascular tone (Ishizaki, 1994). AM activates the PI3K/Akt-dependent pathway in vascular endothelial cells which is considered to regulate multiple critical steps in angiogenesis, including endothelial cell survival, proliferation, migration, and capillary-like structure formation (Jiang, 2000; Nishimatsu, 2001). *In vitro*, AM enhances endothelial cell migration and neovessel formation, a function that is attenuated by PI3K and ERK inhibition (Kim, 2003). Similarly, AM promotes re-endothelialization in an injury model via PKA and PI3K-dependent Akt activation in HUVECs (Miyashita, 2003). Interestingly, AM inhibits the proliferation and migration of vascular SMCs (Horio, 1995; Kano, 1996).

A recent study demonstrated that heterozygous AM+/− mice show significantly less blood flow recovery with less collateral capillary development than their wildtype counterparts (Iimuro, 2004). AM gene transfer promotes blood flow recovery and capillary formation in a murine model of chronic hind limb ischemia (Tokunaga, 2004). Taken together, these findings raise the possibility that AM plays a role in modulating angiogenesis and neovascularization.

Antioxidant effects

Oxidative stress injury is induced by reactive oxygen species (ROS), the most common being free radicals and anions containing reactive oxygen atoms. Damage by

excess oxidative stress is a major metabolic abnormality in atherosclerosis as well as myocardial infarction. Oxidative stress caused by H_2O_2 in endothelial cells can trigger AM transcription and increased expression (Chun, 2000). Such expression may be protective as angiotensin II-stimulated intracellular reactive oxygen species generation was directly blocked by AM, possibly in a cAMP-dependent manner (Yoshimoto, 2005).

Pro- and anti-inflammatory effects of AM

The effects of AM on inflammation are still unclear and appear to differ based on the disease model. For instance, ocular inflammation is increased with AM whereas acetic acid-induced colonitis is attenuated (Ashizuka, 2005; Clementi, 1999). In a cultured macrophage cell line, AM increased secretion of IL-6 but slightly reduced TNF-α levels (Wong, 2005). Furthermore, AM can induce expression of the cell surface adhesion molecules E-selection, VCAM-1, and ICAM-1, critical for leukocyte migration, on cultured endothelial cells (Hagi-Pavli, 2004). However, Kim et al. (2003) showed that AM inhibits VEGF-induced expression of these adhesion molecules. Thus, it appears that AM can act to both promote and inhibit inflammation depending on the cell type and disease of interest.

Conclusion and future studies

Since its discovery, there has been great interest in AM as a promising endogenous peptide for the treatment of cardiovascular diseases. Considering that AM is an endogenous neurohormonal peptide, it may be more readily accepted as a therapeutic agent. Its vasodilatory, inotropic, antiapoptotic, natuiretic and diuretic actions afford this molecule a significant potential clinical advantage in terms of cardiac injury and vascular alterations seen in cardiac diseases. However, it is still unclear which combination of effects of AM is triggered to produce the observed beneficial effects in experimental models and in patients. Several clinical studies have tested the effect of AM in hypertension, ischemic heart disease and pulmonary hypertension. Although their data looks promising, a prospective randomized control trial is needed to reinforce the pharmaceutical prospects of AM.

The optimum timing, amount and method of AM delivery are still under investigation. Although AM has a relatively short half-life compared to other peptides, it is still vulnerable to rapid degradation by renal neural endopeptidases *in vivo*. Thus, in a clinical setting, AM is likely to have an optimal benefit in acute diseases. To address this point, Nagaya et al. (2004) have investigated the efficacy of three types of AM delivery systems: intravenous administration, inhalation, and cell-based gene transfer. Interestingly, ionically-linked DNA-gelatin complexes were able to block chronic hind limb ischemia when delivered by direct muscular injection (Tokunaga, 2004).

Despite unanswered questions regarding the precise mechanism and optimum method of delivery of AM in a clinical setting, the data thus far fully warrants the excitement felt about its potential benefit as a treatment for cardiac diseases. We greatly look forward to the prospects of clinical trials using AM in the setting of cardiovascular illnesses.

Acknowledgements

This work was supported by a postdoctoral fellowship from the Japan Society for the Promotion of Science (BY) and a research grant for Cardiovascular Disease (16C-6) from the Ministry of Health, Labor and Welfare, Japan.

References

Ashizuka S, Ishikawa N, Kato J, Yamaga J, Inatsu H, Eto T, Kitamura K (2005) Effect of adrenomedullin administration on acetic acid-induced colitis in rats. Peptides 26: 2610–2615

Autelitano DJ, Ridings R (2001) Adrenomedullin signalling in cardiomyocytes is dependent upon CRLR and RAMP2 expression. Peptides 22: 1851–1857

Baines CP, Molkentin JD (2005) STRESS signaling pathways that modulate cardiac myocyte apoptosis. J Mol Cell Cardiol 38: 47–62

Caron KM, Smithies O (2001) Extreme hydrops fetalis and cardiovascular abnormalities in mice lacking a functional adrenomedullin gene. Proc Natl Acad Sci USA 98: 615–619

Chun TH, Itoh H, Saito T, Yamahara K, Doi K, Mori Y, Ogawa Y, Yamashita J, Tanaka T, Inoue M, Masatsugu K, Sawada N, Fukunaga Y, Nakao K (2000) Oxidative stress augments secretion of endothelium-derived relaxing peptides, C-type natriuretic peptide and adrenomedullin. J Hypertension 18: 575–580

Clementi G, Caruso A, Cutuli VM, Prato A, Mangano NG, Amico-Roxas M (1999) Antiinflammatory activity of adrenomedullin in the acetic acid peritonitis in rats. Life Sci 65: 203–208

Cueille C, Pidoux E, de Vernejoul MC, Ventura-Clapier R, Garel JM (2002) Increased myocardial expression of RAMP1 and RAMP3 in rats with chronic heart failure. Biochem Biophys Res Commun 294: 340–346

Eguchi S, Hirata Y, Kano H, Sato K, Watanabe Y, Watanabe TX, Nakajima K, Sakakibara S, Marumo F (1994) Specific receptors for adrenomedullin in cultured rat vascular smooth muscle cells. FEBS Lett 340: 226–230

Elhawary AM, Poon J, Pang CC (1995) Effects of calcitonin gene-related peptide receptor antagonists on renal actions of adrenomedullin. Br J Pharmacol 115: 1133–1140

Fujio Y, Nguyen T, Wencker D, Kitsis RN, Walsh K (2000) Akt promotes survival of cardiomyocytes in vitro and protects against ischemia-reperfusion injury in mouse heart. Circulation 101: 660–667

Fukai N, Yoshimoto T, Sugiyama T, Ozawa N, Sato R, Shichiri M, Hirata Y (2004) Concominant expression of adrenomedullin and its receptor components in rat adipose tissues. Am J Physiol Endocrinol Metab 288: 56–62

Garayoa M, Martinez A, Lee S, Pio R, An WG, Neckers L, Trepel J, Montuenga LM, Ryan H, Johnson R, Gassmann M, Cuttitta F (2000) Hypoxia-inducible factor-1 (HIF-1) up-regulates adrenomedullin expression in human tumor cell lines during oxygen deprivation: A possible promotion mechanism of carcinogenesis. Mol Endocrinol 14: 848–862

Hagi-Pavli E, Farthing PM, Kapas S (2004) Stimulation of adhesion molecule expression in human endothelial cells (HUVEC) by adrenomedullin and corticotrophin. Am J Physiol Cell Physiol 286: 239–246

Hayakawa H, Hirata Y, Kakoki M, Suzuki Y, Nishimatsu H, Nagata D, Suzuki E, Kikuchi K, Nagano T, Kangawa K, Matsuo H, Sugimoto T, Omata M (1999) Role of nitric oxide-cGMP pathway in adrenomedullin-induced vasodilation in the rat. Hypertension 33: 689–693

Hirata Y, Hayakawa H, Suzuki Y, Suzuki E, Ikenouchi H, Kohmoto O, Kimura K, Kitamura K, Eto T, Kangawa K, Matsuo H, Omata M (1995) Mechanisms of adrenomedullin-induced vasodilation in the rat kidney. Hypertension 25: 790–795

Hofbauer KH, Jensen BL, Kurtz A, Sandner P (2000) Tissue hypoxygenation activates the adrenomedullin system in vivo. Am J Physiol Regul Integr Comp Physiol 278: 513–519

Horio T, Kohno M, Kano H, Ikeda M, Yasunari K, Yokokawa K, Minami M, Takeda T (1995) Adrenomedullin as a novel antimigration factor of vascular smooth muscle cells. Circ Res 77: 660–664

Ichiki Y, Kitamura K, Kangawa K, Kawamoto M, Matsuo H, Eto T (1994) Distribution and characterization of immunoreactive adrenomedullin in human tissue and plasma. FEBS Lett 338: 6–10

Ihara T, Ikeda U, Tate Y, Ishibashi S, Shimada K (2000) Positive inotropic effects of adrenomedullin on rat papillary muscle. Eur J Pharmacol 390: 167–172

Ikenouchi H, Kangawa K, Matsuo H, Hirata Y (1997) Negative inotropic effect of adrenomedullin in isolated adult rabbit cardiac ventricular myocytes. Circulation 95: 2318–2324

Iimuro S, Shindo T, Moriyama N, Amaki T, Niu P, Takeda N, Iwata H, Zhang Y, Ebihara A, Nagai R (2004) Angiogenic effects of adrenomedullin in ischemia and tumor growth. Circ Res 95: 415–423

Imai Y, Shiindo T, Maemura K, Kurihara Y, Nagai R, Kurihara H (2001) Evidence for the physiological and pathological roles of adrenomedullin from genetic engineering in mice. Ann NY Acad Sci 947: 26–33

Ishimitsu T, Nishikimi T, Saito Y, Kitamura K, Eto T, Kangawa K, Matsuo H, Omae T, Matsuoka H (1994) Plasma levels of adrenomedullin, a newly identified hypotensive peptide, in patients with hypertension and renal failure. J Clin Invest 94: 2158–2161

Ishizaka Y, Ishizaka Y, Tanaka M, Kitamura K, Kangawa K, Minamino N, Matsuo H, Eto T (1994) Adrenomedullin stimulates cyclic AMP formation in rat vascular smooth muscle cells. Biochem Biophys Res Commun 200: 642–646

Iwasaki H, Shichiri M, Marumo F, Hirata Y (2001) Adrenomedullin stimulates proline-rich tyrosine kinase 2 in vascular smooth muscle cells. Endocrinology 142: 564–572

Jiang BH, Zheng JZ, Aoki M, Vogt PK (2000) Phosphatidylinositol 3-kinase signaling mediates angiogenesis and expression of vascular endothelial growth factor in endothelial cells. Proc Natl Acad Sci USA 97: 1749–1753

Jougasaki M, Wei CM, McKinley LJ, Burnett JC Jr (1995) Elevation of circulating and ventricular adrenomedullin in human congestive heart failure. Circulation 92: 286–289

Kano H, Kohno M, Yasunari K, Yokokawa K, Horio T, Ikeda M, Minami M, Hanehira T, Takeda T, Yoshikawa J (1996) Adrenomedullin as a novel antiproliferative factor of vascular smooth muscle cells. J Hypertension 14: 209–213

Katayama T, Nakashima H, Furudono S, Honda Y, Suzuki S, Yano K (2004) Evaluation of neurohumoral activation (adrenomedullin, BNP, catecholamines, etc.) in patients with acute myocardial infarction. Intern Med 43: 1015–1022

Kato H, Shichiri M, Marumo F, Hirata Y (1997) Adrenomedullin as an autocrine/paracrine apoptosis survival factor for rat endothelial cells. Endocrinology 138: 2615–2620

Kim W, Moon SO, Sung MJ, Kim SH, Lee S, Kim HJ, Koh GY, Park SK (2002) Protective effect of adrenomedullin in mannitol-induced apoptosis. Apoptosis 7: 527–536

Kim W, Moon SO, Sung MJ, Kim SH, Lee S, So JN, Park SK (2003) Angiogenic role of adrenomedullin through activation of Akt, mitogen-activated protein kinase, and focal adhesion kinase in endothelial cells. FASEB J 13: 1937–1939

Kim W, Moon SO, Lee S, Sung MJ, Kim SH, Park SK (2003a) Adrenomedullin reduces VEGF-induced endothelial adhesion molecules and adhesiveness through a phosphatidylinositol 3′-kinase pathway. Arterioscler Thromb Vasc Biol 23: 1377–1383

Nikitenko LL, Smith DM, Bicknell R, Rees MC (2003) Transcriptional regulation of the CRLR gene in human microvascular endothelial cells by hypoxia. FASEB J 17: 1499–1501

Kitamura K, Kangawa K, Kawamoto M, Ichiki Y, Nakamura S, Matsuo H, Eto T (1993) Adrenomedullin: A novel hypotensive peptide isolated from human pheochromocytoma. Biochem Biophys Res Commun 192: 553–560

Kobayashi K, Kitamura K, Hirayama N, Date H, Kashiwagi T, Ikushima I, Hanada Y, Nagatomo Y, Takenaga M, Ishikawa T, Imamura T, Koiwaya Y, Eto T (1996) Increased plasma adrenomedullin in acute myocardial infarction. Am Heart J 131: 676–680

Krzeminski K, Kruk B, Wojcik-Ziolkowska E, Kozera J, Cybulski G, Nazar K (2002) Effect of static handgrip on plasma adrenomedullin concentration in patients with heart failure and in healthy subjects. J Physiol Pharmacol 53: 199–210

Kubo A, Minamino N, Isumi Y, Katafuchi T, Kangawa K, Dohi K, Matsuo H (1998) Production of adrenomedullin in macrophage cell line and peritoneal macrophage. J Biol Chem 273: 16730–16738

Majid DSA, Kadowitz PJ, Coy DH, Navar LG (1996) Renal responses to intra-arterial administration of adrenomedullin in dogs. Am J Physiol 270: F200–F205

Matsui T, Li L, del Monte F, Fukui Y, Franke TF, Hajjar RJ, Rosenzweig A (1999) Adenoviral gene transfer of activated phosphatidylinositol 3′-kinase and Akt inhibits apoptosis of hypoxic cardiomyocytes in vitro. Circulation 100: 2373–2379

McLatchie LM, Fraser NJ, Main MJ, Wise A, Brown J, Thompson N, Solari R, Lee MG, Foord SM (1998) RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. Nature 393: 333–339

Mittra S, Hyvelin JM, Shan Q, Tang F, Bourreau JP (2004) Role of cyclooxygenase in ventricular effects of adrenomedullin: is adrenomedullin a double-edged sword in sepsis? Am J Physiol Heart Circ Physiol 286: 1034–1042

Miyao Y, Nishikimi T, Goto Y, Miyazaki S, Daikoku S, Morii I, Matsumoto T, Takishita S, Miyata A, Matsuo H, Kangawa K, Nonogi H (1998) Increased plasma adrenomedullin levels in patients with acute myocardial infarction in proportion to the clinical severity. Heart 79: 39–44

Nagaya N, Goto Y, Satoh T, Sumida H, Kojima S, Miyatake K, Kangawa K (2002) Intravenous adrenomedullin in myocardial function and energy metabolism in patients after myocardial infarction. J Cardiovasc Pharmacol 39: 754–760

Nagaya N, Kangawa K (2004) Adrenomedullin in the treatment of pulmonary hypertension. Peptides 25: 2013–2018

Nagaya N, Nishikimi T, Horio T, Yoshihara F, Kanazawa A, Matsuo H, Kangawa K (1999) Cardiovascular and renal effects of adrenomedullin in rats with heart failure. Am J Physiol 276: 213–218

Nagaya N, Nishikimi T, Uematsu M, Satoh T, Oya H, Kyotani S, Sakamaki F, Ueno K, Nakanishi N, Miyatake K, Kangawa K (2000) Haemodynamic and hormonal effects of adrenomedullin in patients with pulmonary hypertension. Heart 84: 653–658

Nagaya N, Nishikimi T, Uematsu M, Yoshitomi Y, Miyao Y, Miyazaki S, Goto Y, Kojima S, Kuramochi M, Matsuo H, Kangawa K, Nonogi H (1999) Plasma adrenomedullin as an indicator of prognosis after acute myocardial infarction. Heart 81: 483–487

Nagaya N, Nishikimi T, Yoshihara F, Horio T, Morimoto A, Kangawa K (2000) Cardiac adrenomedullin gene expression and peptide accumulation after acute myocardial infarction in rats. Am J Physiol Regul Integr Comp Physiol 278: 1019–1026

Nagaya N, Satoh T, Nishikimi T, Uematsu M, Furuichi S, Sakamaki F, Oya H, Kyotani S, Nakanishi N, Goto Y, Masuda Y, Miyatake K, Kangawa K (2000) Hemodynamic, renal, and hormonal effects of adrenomedullin infusion in patients with congestive heart failure. Circulation 101: 498–503

Nakamura M, Yoshida H, Makita S, Arakawa N, Niinuma H, Hiramori K (1997) Potent and long-lasting vasodilatory effects of adrenomedullin in humans: Comparisons between normal subjects and patients with chronic heart failure. Circulation 95: 1214–1221

Nikitenko LL, Smith DM, Bicknell R, Rees MC (2003) Transcriptional regulation of the CRLR gene in human microvascular endothelial cells by hypoxia. FASEB J 17: 1499–1501

Nishikimi T, Saito Y, Kitamura K, Ishimitsu T, Eto T, Kangawa K, Matsuo H, Omae T, Matsuoka H (1995) Increased plasma levels of adrenomedullin in patients with heart failure. J Am Coll Cardiol 26: 1424–1431

Nishikimi T, Tadokoro K, Akimoto K, Mori Y, Ishikawa Y, Ishimura K, Horio T, Kangawa K, Matsuoka H (2005) Response of adrenomedullin system to cytokine in cardiac fibroblasts-role of adrenomedullin as an antifibrotic factor. Cardiovasc Res 66: 104–113

Nishikimi T, Tadokoro K, Mori Y, Wang X, Akimoto K, Yoshihara F, Minamino N, Kangawa K, Matsuoka H (2003) Ventricular adrenomedullin system in the transition from LVH to heart failure in rats. Hypertension 41: 512–518

Nishikimi T, Yoshihara F, Mori Y, Kangawa K, Matsuoka H (2003) Cardioprotective effect of adrenomedullin in heart failure. Hypertens Res [Suppl] 26: 121–127

Nishimatsu H, Suzuki E, Nagata D, Moriyama N, Satonaka H, Walsh K, Sata M, Kangawa K, Matsuo H, Goto A, Kitamura T, Hirata Y (2001) Adrenomedullin induces endothelium-dependent vasorelaxation via the phosphatidylinositol 3-kinase/Akt-dependent pathway in rat aorta. Circ Res 89: 63–70

Niu P, Shindo T, Iwata H, Iimuro S, Takeda N, Zhang Y, Ebihara A, Suematsu Y, Kangawa K, Hirata Y, Nagai R (2004) Protective effects of endogenous adrenomedullin on cardiac hypertrophy, fibrosis, and renal damage. Circulation 109: 1789–1794

Oehler MK, Hague S, Rees MC, Bicknell R (2002) Adrenomedullin promotes formation of xenografted endometrial tumors by stimulation of autocrine growth and angiogenesis. Oncogene 21: 2815–2821

Okumura H, Nagaya N, Itoh T, Okano I, Hino J, Mori K, Tsukamoto Y, Ishibashi-Ueda H, Miwa S, Tambara K, Toyokuni S, Yutani C, Kangawa K (2004) Adrenomedullin infusion attenuates myocardial ischemia/reperfusion injury through the phosphatidylinositol 3-kinase/Akt-dependent pathway. Circulation 109: 242–248

Owji AA, Smith DM, Coppock HA, Morgan DG, Bhogal R, Ghatei MA, Bloom SR (1995) An abundant and specific binding site for the novel vasodilator adrenomedullin in the rat. Endocrinology 136: 2127–2134

Oya H, Nagaya N, Furuichi S, Nishikimi T, Ueno K, Nakanishi N, Yamagishi M, Kangawa K, Miyatake K (2000) Comparison of intravenous adrenomedullin with atrial natriuretic peptide in patients with congestive heart failure. Am J Cardiol 86: 94–98

Parkes DG, May CN (1997) Direct cardiac and vascular actions of adrenomedullin in conscious sheep. Br J Pharmacol 120: 1179–1185

Perret M, Broussard H, LeGros T, Burns A, Chang JK, Summer W, Hyman A, Lippton H (1993) The effect of adrenomedullin on the isolated heart. Life Sci 53: 377–379

Rademaker MT, Charles CJ, Lewis LK, Yandle TG, Cooper GJS, Coy DH, Richards AM, Nicholls MG (1997) Beneficial hemodynamic and renal effects of adrenomedullin in an ovine model of heart failure. Circulation 96: 1983–1990

Saito Y, Nakao K, Nishimura K, Sugawara A, Okumura K, Obata K, Sonoda R, Ban T, Yasue H, Imura H (1987) Clinical application of atrial natriuretic peptide in patients with congestive heart failure: Beneficial effects on left ventricular function. Circulation 76: 115–124

Sakata J, Shimokubo T, Kitamura K, Nishizono M, Iehiki Y, Kangawa K, Matsuo H, Eto T (1994) Distribution and characterization of immunoreactive rat adrenomedullin in tissue and plasma. FEBS Lett 352: 105–108

Shichiri M, Fukai N, Ozawa N, Iwasaki H, Hirata Y (2003) Adrenomedullin is an autocrine/paracrine growth factor for rat vascular smooth muscle cells. Regul Pept 112: 167–173

Shimekake Y, Nagata K, Ohta S, Kambayashi Y, Teraoka H, Kitamura K, Eto T, Kangawa K, Matsuo H (1995) Adrenomedullin stimulates two signal transduction pathways, cAMP accumulation and Ca^{2+} mobilization, in bovine aortic endothelial cells. J Biol Chem 270: 4412–4417

Shindo T, Kurihara Y, Nishimatsu H, Moriyama N, Kakoki M, Wang Y, Imai Y, Ebihara A, Kuwaki T, Ju KH, Minamino N, Kangawa K, Ishikawa T, Fukuda M, Akimoto Y, Kawakami H, Imai T, Morita H, Yazaki Y, Nagai R, Hirata Y, Kurihara H (2001) Vascular abnormalities and elevated blood pressure in mice lacking adrenomedullin gene. Circulation 104: 1964–1971

Stangl V, Dschietzig T, Bramlage P, Boye P, Kinkel HT, Staudt A, Baumann G, Felix SB, Stangl K (2000) Adrenomedullin and myocardial contractility in the rat. Eur J Pharmacol 408: 83–89

Sugo S, Minamino N, Shoji H, Kangawa K, Kitamura K, Eto T, Matsuo H (1994) Interleukin-1, tumor necrosis factor and lipopolysaccharide additively stimulate production of adrenomedullin in vascular smooth muscle cells. Biochem Biophys Res Commun 207: 25–32

Sugo S, Minamino N, Shoji H, Kangawa K, Kitamura K, Eto T, Matsuo H (1994a) Production and secretion of adrenomedullin from vascular smooth muscle cells: Augmented production by tumor necrosis factor-alpha. Biochem Biophys Res Commun 203: 719–726

Suzuki Y, Horio T, Hayashi T, Nonogi H, Kitamura K, Eto T, Kangawa K, Kawano Y (2004) Plasma adrenomedullin concentration is increased in patients with peripheral arterial occlusive disease associated with vascular inflammation. Regul Pept 118: 99–104

Szokodi I, Kinnunen P, Tavi P, Weckstrom M, Toth M, Ruskoaho H (1998) Evidence for cAMP-independent mechanisms mediating the effects of adrenomedullin, a new inotropic peptide. Circulation 97: 1062–1070

Tadokoro K, Nishikimi T, Mori Y, Wang X, Akimoto K, Matsuoka H (2003) Altered gene expression of adrenomedullin and its receptor system and molecular forms of tissue adrenomedullin in left ventricular hypertrophy induced by malignant hypertension. Regul Pept 112: 71–78

Tokunaga N, Nagaya N, Shirai M, Tanaka E, Ishibashi-Ueda H, Harada-Shiba M, Kanda M, Ito T, Shimizu W, Tabata Y, Uematsu M, Nishigami K, Sano S, Kangawa K, Mori H (2004) Adrenomedullin gene transfer induces therapeutic angiogenesis in a rabbit model of chronic hind limb ischemia: benefits of a novel nonviral vector, gelatin. Circulation 109: 526–531

Totsune K, Takahashi K, Mackenzie HS, Murakami O, Arihara Z, Sone M, Mouri T, Brenner BM, Ito S (2000) Increased gene expression of adrenomedullin and adrenomedullin-receptor complexes, receptor-activity modifying protein (RAMP)2 and calcitonin-receptor-like receptor (CRLR) in the hearts of rats with congestive heart failure. Clin Sci 99: 541–546

Tsuruda T, Jougasaki M, Boerrigter G, Costello-Boerrigter LC, Cataliotti A, Lee SC, Salz-Gilman L, Nordstrom LJ, McGregor CG, Burnett JC (2003) Ventricular adrenomedullin is associated with myocyte hypertrophy in human transplanted heart. Regul Pept 112: 161–166

Tsuruda T, Kato J, Kitamura K, Kuwasako K, Imamura T, Koiwaya Y, Tsuji T, Kangawa K, Eto T (1998) Adrenomedullin: a possible autocrine or paracrine inhibitor of hypertrophy of cardiomyocytes. Hypertension: 505–510

Tsuruda T, Kato J, Kitamura K, Kawamoto M, Kuwasako K, Imamura T, Koiwaya Y, Tsuji T, Kangawa K, Eto T (1999) An autocrine or a paracrine role of adrenomedullin in modulating cardiac fibroblast growth. Cardiovasc Res 43: 958–967

Wong LY, Cheung BM, Li YY, Tang F (2005) Adrenomedullin is both proinflammatory and antiinflammatory: Its effects on gene expression and secretion of cytokines and macrophage migration inhibitory factor in NR8383 macrophage cell line. Endocrinology 146: 1321–1327

Yamaguchi T, Baba K, Doi Y, Yano K (1995) Effect of adrenomedullin on aldosterone secretion by dispersed rat adrenal zona glomerulosa cells. Life Sci 56: 379–387

Yin H, Chao L, Chao J (2004) Adrenomedullin protects against myocardial apoptosis after ischemia/reperfusion through activation of Akt-GSK signaling. Hypertension 43: 109–116

Yoshimoto T, Gochou N, Fukai N, Sugiyama T, Shichiri M, Hirata Y (2005) Adrenomedullin inhibits angiotensin II-induced oxidative stress and gene expression in rat endothelial cells. Hypertension Res 28: 165–172

Authors' address: Dr. Noritoshi Nagaya, National Cardiovascular Center Research Institute, 5-7-1 Fujishirodai, Suita-City, 565-8565 Osaka, Japan, Fax: +81-6-6833-9865, E-mail: nnagaya@ri.ncvc.go.jp

Verleger: Springer-Verlag GmbH, Sachsenplatz 4–6, 1201 Wien, Austria – Herausgeber: Prof. Dr. Gert Lubec, Universitäts-Kinderklinik, Währinger Gürtel 18–20, 1090 Wien, Austria – Redaktion: Sachsenplatz 4–6, 1201 Wien, Austria – Hersteller: Satz und Umbruch: Thomson Press (India) Ltd., Chennai; Offsetdruck: Krips bv, Kaapweg 6, 7944 HV Meppel, The Netherlands – Verlagsort: Wien – Herstellungsort: Meppel – Printed in The Netherlands.

Offenlegung gem. § 25 Abs. 1 bis 3 Mediengesetz: Unternehmensgegenstand: Verlag von wissenschaftlichen Büchern und Zeitschriften. An der Springer-Verlag GmbH ist beteiligt: Springer Austria GmbH, Sachsenplatz 4–6, 1201 Wien, Austria, zu 99,8%. Geschäftsführer: Dr. Sven Fund, Sachsenplatz 4–6, 1201 Wien, Austria.