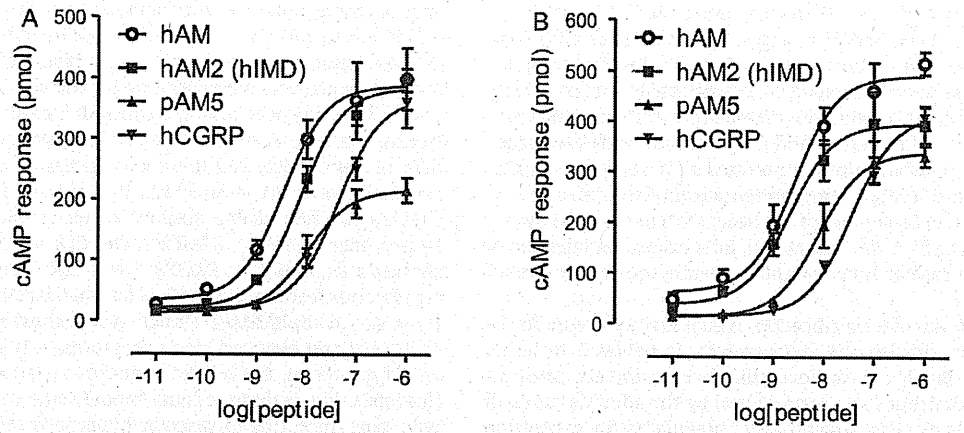


Fig. 3. AM-evoked cAMP production in HEK-293 cells transiently overexpressing hCLR/hRAMP2 (A) or hCLR/hRAMP3 (B). Following transient transfection, the cells were incubated for 15 min at 37 °C in Hanks' buffer with 20 mM HEPES, 0.2% BSA, 0.5 mM 3-isobutyl-1-methylxanthine and the indicated concentrations of hAM, hAM2, haCGRP or porcine (p)AM5 and were then lysed. The cell lysates were then analyzed for intracellular cAMP content using a commercial enzyme immunoassay kit. None of the agonists elicited cAMP production in HEK-293 cells transfected with empty vector up to a concentration of 10^{7} M (data not shown). Symbols represent the means ±S.E. from three separate identical experiments. Data were analyzed using Prism 5.02 (GrapghPad Software Inc., San Diego, CA, USA).

RAMP3 mRNA levels were unaffected, despite significantly elevated levels of AM and AM2 mRNA [52]. In addition, no increases in the expression of AM receptor mRNA were seen in the right ventricles of the MI rats, although AM and AM2 mRNAs were both significantly increased (AM2 > AM) [52]. Renal expression of mRNA for AMs and their receptors was not upregulated during heart failure in MI rats [52], despite the fact that intra-renal infusion of AM or AM2 causes diuresis and natriuresis without a significant reduction in systolic blood pressure in rats [33,38,93]. Interestingly, severe MI-induced heart failure diminished the expression of mRNA encoding the AM_1 receptor but not the AM_2 receptor in the rat lung. As a result, the numbers of pulmonary AM receptors were greatly reduced, whereas pulmonary AM mRNA and peptide expression were greatly increased [98]. In fact, the release of AM from the lungs into circulation has been observed in patients with severe systolic dysfunction [98]. Alveolar macrophages are likely the main source of increased AM expression in the lungs, as their expression of AM mRNA is substantially elevated in rats and humans with severe heart failure [98]. The elevated AM levels may limit pulmonary infiltration of neutrophils because AM inhibits the cytokine-induced secretion of neutrophil chemoattractant from rat alveolar macrophages *in vitro* [62].

Hemodynamic stress and angiotensin II are both key mediators of cardiac hypertrophy induced by pressure overload (POL; by aortic banding) or volume overload (VOL; by aortocaval shunt). Angiotensin II, rather than hemodynamic stress, is reportedly a critical stimulator of the left ventricular mRNA expression of AM and its receptors in rat POL and VOL models [101]. In this report, acute POL increasedAM_1 and AM_2 receptor mRNA expression, whereas acute VOL increased only AM_1 receptor mRNA; moreover, the induction

Table 2
Changes in gene expression of AM and its receptor components under various disease conditions in rat models.

Pathology	Experimental model	Tissue	AM	AM2	CLR	RAMP2	RAMP3	Reference
Hypertension with cardiac hypertrophy	+ Chronic pressure loading (SHRs, 11-week-old)	Myocardium	↑	ND	↑	↑	↑	[104]
		Aorta	↑	ND	↑	↑	↑	[104]
	+ Chronic pressure loading (SHRs, 20-week-old)	Left ventricle	→	↑	→	→	↑	[9]
		Right ventricle	→	→	→	→	→	[9]
	+ L-NAME (SD rats)	Left ventricle	↑	↑	↑	↑	↑	[11,12,145]
		Right ventricle	→	↑	→	→	→	[145]
		Aorta	↑	ND	↑	↑	↑	[145]
	+ 8% NaCl (DS rats, 11-week-old)	Left ventricle	↑	ND	↑	↑	↑	[93]
Malignant hypertension	+ DOCA-salt (SHRs)	Left ventricle	↑	ND	↑	↑	↑	[127]
Heart failure	+ 8% NaCl (DS rats, 18-week-old)	Left ventricle	↑	ND	↑	↑	↑	[93]
	+ Myocardial infarction	Left ventricle	↑	↑	↑	↑	↑	[52]
		Right ventricle	↑	↑	→	→	→	[52]
		Atrium	↑	↑	↑	↑	→	[52]
		Lung	↑	ND	↓	↓	→	[98]
	+ Aortic banding	Left ventricle	↑	ND	↑	↑	↑	[62]
	+ Aortocaval shunt	Left ventricle	↑	ND	↑	↑	↑	[62]
Cardiomyopathy	+ Isoproterenol	Myocardium	↑	ND	↑	↑	↑	[60,112]
Cardiovascular calcification	+ Vitamin D_3 plus nicotine	Myocardium	↑	ND	↑	↑	↓	[106]
		Aorta	↑	ND	↑	↑	↑	[106]
Renal failure	+ 5/6 nephrectomy	Kidney	→	ND	↓	→	↓	[138]
Nephropathy	+ Ureteral obstruction	Obstructed kidney	→	ND	↑	↑	→	[87]
Diabetes	+ Streptozotocin (STZ)	Kidney	↑	ND	ND	↑	→	[50]
Salt loading	+ 8% NaCl (Wistar rats)	Adrenal gland	↑	ND	↑	↑	→	[17
		Kidney	↑	ND	↑	→	↑	[17]
Sepsis	+ Lipopolysaccharide	Lung	↑	ND	↓	↓	↑	[102]

Abbreviations: AM, adrenomedullin; CLR, calcitonin-receptor-like receptor; RAMP, receptor activity-modifying protein; SHR, spontaneously hypertensive rat; L-NAME, N^{G}-nitro-L-arginine methyl ester; SD, Sprague–Dawley; DS, Dahl salt-sensitive; DOCA, Deoxycorticosterone acetate; ND, not determined.

of AM receptor mRNAs in POL models appears to be greater than in VOL models. These findings suggest that the effect of VOL on RAMP3 transcription differs from its effect on RAMP2 transcription and that the two AM receptors have different functions under the pathophysiological conditions created by cardiac overload. Significant induction of both AM_1 and AM_2 receptor mRNAs was also observed in the myocardium of rats treated with isoproterenol, a β-adrenergic agonist [60,112]. Isoproterenol administration is known to cause marked enlargement of the heart with myocardial hypertrophy and necrosis. In this rat model, intraperitoneal injection of AM2 improved cardiac function and prevented myocardial injury [60].

Cardiac and vascular calcification, which mainly occurs in the aorta, coronary arteries and myocardium, is believed to be an important risk factor for cardiovascular events. In rats, calcification of the myocardium and aorta caused by the administration of Vitamin D_3 plus nicotine significantly upregulated the expression of AM and AM_1 receptor mRNAs [106]. Notably, myocardial and aortic calcification had the opposite effect on RAMP3 mRNA [106].

Local levels of RAMP2 and -3 mRNAs are also affected by renal failure. Expression of CLR and RAMP3 mRNAs is downregulated in the remnant kidney after 5/6 nephrectomy, which causes acute renal failure, although expression of RAMP2 mRNA is unaltered [138]. CLR and RAMP2 mRNAs, but not RAMP3 mRNA, are upregulated in kidneys with ureteral obstruction, despite demonstrating no change in the expression of AM mRNA [87]. The upregulation of AM_1 receptors might provide counter-regulatory actions against the proliferative and/or fibrotic changes in the obstructed kidney. Streptozocin (STZ)-induced diabetic rats show upregulated expression of AM and RAMP2 mRNAs, but not RAMP3 mRNA, in hypertrophied glomeruli and in afferent arterioles and enhanced urinary excretion of nitric oxide (NO_2^- and NO_3^-), probably due to AM stimulation [50]. In this model, adenovirus-mediated AM gene transfer improved cardiac function and prevented renal damage [30]. In addition, chronic salt loading led to the increased expression of AM and CLR mRNAs in rat adrenal glands and kidneys, without a significant rise in blood pressure [17]. In that experiment, RAMP1 and -2 mRNAs were upregulated in the adrenal glands, while RAMP3 mRNA was increased in the kidneys. In addition to its diuretic and natriuretic effects, AM can also inhibit angiotensin II- or potassium-induced aldosterone secretion from the adrenal glands [143,144]. Following salt loading, some secondary mediators may differentially regulate AM receptor expression to restore water and electrolyte balance.

During sepsis, which induces the most conspicuous increases in both circulating and local AM levels in both humans and rats [83,97], mRNA expression of CLR and the three RAMPs is markedly downregulated in a number of tissues [102]. This most likely reflects substantial increases in the levels of various agonists, cytokines or both. Most notably, RAMP3 mRNA in the lung, spleen and thymus show accelerated upregulation during the late stage of sepsis [102], suggesting that RAMP3 may be involved in some aspect of immune function *in vivo*.

Taken together, the findings summarized in this section suggest that the shared and separate functions of the AM_1 and AM_2 receptors in disease are dependent on their location *in vivo* and the pathophysiological conditions.

6. Knockout mouse models for RAMP2 or RAMP3

Two research groups have now characterized homozygous and heterozygous RAMP2 knockout (KO) mice (*RAMP2*$^{-/-}$ and *RAMP2*$^{-/+}$, respectively) [26,37,57]. *RAMP2*$^{-/-}$ embryos die *in utero* by mid-gestation after developing severe interstitial edema due to abnormalities in their vascular development [37,57]. Similar findings were obtained with homozygous AM [18,118] and CLR [27] KO mice. *RAMP2*$^{-/-}$ mice generated by Ichikawa-Shindo et al. [57] exhibited vascular fragility that resulted in severe hemorrhaging, which was observable under the skin and within organs. Severe bleeding was also seen in *AM*$^{-/-}$ embryos [118], but the systemic edema seen in *RAMP2*$^{-/-}$ mice was much more severe than in *AM*$^{-/-}$ mice, and there was excessive pericardial effusion suggestive of heart failure [57]. By contrast, Fritz-Six et al. [37] showed the remarkable absence of heart failure and embryonic hemorrhage in *AM*$^{-/-}$, *RAMP2*$^{-/-}$ and *CLR*$^{-/-}$ mice [37]. They also showed that, in *AM*$^{-/-}$, *RAMP2*$^{-/-}$ and *CLR*$^{-/-}$ embryos, there were significantly fewer proliferative endothelial cells in the lymph sacs. However, in adult *RAMP2*$^{-/+}$ mice, which show reduced expression of RAMP2 and elevated blood pressures (~10 mmHg higher than wild-type mice), the edema induced in various disease models (footpad, skin or brain edema model) was more severe than in wild-type mice, due to vascular hyperpermeability and impaired neovascularization [57]. Human dermal lymphatic microvascular endothelial cells (HLMVECs) strongly express AM, CLR and RAMP2 mRNAs, such that their expression is ~4-fold higher than in human umbilical vein endothelial cells (HUVECs) [37]. AM promoted cell proliferation and migration as well as network formation in cultured HLMVECs. The increased cell proliferation was cAMP- and MEK/ERK-dependent, and *in vivo*, AM treatment increased the numbers of lymphatic vessels and blood vessels in injured mouse tails, thereby reducing lymphedema [61]. This suggests that the AM-AM_1 receptor system could be a novel therapeutic target for patients with secondary lymphedema. Although AM2 has also emerged as a powerful angiogenic growth factor [88,122], it remains unknown whether AM2 is required for lymphangiogenesis.

Interestingly, AM2 did not rescue the deficiency of AM in *AM*$^{-/-}$ mice. It has been proposed that *AM*$^{-/-}$ embryos die *in utero* at mid-gestation due to vascular fragility, which leads to severe edema and particularly hydrocephalus resulting from the immature blood–brain barrier (BBB) [18,118]. Indeed, AM is actively secreted from cerebral endothelial cells (CECs) [65,66], the major cellular component of the BBB, and improves the BBB function through the expression of Claudin-5 [54]. It is well known cAMP is an important second messenger in the regulation of BBB functions. AM2 has been shown to induce cAMP production in the CECs, which is comparable to AM [22]. This suggests that exogenous AM2 can interact with the same receptor as AM. In general, AM2-staining in the peripheral vascular endothelial cells is weaker than that of AM2; however, little is known about AM2 production in the CECs. There is a possibility that the CECs produce little or no AM2, despite the complete loss of the AM gene *in vivo*. Another possibility is that endogenous AM2 in the CECs may bind to another unique receptor that is not involved in the development of the BBB during fetal life. Studies of AM2 KO mice will be necessary to investigate the embryonic functions of AM2.

Surprisingly, a complete absence of RAMP3 has no affect the survival of *RAMP3*$^{-/-}$ mice, at least up to ~6 months of age [26]. Older *RAMP3*$^{-/-}$ mice (9–10 months old) weigh ~25% less than age-matched wild-type mice, but they survive to at least 18 months of age with no obvious decline in health [26]. In addition, aged *RAMP2*$^{-/+}$ and *CLR*$^{-/+}$ mice do not differ significantly in body weight from their respective wild-type littermates [26]. Although RAMP3, as with AM and AM2 [128], is strongly expressed in the proximal renal tubule, there are no obvious differences in urine volume or protein/creatinine between *RAMP3*$^{-/-}$ and wild-type mice [26].

Thus, studies of RAMP KO mice have shown that RAMP2 and RAMP3 have distinct physiological functions throughout embryogenesis, adulthood and old age, despite mediating similar AM and AM2 signaling in complexes with CLR.

7. Transgenic mouse models for RAMP2

RAMP function has also been studied using transgenic (TG) mice. So far, there have been two reports of myc epitope-tagged mouse RAMP2 (myc-mRAMP2) overexpression in smooth muscle cells induced by the mouse α-actin gene [82,132]. AM is reported to be actively secreted from cultured vascular endothelial cells and VSMCs [136], while CLR is mainly expressed in the vascular endothelium [40]. Expression of myc-mRAMP2 was identified in the smooth muscle cell layers of the aorta, stomach and urinary bladder [132], and the morphology of the aorta and mesenteric microvessels in wild-type and RAMP2 TG mice were histologically indistinguishable. Basal blood pressure and cardiac hemodynamics were also similar in wild-type and RAMP2 TG mice. However, the hypotensive effects of intravenous bolus injection of AM were significantly enhanced in conscious RAMP2 TG mice compared to wild-type mice, whereas the hypotensive effects of αCGRP did not differ between TG and wild-type mice [132]. In addition, exogenous AM more potently relaxed noradrenaline-precontracted aortic rings from RAMP2 TG mice than from wild-type mice. These responses were inhibited by AM_{22-52}, but not by $\alpha CGRP_{8-37}$, in both wild-type and TG mice [132], reflecting the higher numbers of functionally active AM_1 receptors in the aortas of RAMP2 TG mice. This is consistent with the findings that the adult rat aorta expresses RAMP2 mRNA but not RAMP3 mRNA [143].

This RAMP2 TG mouse model remains susceptible to angiotensin II-induced increases in blood pressure and cardiac hypertrophy [82]. Nevertheless, the RAMP2 TG mice were almost completely protected from aortic vascular hypertrophy and inflammation caused by chronic angiotensin II infusion [82]. Moreover, cultured VSMCs from aortic explants from RAMP2 TG mice grew more slowly than those from wild-type mice, even in the presence of angiotensin II [82], and AM_{22-52} was able to enhance angiotensin II-stimulated proliferation of RAMP2 TG VSMCs to a greater degree than wild-type cells [82]. This suggests that, in the TG cells, endogenous AM is acting more effectively to inhibit aortic VSMC proliferation, thanks to the greater numbers of functional AM_1 receptors. Thus, the overexpression of RAMP2 in VSMCs exerts a powerful protective effect against vascular hypertrophy and inflammation by enhancing the vascular response to AM. This suggests that the vascular AM-AM_1 receptor system could be an important target for novel therapeutic approaches. In addition, AM2 was recently shown to be expressed in VSMCs from human renal arterioles [128], suggesting that AM2 may also exert protective effects via the AM_1 receptors.

8. Molecular basis of RAMP ECD function

Since their discovery in 1998, the structure–function relationships of hRAMPs have been extensively investigated using various point and deletion mutants and chimeras. The hRAMP ECD is known to be the major determinant of hCLR surface delivery and agonist binding specificity [43,75,77,78,109,119], the details of which are well summarized by Qi and Hay [110]. They reported the crystal structure of the RAMP1 ECD in complex with the CLR ECD and revealed the hydrophobic and electrostatic interactions between the N-terminal α-helix of CLR and α-helices 2 and 3 of RAMP1 [134]. In addition, studies of the small molecule CGRP receptor antagonistsolcegepant (BIBN409BS6) [99] and telcagepant (MK0974) [53], which are both used in the treatment of acute migraine, form hydrophobic interactions with Trp74 in α-helix 2 of the RAMP1 ECD and with Trp72 in the CLR ECD [134]. Interestingly, however, Trp74 in hRAMP1 does not affect the affinity of αCGRP for the CLR/RAMP1 complex, suggesting that Trp74 does not participate in CGRP binding, although it may be in close proximity to the CGRP binding site. Reciprocal replacement of the equivalent residue (Glu74) in hRAMP3 with Trp (CLR/RAMP3-Glu74Trp) elicited a ~10-fold reduction in AM potency without affecting αCGRP potency in humans [43,109]. The opposite effect was seen with the reciprocal hRAMP1-Trp74Glu mutant in complex with CLR; that is, AM potency increased ~10-fold, while αCGRP potency was unaffected [109]. These findings are indicative of a direct interaction between residue 74 and AM. Subsequent analysis showed that the most important requirement for this interaction is a residue with a full negative charge at position 74 in RAMP1 or RAMP3 [111]. The CLR/RAMP1-Trp74Glu mutant also enhanced the potency of AM2 to the same degree as AM, while CLR/RAMP3-Glu74Trp slightly reduced AM2 potency. This suggests that AM2 may interact with CGRP and AM_2 receptors differently than AM. Human RAMP2 also possesses a Glu residue at the position equivalent to Glu74 (Glu101) in hRAMP3 [46]. It remains to be determined whether CLR/RAMP2-Glu101Trp behaves pharmacologically similar to CLR/RAMP3-Glu74Trp. There are another 7 residues that are conserved in hRAMP2 and hRAMP3 but not in hRAMP1 [109]. However, these residues (Glu35, Asp46, Pro87, Leu88, Ala89, Ile93 and Asn103) in hRAMP3 are not involved in AM binding to the AM_2 receptor [109,110].

So far, Glu74 is the only residue in hRAMP3 known to be involved in AM binding. However, recent data [111] suggest that this residue does not play a key role in determining the selectivity of agonist binding (AM vs. CGRP) to AM_2 and CGRP receptors. In many family B GPCRs, agonist specificity is primarily associated with the ECD, with secondary recognition by a TM domain; the N-terminal and C-terminal portions of the agonist are recognized by the TM and ECD, respectively [121]. This "two domain model" is thought to apply to CLR/RAMP complexes because the N-terminus of CLR is known to be important for agonist binding [6,7,21,69,70]. It is noteworthy that the CLR N-terminus contains a sequence that contributes to the selective interaction of AM with the AM_1 receptor but not CGRP with its receptor [69,70]. How then do the three RAMP ECDs critically govern the CGRP vs. AM specificity of CLR? An earlier cross-linking study showed that ^{125}I-αCGRP is incorporated into RAMP1, while ^{125}I-AM is incorporated into RAMP2 or RAMP3 [49]. This suggests that the three RAMPs lie close to the agonist binding pocket within the CLR/RAMP complex. From the analysis of the crystal structure of hRAMP1 ECD, it was predicted that Arg67, Asp71, Trp74, Glu78 and Trp84, which, except for Trp84, are all situated in α-helix 2, comprise part of the agonist binding pocket [71]. Subsequent analysis revealed that the formation of a heterodimer with the RAMP1 ECD does not cause a significant conformational rearrangement of the structural features of the CLR ECD [134]. Additional information on the crystal structure of the entire CLR protein in complex with RAMP1 will be needed to clarify whether RAMP1 confers CGRP vs. AM selectivity through the allosteric modulation of the conformation of CLR or by directly contributing to the structure of the agonist binding pocket.

The affinity of CGRP for the AM_2 receptor is at least 10-fold greater than for the AM_1 receptor. It has been suggested that the CGRP vs. AM selectivity of the RAMPs may be regulated in part by the disulfide bond linking Cys27 and Cys82, which is strictly conserved in RAMP1 and RAMP3 but not RAMP2. That is, this bond may create a putative CGRP binding domain. It should be noted that several amino acid residues close to these Cys residues are also highly conserved in RAMP1 and RAMP3, but not RAMP2, and that the N-terminus of the mature RAMP2 sequence is ~20 residues longer than those of the other 2 RAMPs. This sequence variation likely contributes to the different pharmacological features of the AM_1 and AM_2 receptors.

Among the amino acid residues making up RAMPs, His residues exhibit the lowest content percentage; hRAMP1, -2 and -3 possess 2, 4 and 2 His residues, respectively, all of which are located in their ECDs (Fig. 2) [46]. A RAMP1-His71Ala mutant mediated nor-

mal CLR surface delivery, but the resultant heterodimers showed significantly diminished AM binding and potency [77]. It is therefore likely that His71 of RAMP1 is directly or indirectly involved in AM binding, although its contribution appears minor. Expression of RAMP2-H is 124Ala and -His127Ala leads to poor surface expression of CLR, thereby abolishing AM binding and signaling [109]. His124 in hRAMP2 is also conserved at position 97 in hRAMP1 and RAMP3 (Fig. 2). Notably, His97 in RAMP1 was identified as one of the three residues (Phe93, His97 and Phe101), all located in α-helix 3, that constitute the CLR binding interface [71]. Co-expression of CLR with RAMP1-His97Ala or RAMP3-His97Ala also reduced the cell surface expression and function of the receptors, making them less effective than the CLR/RAMP2-His124Ala receptor complex [77,78]. By contrast, RAMP2-His102Ala and RAMP3-His110Ala mutations had little effect on receptor expression or function [77].

9. Receptor trafficking regulated by the RAMP C-tail

Upon binding to their respective agonists, human CLR/RAMP complexes stably expressed in HEK-293 cells are rapidly internalized, without dissociation, via clathrin-coated vesicles [48,80]. CLR/RAMP1 receptor internalization is also reported lydynamin- and β-arrestin 2-dependent [48]. In that regard, it is well known that G protein coupled receptor kinases (GRKs) phosphorylate Ser/Thr sites located in many GPCR C-tails, enabling β-arrestins to bind at these sites [34]. Interestingly, AM_1 receptors display greater internalization than AM_2 receptors when expressed in HEK-293 cells [72], although the underlying mechanism remains unclear. Once internalized, GPCRs are often recycled back to the plasma membrane resulting in resensitization. However, unlike many GPCRs, after internalization endogenous and recombinant CLR/RAMP complexes are targeted to lysosomes where they are degraded [48,80,91]. It has been suggested that although they are short, RAMP C-tails are important for CLR surface expression, internalization and recycling [15,16,35,72,125]. Deletion of the C-tail from hRAMP2 disrupts transport of hCLR to the cell surface, leading to the significant loss of receptor function, while deleting the C-tail from hRAMP3 enhances AM-stimulated receptor internalization with no change in AM affinity or potency [72]. By contrast, deletion of the C-tail from hRAMP1 has little effect on receptor expression, function or intracellular trafficking [35,72].

RAMP C-tails may contain sites of potential interaction with other proteins [46,115]. For instance, the hRAMP3 C-tail, but not the hRAMP1 or RAMP2C-tails, possesses a classical type I PDZ binding motif (Thr-Leu-Leu), and the binding of *N*-ethylmaleimide-sensitive factor (NSF) to the PDZ motif of hRAMP3 was shown to promote slow recycling of internalized AM_2 receptors in HEK-293 cells [15]. Likewise, Na^+/H^+ exchanger regulatory factor-1 can also interact with the PDZ motif of hRAMP3, resulting in the complete inhibition of the internalization of the AM_2 receptor but not the CGRP or AM_1 receptor [16]. The C-tails of RAMPs, similar to that of CLR, also contain potential phosphorylation and ubiquitination sites [46,115]. In HEK-293 cells overexpressing the hCLR/hRAMP1 complex, agonists promote rapid phosphorylation of CLR but not RAMP1 [48]. In addition, deleting the respective C-tails from hRAMPs had no effect on lysosomal sorting of CLR [72]. It is therefore unlikely that the hRAMP C-tails participate in either phosphorylation or ubiquitination.

Taken together, these findings indicate that the hRAMP2 C-tail is critically involved in AM_1 receptor expression and function, while the hRAMP3 C-tail affects AM_2 receptor internalization and recycling. Thus, the C-tails of hRAMP2 and RAMP3 differentially govern AM receptor trafficking.

10. Perspectives

The hypotensive peptides AM and AM2 exert potent protective effects against multiorgan damage. Most notably, both peptides appear to inhibit cardiovascular oxidative stress, remodeling and apoptosis and to promote angiogenesis, although the data for AM2 are limited. Given the efficacy of these peptides, much effort has been devoted to developing practical clinical uses for AM in the treatment of acute MI [63], heart failure [95,103], arteriosclerosis obliterans [126] and pulmonary hypertension [29]. Furthermore, recent studies have clearly shown that the endogenous AM-AM_1 receptor system is required for both lymphangiogenesis and angiogenesis and that AM treatment can significantly improve rat-tail edema caused by injury. Consequently, this receptor system has also been regarded as a potential therapeutic target for patients with secondary lymphedema. On the contrary, selective local blockade of lymphangiogenesis mediated via AM_1 receptors could be an important therapeutic strategy for inhibiting tumor metastasis. Unfortunately, no selective non-peptide or peptide AM receptor agonists or antagonists have been identified. The two non-peptide CGRP receptor antagonists, olcegepant (BIBN409BS6) [99] and telcagepant (MK0974) [53], both of which are currently available for the treatment of migraines, have little or no affinity for AM_1 and AM_2 receptors [116].

So far, there is no evidence of a difference in the affinity of recombinant AM_1 and AM_2 receptors for AM or in their abilities to transduce the AM signal [8,80]. These AM receptors exist together by their location *in vivo* [100,107,115,142], although tissue expression of RAMP2 and RAMP3 mRNAs appears to vary under different pathophysiological conditions. In addition, the effects of AM2 can also be mediated by the 2 AM receptors. To complicate matters further, hRAMP2 and hRAMP3 can also strongly interact with the CT receptor and the vasoactive intestinal peptide (VIP)/pituitary adenylatecyclase-activating polypeptide type 1 receptor (VPAC1), both of which are family B GPCRs [46,116]. Consequently, the development of non-peptide AM receptor-specific agonists and antagonists would be highly desirable, not only to clarify the shared and separate functions of AM_1 and AM_2 receptors *in vivo* but also to realize the clinical application of AM. To that end, further work to elucidate the entire crystal structure of RAMP2 and RAMP3 in complex with CLR would be valuable because it is anticipated that each CLR/RAMP complex contains numerous crevices that could contribute to drug binding and discovery. It would also be interesting to know the structural basis for the preference of the three RAMPs for interaction with CLR, as opposed to other GPCRs (e.g., CT receptor, VPAC1) in the same tissues and cells. If the molecular mechanism(s) underlying the preferential association between RAMPs and GPCRs is solved, it may enable us to provide selective treatment for a targeted GPCR/RAMP.

After internalization in vascular endothelial cells, endogenous hAM_1 receptors are targeted for degradation in lysosomes [91], which is consistent with the intracellular trafficking of hAM_1 receptors stably overexpressed in HEK-293 cells [48,80]. At present, the mechanism of lysosomal sorting of AM_1 receptors remains unknown, but the establishment of strategies for promoting receptor recycling could be an important means of sustaining AM signaling.

11. Conclusion

In summary, AM and AM2 are potent hypotensive, antioxidative and anti-atherosclerotic factors in the cardiovascular system. In particular, much attention has been paid to the clinical application of AM, many of the effects of which are mediated via the RAMP2-based AM_1 receptor. However, the evidence now

suggests that the AM_2 receptor also has distinct functions *in vivo*. Further studies are needed to clarify the shared and differing roles of the 2 AM receptors to realize their potential clinical applications.

Conflict of interest

None.

Acknowledgements

This study was supported in part by grants-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan and by research grants from the Takeda Science Foundation.

References

[1] Aiyar N, Disa J, Ao Z, Xu D, Surya A, Pillarisetti K, et al. Molecular cloning and pharmacological characterization of bovine calcitonin receptor-like receptor from bovine aortic endothelial cells. Biochem Pharmacol 2002;63:1949–59.
[2] Aiyar N, Disa J, Pullen M, Nambi P. Receptor activity modifying proteins interaction with human and porcine calcitonin receptor-like receptor (CRLR) in HEK-293 cells. Mol Cell Biochem 2001;224:123–33.
[3] Aiyar N, Rand K, Elshourbagy NA, Zeng Z, Adamou JE, Bergsma DJ, et al. A cDNA encoding the calcitonin gene-related peptide type 1 receptor. J Biol Chem 1996;271:11325–9.
[4] Amara SG, Jonas V, Rosenfeld MG, Ong ES, Evans RM. Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. Nature 1982;298:240–4.
[5] Bailey RJ, Hay DL. Agonist-dependent consequences of proline to alanine substitution in the transmembrane helices of the calcitonin receptor. Br J Pharmacol 2007;151:678–87.
[6] Banerjee S, Evanson J, Harris E, Lowe SL, Thomasson KA, Porter JE. Identification of specific calcitonin-like receptor residues important for calcitonin gene-related peptide high affinity binding. BMC Pharmacol 2006;6:9.
[7] Barwell J, Miller PS, Donnelly D, Poyner DR. Mapping interaction sites within the N-terminus of the calcitonin gene-related peptide receptor; the role of residues 23-60 of the calcitonin receptor-like receptor. Peptides 2010;31:170–6.
[8] Bell D, McDermott BJ. Intermedin (adrenomedullin-2): a novel counter-regulatory peptide in the cardiovascular and renal systems. Br J Pharmacol 2008;153(Suppl. 1):S247–62.
[9] Bell D, Zhao Y, McCoy FP, Devine A, McDermott BJ. Expression of the counter-regulatory peptide intermedin is augmented in the presence of oxidative stress in hypertrophied cardiomyocytes. Cell Physiol Biochem 2008;21:409–20.
[10] Bell D, Zhao Y, McCoy FP, Devine AB, McDermott BJ. Differential effects of an anti-oxidant intervention on cardiomyocyte expression of adrenomedullin and intermedin and their receptor components in chronic nitric oxide deficiency. Cell Physiol Biochem 2007;20:269–82.
[11] Bell D, Zhao YY, Devine AB, McDermott BJ. Influence of atenolol and nifedipine on nitric-oxide deficient cardiomyocyte hypertrophy and expression of the cardio-endocrine peptide intermedin and its receptor components. Cell Physiol Biochem 2008;21:203–14.
[12] Bell D, Zhao YY, Kelso EJ, McHenry EM, Rush LM, Lamont VM, et al. Upregulation of adrenomedullin and its receptor components during cardiomyocyte hypertrophy induced by chronic inhibition of nitric oxide synthesis in rats. Am J Physiol Heart Circ Physiol 2006;290:H904–14.
[13] Beltowski J, Jamroz A. Adrenomedullin—what do we know 10 years since its discovery? Pol J Pharmacol 2004;56:5–27.
[14] Bernatova I, Pechanova O, Simko F. Effect of captopril in L-NAME-induced hypertension on the rat myocardium, aorta, brain and kidney. Exp Physiol 1999;84:1095–105.
[15] Bomberger JM, Parameswaran N, Hall CS, Aiyar N, Spielman WS. Novel function for receptor activity-modifying proteins (RAMPs) in post-endocytic receptor trafficking. J Biol Chem 2005;280:9297–307.
[16] Bomberger JM, Spielman WS, Hall CS, Weinman EJ, Parameswaran N. Receptor activity-modifying protein (RAMP) isoform-specific regulation of adrenomedullin receptor trafficking by NHERF-1. J Biol Chem 2005;280:23926–35.
[17] Cao YN, Kitamura K, Kato J, Kuwasako K, Ito K, Onitsuka H, et al. Chronic salt loading upregulates expression of adrenomedullin and its receptors in adrenal glands and kidneys of the rat. Hypertension 2003;42:369–72.
[18] Caron KM, Smithies O. Extreme hydrops fetalis and cardiovascular abnormalities in mice lacking a functional Adrenomedullin gene. Proc Natl Acad Sci U S A 2001;98:615–9.
[19] Chang CL, Roh J, Hsu SY. Intermedin, a novel calcitonin family peptide that exists in teleosts as well as in mammals: a comparison with other calcitonin/intermedin family peptides in vertebrates. Peptides 2004;25:1633–42.
[20] Chao J, Jin L, Lin KF, Chao L. Adrenomedullin gene delivery reduces blood pressure in spontaneously hypertensive rats. Hypertens Res 1997;20:269–77.
[21] Chauhan M, Rajarathnam K, Yallampalli C. Role of the N-terminal domain of the calcitonin receptor-like receptor in ligand binding. Biochemistry 2005;44:782–9.
[22] Chen L, Kis B, Hashimoto H, Busija DW, Takei Y, Yamashita H, et al. Adrenomedullin 2 protects rat cerebral endothelial cells from oxidative damage in vitro. Brain Res 2006;1086:42–9.
[23] Chiba T, Yamaguchi A, Yamatani T, Nakamura A, Morishita T, Inui T, et al. Calcitonin gene-related peptide receptor antagonist human CGRP-(8-37). Am J Physiol 1989;256:E331–5.
[24] Choksi T, Hay DL, Legon S, Poyner DR, Hagner S, Bloom SR, et al. Comparison of the expression of calcitonin receptor-like receptor (CRLR) and receptor activity modifying proteins (RAMPs) with CGRP and adrenomedullin binding in cell lines. Br J Pharmacol 2002;136:784–92.
[25] Conner AC, Hay DL, Howitt SG, Kilk K, Langel U, Wheatley M, et al. Interaction of calcitonin-gene-related peptide with its receptors. Biochem Soc Trans 2002;30:451–5.
[26] Dackor R, Fritz-Six K, Smithies O, Caron K. Receptor activity-modifying proteins 2 and 3 have distinct physiological functions from embryogenesis to old age. J Biol Chem 2007;282:18094–9.
[27] Dackor RT, Fritz-Six K, Dunworth WP, Gibbons CL, Smithies O, Caron KM. Hydrops fetalis, cardiovascular defects, and embryonic lethality in mice lacking the calcitonin receptor-like receptor gene. Mol Cell Biol 2006;26:2511–8.
[28] Deville JL, Salas S, Figarella-Branger D, Ouafik L, Daniel L. Adrenomedullin as a therapeutic target in angiogenesis. Expert Opin Ther Targets 2010;14:1059–72.
[29] Dewachter L, Dewachter C, Naeije R. New therapies for pulmonary arterial hypertension: an update on current bench to bedside translation. Expert Opin Investigational Drugs 2010;19:469–88.
[30] Dobrzynski E, Montanari D, Agata J, Zhu J, Chao J, Chao L. Adrenomedullin improves cardiac function and prevents renal damage in streptozotocin-induced diabetic rats. Am J Physiol Endocrinol Metab 2002;283:E1291–8.
[31] Eguchi S, Hirata Y, Iwasaki H, Sato K, Watanabe TX, Inui T, et al. Structure–activity relationship of adrenomedullin, a novel vasodilatory peptide, in cultured rat vascular smooth muscle cells. Endocrinology 1994;135:2454–8.
[32] Eguchi S, Hirata Y, Kano H, Sato K, Watanabe Y, Watanabe TX, et al. Specific receptors for adrenomedullin in cultured rat vascular smooth muscle cells. FEBS Lett 1994;340:226–30.
[33] Eto T. A review of the biological properties and clinical implications of adrenomedullin and proadrenomedullin N-terminal 20 peptide (PAMP), hypotensive and vasodilating peptides. Peptides 2001;22:1693–711.
[34] Ferguson SS. Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling. Pharmacol Rev 2001;53:1–24.
[35] Fitzsimmons TJ, Zhao X, Wank SA. The extracellular domain of receptor activity-modifying protein 1 is sufficient for calcitonin receptor-like receptor function. J Biol Chem 2003;278:14313–20.
[36] Fraser NJ, Wise A, Brown J, McLatchie LM, Main MJ, Foord SM. The amino terminus of receptor activity modifying proteins is a critical determinant of glycosylation state and ligand binding of calcitonin receptor-like receptor. Mol Pharmacol 1999;55:1054–9.
[37] Fritz-Six KL, Dunworth WP, Li M, Caron KM. Adrenomedullin signaling is necessary for murine lymphatic vascular development. J Clin Invest 2008;118:40–50.
[38] Fujisawa Y, Nagai Y, Miyatake A, Takei Y, Miura K, Shoukouji T, et al. Renal effects of a new member of adrenomedullin family, adrenomedullin2, in rats. Eur J Pharmacol 2004;497:75–80.
[39] Gibbons C, Dackor R, Dunworth W, Fritz-Six K, Caron KM. Receptor activity-modifying proteins: RAMPing up adrenomedullin signaling. Mol Endocrinol 2007;21:783–96.
[40] Hagner S, Stahl U, Knoblauch B, McGregor GP, Lang RE. Calcitonin receptor-like receptor: identification and distribution in human peripheral tissues. Cell Tissue Res 2002;310:41–50.
[41] Hasbak P, Sams A, Schifter S, Longmore J, Edvinsson L. CGRP receptors mediating CGRP-, adrenomedullin- and amylin-induced relaxation in porcine coronary arteries. Characterization with 'Compound 1' (WO98/11128), a non-peptide antagonist. Br J Pharmacol 2001;133:1405–13.
[42] Hay DL, Christopoulos G, Christopoulos A, Poyner DR, Sexton PM. Pharmacological discrimination of calcitonin receptor: receptor activity-modifying protein complexes. Mol Pharmacol 2005;67:1655–65.
[43] Hay DL, Christopoulos G, Christopoulos A, Sexton PM. Determinants of 1-piperidinecarboxamide, *N*-[2-[[5-amino-1-[[4-(4-pyridinyl)-1-piperazinyl]carbonyl]pentyl]amino]-1-[(3,5-dibromo-4-hydroxyphenyl)methyl]-2-oxoethyl]-4-(1,4-dihydro-2-oxo-3(2H)-quinazolinyl) (BIBN4096BS) affinity for calcitonin gene-related peptide and amylin receptors—the role of receptor activity modifying protein 1. Mol Pharmacol 2006;70:1984–91.
[44] Hay DL, Conner AC, Howitt SG, Smith DM, Poyner DR. The pharmacology of adrenomedullin receptors and their relationship to CGRP receptors. J Mol Neurosci 2004;22:105–13.
[45] Hay DL, Howitt SG, Conner AC, Doods H, Schindler M, Poyner DR. A comparison of the actions of BIBN4096BS and CGRP(8-37) on CGRP and adrenomedullin receptors expressed on SK-N-MC, L6, Col 29 and Rat 2 cells. Br J Pharmacol 2002;137:80–6.
[46] Hay DL, Poyner DR, Sexton PM. GPCR modulation by RAMPs. Pharmacol Ther 2006;109:173–97.

[47] Hay DL, Walker CS, Poyner DR. Adrenomedullin and calcitonin gene-related peptide receptors in endocrine-related cancers: opportunities and challenges. Endocr Relat Cancer 2011;18:C1–14.
[48] Hilairet S, Belanger C, Bertrand J, Laperriere A, Foord SM, Bouvier M. Agonist-promoted internalization of a ternary complex between calcitonin receptor-like receptor, receptor activity-modifying protein 1 (RAMP1), and beta-arrestin. J Biol Chem 2001;276:42182–90.
[49] Hilairet S, Foord SM, Marshall FH, Bouvier M. Protein-protein interaction and not glycosylation determines the binding selectivity of heterodimers between the calcitonin receptor-like receptor and the receptor activity-modifying proteins. J Biol Chem 2001;276:29575–81.
[50] Hiragushi K, Wada J, Eguchi J, Matsuoka T, Yasuhara A, Hashimoto I, et al. The role of adrenomedullin and receptors in glomerular hyperfiltration in streptozotocin-induced diabetic rats. Kidney Int 2004;65:540–50.
[51] Hirose T, Totsune K, Mori N, Mori T, Morimoto R, Metoki H, et al. Expression of adrenomedullin 2/intermedin, a possible reno-protective peptide, is decreased in the kidneys of rats with hypertension or renal failure. Am J Physiol Renal Physiol 2010;299:F128–34.
[52] Hirose T, Totsune K, Mori N, Morimoto R, Hashimoto M, Nakashige Y, et al. Increased expression of adrenomedullin 2/intermedin in rat hearts with congestive heart failure. Eur J Heart Fail 2008;10:840–9.
[53] Ho TW, Ferrari MD, Dodick DW, Galet V, Kost J, Fan X, et al. Efficacy and tolerability of MK-0974 (telcagepant), a new oral antagonist of calcitonin gene-related peptide receptor, compared with zolmitriptan for acute migraine: a randomised, placebo-controlled, parallel-treatment trial. Lancet 2008;372:2115–23.
[54] Honda M, Nakagawa S, Hayashi K, Kitagawa N, Tsutsumi K, Nagata I, et al. Adrenomedullin improves the blood–brain barrier function through the expression of claudin-5. Cell Mol Neurobiol 2006;26:109–18.
[55] Husmann K, Born W, Fischer JA, Muff R. Three receptor-activity-modifying proteins define calcitonin gene-related peptide or adrenomedullin selectivity of the mouse calcitonin-like receptor in COS-7 cells. Biochem Pharmacol 2003;66:2107–15.
[56] Husmann K, Sexton PM, Fischer JA, Born W. Mouse receptor-activity-modifying proteins 1, -2 and -3: amino acid sequence, expression and function. Mol Cell Endocrinol 2000;162:35–43.
[57] Ichikawa-Shindo Y, Sakurai T, Kamiyoshi A, Kawate H, Iinuma N, Yoshizawa T, et al. The GPCR modulator protein RAMP2 is essential for angiogenesis and vascular integrity. J Clin Invest 2008;118:29–39.
[58] Iimuro S, Shindo T, Moriyama N, Amaki T, Niu P, Takeda N, et al. Angiogenic effects of adrenomedullin in ischemia and tumor growth. Circ Res 2004;95:415–23.
[59] Ishimitsu T, Ono H, Minami J, Matsuoka H. Pathophysiologic and therapeutic implications of adrenomedullin in cardiovascular disorders. Pharmacol Ther 2006;111:909–27.
[60] Jia YX, Yang JH, Pan CS, Geng B, Zhang J, Xiao Y, et al. Intermedin1-53 protects the heart against isoproterenol-induced ischemic injury in rats. Eur J Pharmacol 2006;549:117–23.
[61] Jin D, Harada K, Ohnishi S, Yamahara K, Kangawa K, Nagaya N. Adrenomedullin induces lymphangiogenesis and ameliorates secondary lymphoedema. Cardiovasc Res 2008;80:339–45.
[62] Kamoi H, Kanazawa H, Hirata K, Kurihara N, Yano Y, Otani S. Adrenomedullin inhibits the secretion of cytokine-induced neutrophil chemoattractant, a member of the interleukin-8 family, from rat alveolar macrophages. Biochem Biophys Res Commun 1995;211:1031–5.
[63] Kataoka Y, Miyazaki S, Yasuda S, Nagaya N, Noguchi T, Yamada N, et al. The first clinical pilot study of intravenous adrenomedullin administration in patients with acute myocardial infarction. J Cardiovasc Pharmacology 2010;56:413–9.
[64] Kato J, Tsuruda T, Kita T, Kitamura K, Eto T. Adrenomedullin: a protective factor for blood vessels. Arterioscler Thromb Vasc Biol 2005;25:2480–7.
[65] Kis B, Abraham CS, Deli MA, Kobayashi H, Niwa M, Yamashita H, et al. Adrenomedullin, an autocrine mediator of blood–brain barrier function. Hypertens Res: Official J Jpn Soc Hypertens 2003;26(Suppl.):S61–70.
[66] Kis B, Kaiya H, Nishi R, Deli MA, Abraham CS, Yanagita T, et al. Cerebral endothelial cells are a major source of adrenomedullin. J Neuroendocrinol 2002;14:283–93.
[67] Kitamura K, Kangawa K, Eto T. Adrenomedullin and PAMP: discovery, structures, and cardiovascular functions. Microsc Res Tech 2002;57:3–13.
[68] Kitamura K, Kangawa K, Kawamoto M, Ichiki Y, Nakamura S, Matsuo H, et al. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. Biochem Biophys Res Commun 1993;192:553–60.
[69] Koller D, Born W, Leuthauser K, Fluhmann B, McKinney RA, Fischer JA, et al. The extreme N-terminus of the calcitonin-like receptor contributes to the selective interaction with adrenomedullin or calcitonin gene-related peptide. FEBS Lett 2002;531:464–8.
[70] Koller D, Ittner LM, Muff R, Husmann K, Fischer JA, Born W. Selective inactivation of adrenomedullin over calcitonin gene-related peptide receptor function by the deletion of amino acids 14-20 of the mouse calcitonin-like receptor. J Biol Chem 2004;279:20387–91.
[71] Kusano S, Kukimoto-Niino M, Akasaka R, Toyama M, Terada T, Shirouzu M, et al. Crystal structure of the human receptor activity-modifying protein 1 extracellular domain. Protein Sci 2008;17:1907–14.
[72] Kuwasako K, Cao YN, Chu CP, Iwatsubo S, Eto T, Kitamura K. Functions of the cytoplasmic tails of the human receptor activity-modifying protein components of calcitonin gene-related peptide and adrenomedullin receptors. J Biol Chem 2006;281:7205–13.
[73] Kuwasako K, Cao YN, Nagoshi Y, Kitamura K, Eto T. Adrenomedullin receptors: pharmacological features and possible pathophysiological roles. Peptides 2004;25:2003–12.
[74] Kuwasako K, Cao YN, Nagoshi Y, Tsuruda T, Kitamura K, Eto T. Characterization of the human calcitonin gene-related peptide receptor subtypes associated with receptor activity-modifying proteins. Mol Pharmacol 2004;65:207–13.
[75] Kuwasako K, Kitamura K, Ito K, Uemura T, Yanagita Y, Kato J, et al. The seven amino acids of human RAMP2 (86) and RAMP3 (59) are critical for agonist binding to human adrenomedullin receptors. J Biol Chem 2001;276:49459–65.
[76] Kuwasako K, Kitamura K, Nagata S, Hikosaka T, Kato J. Function of the cytoplasmic tail of human calcitonin receptor-like receptor in complex with receptor activity-modifying protein 2. Biochem Biophys Res Commun 2010;392:380–5.
[77] Kuwasako K, Kitamura K, Nagata S, Kato J. Functions of the extracellular histidine residues of receptor activity-modifying proteins vary within adrenomedullin receptors. Biochem Biophys Res Commun 2008;377:109–13.
[78] Kuwasako K, Kitamura K, Nagoshi Y, Cao YN, Eto T. Identification of the human receptor activity-modifying protein 1 domains responsible for agonist binding specificity. J Biol Chem 2003;278:22623–30.
[79] Kuwasako K, Kitamura K, Uemura T, Nagoshi Y, Kato J, Eto T. The function of extracellular cysteines in the human adrenomedullin receptor. Hypertens Res 2003;26(Suppl.):S25–31.
[80] Kuwasako K, Shimekake Y, Masuda M, Nakahara K, Yoshida T, Kitaura M, et al. Visualization of the calcitonin receptor-like receptor and its receptor activity-modifying proteins during internalization and recycling. J Biol Chem 2000;275:29602–9.
[81] Li Y, Jiang C, Wang X, Zhang Y, Shibahara S, Takahashi K. Adrenomedullin is a novel adipokine: adrenomedullin in adipocytes and adipose tissues. Peptides 2007;28:1129–43.
[82] Liang L, Tam CW, Pozsgai G, Siow R, Clark N, Keeble J, et al. Protection of angiotensin II-induced vascular hypertrophy in vascular smooth muscle-targeted receptor activity-modifying protein 2 transgenic mice. Hypertension 2009;54:1254–61.
[83] Matsui E, Kitamura K, Yoshida M, Kato J, Asada Y, Sumiyoshi A, et al. Biosynthesis and secretion of adrenomedullin and proadrenomedullin N-terminal 20 peptide in a rat model of endotoxin shock. Hypertens Res 2001;24:543–9.
[84] McLatchie LM, Fraser NJ, Main MJ, Wise A, Brown J, Thompson N, et al. RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. Nature 1998;393:333–9.
[85] Miret JJ, Rakhilina L, Silverman L, Oehlen B. Functional expression of heteromeric calcitonin gene-related peptide and adrenomedullin receptors in yeast. J Biol Chem 2002;277:6881–7.
[86] Muff R, Born W, Fischer JA. Adrenomedullin selectivity of calcitonin-like receptor/receptor activity modifying proteins. Hypertens Res 2003;26(Suppl.):S3–8.
[87] Nagae T, Mukoyama M, Sugawara A, Mori K, Yahata K, Kasahara M, et al. Rat receptor-activity-modifying proteins (RAMPs) for adrenomedullin/CGRP receptor: cloning and upregulation in obstructive nephropathy. Biochem Biophys Res Commun 2000;270:89–93.
[88] Nagaya N, Mori H, Murakami S, Kangawa K, Kitamura S. Adrenomedullin: angiogenesis and gene therapy. Am J Physiol Regul, Integr Comp Physiol 2005;288:R1432–7.
[89] Nagoshi Y, Kuwasako K, Ito K, Uemura T, Kato J, Kitamura K, et al. The calcitonin receptor-like receptor/receptor activity-modifying protein 1 heterodimer can function as a calcitonin gene-related peptide-(8-37)-sensitive adrenomedullin receptor. Eur J Pharmacol 2002;450:237–43.
[90] Nakamura R, Kato J, Kitamura K, Onitsuka H, Imamura T, Cao Y, et al. Adrenomedullin administration immediately after myocardial infarction ameliorates progression of heart failure in rats. Circulation 2004;110:426–31.
[91] Nikitenko LL, Blucher N, Fox SB, Bicknell R, Smith DM, Rees MC. Adrenomedullin and CGRP interact with endogenous calcitonin-receptor-like receptor in endothelial cells and induce its desensitisation by different mechanisms. J Cell Sci 2006;119:910–22.
[92] Nikitenko LL, Fox SB, Kehoe S, Rees MC, Bicknell R. Adrenomedullin and tumour angiogenesis. Br J Cancer 2006;94:1–7.
[93] Nishikimi T. Adrenomedullin in the kidney-renal physiological and pathophysiological roles. Curr Med Chem 2007;14:1689–99.
[94] Nishikimi T, Horio T, Yoshihara F, Nagaya N, Matsuo H, Kangawa K. Effect of adrenomedullin on cAMP and cGMP levels in rat cardiac myocytes and nonmyocytes. Eur J Pharmacol 1998;353:337–44.
[95] Nishikimi T, Karasawa T, Inaba C, Ishimura K, Tadokoro K, Koshikawa S, et al. Effects of long-term intravenous administration of adrenomedullin (AM) plus hANP therapy in acute decompensated heart failure: a pilot study. Circulation J: Official J Jpn Circulation Soc 2009;73:892–8.
[96] Nishikimi T, Tadokoro K, Mori Y, Wang X, Akimoto K, Yoshihara F, et al. Ventricular adrenomedullin system in the transition from LVH to heart failure in rats. Hypertension 2003;41:512–8.
[97] Nishio K, Akai Y, Murao Y, Doi N, Ueda S, Tabuse H, et al. Increased plasma concentrations of adrenomedullin correlate with relaxation of vascular tone in patients with septic shock. Crit Care Med 1997;25:953–7.
[98] Oie E, Ahmed MS, Ueland T, Sikkeland LI, Dahl CP, Hagelin EM, et al. Adrenomedullin is increased in alveolar macrophages and released from the lungs into the circulation in severe heart failure. Basic Res Cardiol 2010;105:89–98.

[99] Olesen J, Diener HC, Husstedt IW, Goadsby PJ, Hall D, Meier U, et al. Calcitonin gene-related peptide receptor antagonist BIBN 4096 BS for the acute treatment of migraine. N Engl J Med 2004;350:1104–10.
[100] Oliver KR, Kane SA, Salvatore CA, Mallee JJ, Kinsey AM, Koblan KS, et al. Cloning, characterization and central nervous system distribution of receptor activity modifying proteins in the rat. Eur J Neurosci 2001;14:618–28.
[101] Onitsuka H, Imamura T, Ito K, Kuwasako K, Yamakawa H, Hirano S, et al. Differential gene expression of adrenomedullin receptors in pressure and volumeoverloaded heart role of angiotensin II. Peptides 2004;25:1107–14.
[102] Ono Y, Okano I, Kojima M, Okada K, Kangawa K. Decreased gene expression of adrenomedullin receptor in mouse lungs during sepsis. Biochem Biophys Res Commun 2000;271:197–202.
[103] Oya H, Nagaya N, Furuichi S, Nishikimi T, Ueno K, Nakanishi N, et al. Comparison of intravenous adrenomedullin with atrial natriuretic peptide in patients with congestive heart failure. Am J Cardiol 2000;86:94–8.
[104] Pan Ch S, Jiang W, Wu Sh Y, Zhao J, Pang YZ, Tang Ch S, et al. Potentiated response to adrenomedullin in myocardia and aortas in spontaneously hypertensive rat. Basic Res Cardiol 2006;101:193–203.
[105] Pan CS, Jiang W, Zhong GZ, Zhao J, Pang YZ, Tang CS, et al. Hypertension induced by nitric oxide synthase inhibitor increases responsiveness of ventricular myocardium and aorta of rat tissue to adrenomedullin stimulation in vitro. Life Sci 2005;78:398–405.
[106] Pan CS, Qi YF, Wu SY, Jiang W, Li GZ, Tang CS. The role of adrenomedullin and its receptor system in cardiovascular calcification of rat induced by Vitamin D(3) plus nicotine. Peptides 2004;25:601–8.
[107] Parameswaran N, Spielman WS. RAMPs: the past, present and future. Trends Biochem Sci 2006;31:631–8.
[108] Poyner DR, Sexton PM, Marshall I, Smith DM, Quirion R, Born W, et al. International Union of Pharmacology, XXXII. The mammalian calcitonin gene-related peptides, adrenomedullin, amylin, and calcitonin receptors. Pharmacol Rev 2002;54:233–46.
[109] Qi T, Christopoulos G, Bailey RJ, Christopoulos A, Sexton PM, Hay DL. Identification of N-terminal receptor activity-modifying protein residues important for calcitonin gene-related peptide, adrenomedullin, and amylin receptor function. Mol Pharmacol 2008;74:1059–71.
[110] Qi T, Hay DL. Structure–function relationships of the N-terminus of receptor activity-modifying proteins. Br J Pharmacol 2010;159:1059–68.
[111] Qi T, Ly K, Poyner DR, Christopoulos G, Sexton PM, Hay DL. Structure–function analysis of amino acid 74 of human RAMP1 and RAMP3 and its role in peptide interactions with adrenomedullin and calcitonin gene-related peptide receptors. Peptides 2011.
[112] Qi YF, Shi YR, Bu DF, Pang YZ, Tang CS. Changes of adrenomedullin and receptor activity modifying protein 2 (RAMP2) in myocardium and aorta in rats with isoproterenol-induced myocardial ischemia. Peptides 2003;24:463–8.
[113] Robinson SD, Aitken JF, Bailey RJ, Poyner DR, Hay DL. Novel peptide antagonists of adrenomedullin and calcitonin gene-related peptide receptors: identification, pharmacological characterization, and interactions with position 74 in receptor activity-modifying protein 1/3. J Pharmacol Exp Ther 2009;331:513–21.
[114] Roh J, Chang CL, Bhalla A, Klein C, Hsu SY. Intermedin is a calcitonin/calcitonin gene-related peptide family peptide acting through the calcitonin receptor-like receptor/receptor activity-modifying protein receptor complexes. J Biol Chem 2004;279:7264–74.
[115] Sexton PM, Albiston A, Morfis M, Tilakaratne N. Receptor activity modifying proteins. Cell Signal 2001;13:73–83.
[116] Sexton PM, Poyner DR, Simms J, Christopoulos A, Hay DL. Modulating receptor function through RAMPs: can they represent drug targets in themselves? Drug Discov Today 2009;14:413–9.
[117] Shimosawa T, Fujita T. Adrenomedullin as a potent antioxidative and antiatherosclerotic substance. Drug News Perspect 2005;18:185–9.
[118] Shindo T, Kurihara Y, Nishimatsu H, Moriyama N, Kakoki M, Wang Y, et al. Vascular abnormalities and elevated blood pressure in mice lacking adrenomedullin gene. Circulation 2001;104:1964–71.
[119] Simms J, Hay DL, Bailey RJ, Konycheva G, Bailey G, Wheatley M, et al. Structure–function analysis of RAMP1 by alanine mutagenesis. Biochemistry 2009;48:198–205.
[120] Simms J, Hay DL, Wheatley M, Poyner DR. Characterization of the structure of RAMP1 by mutagenesis and molecular modeling. Biophys J 2006;91:662–9.
[121] Siu FY, Stevens RC. RAMP-ing up class-B GPCR ECD structural coverage. Structure 2010;18:1067–8.
[122] Smith Jr RS, Gao L, Bledsoe G, Chao L, Chao J. Intermedin is a new angiogenic growth factor. Am J Physiol Heart Circulatory Physiol 2009;297:H1040–7.
[123] Steenbergh PH, Hoppener JW, Zandberg J, Lips CJ, Jansz HS. A second human calcitonin/CGRP gene. FEBS Lett 1985;183:403–7.
[124] Steiner S, Born W, Fischer JA, Muff R. The function of conserved cysteine residues in the extracellular domain of human receptor-activity-modifying protein. FEBS Lett 2003;555:285–90.
[125] Steiner S, Muff R, Gujer R, Fischer JA, Born W. The transmembrane domain of receptor-activity-modifying protein 1 is essential for the functional expression of a calcitonin gene-related peptide receptor. Biochemistry 2002;41:11398–404.
[126] Tachi Y, Fukui D, Wada Y, Koshikawa M, Shimodaira S, Ikeda U, et al. Changes in angiogenesis-related factors in serum following autologous bone marrow cell implantation for severe limb ischemia. Expert Opin Biol Therapy 2008;8:705–12.
[127] Tadokoro K, Nishikimi T, Mori Y, Wang X, Akimoto K, Matsuoka H. 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 2003;112:71–8.
[128] Takahashi K, Hirose T, Mori N, Morimoto R, Kohzuki M, Imai Y, et al. The renin-angiotensin system, adrenomedullins and urotensin II in the kidney: possible renoprotection via the kidney peptide systems. Peptides 2009;30:1575–85.
[129] Takahashi K, Morimoto R, Hirose T, Satoh F, Totsune K. Adrenomedullin 2/intermedin in the hypothalamo-pituitary-adrenal axis. J Mol Neurosci 2011;43:182–92.
[130] Takei Y, Hyodo S, Katafuchi T, Minamino N. Novel fish-derived adrenomedullin in mammals: structure and possible function. Peptides 2004;25:1643–56.
[131] Takei Y, Inoue K, Ogoshi M, Kawahara T, Bannai H, Miyano S. Identification of novel adrenomedullin in mammals: a potent cardiovascular and renal regulator. FEBS Lett 2004;556:53–8.
[132] Tam CW, Husmann K, Clark NC, Clark JE, Lazar Z, Ittner LM, et al. Enhanced vascular responses to adrenomedullin in mice overexpressing receptor-activity-modifying protein 2. Circ Res 2006;98:262–70.
[133] Taylor MM, Bagley SL, Samson WK. Intermedin/Adrenomedullin-2 inhibits growth hormone release from cultured, primary anterior pituitary cells. Endocrinology 2006;147:859–64.
[134] ter Haar E, Koth CM, Abdul-Manan N, Swenson L, Coll JT, Lippke JA, et al. Crystal structure of the ectodomain complex of the CGRP receptor, a class-B GPCR, reveals the site of drug antagonism. Structure 2010;18:1083–93.
[135] Tilakaratne N, Christopoulos G, Zumpe ET, Foord SM, Sexton PM. Amylin receptor phenotypes derived from human calcitonin receptor/RAMP coexpression exhibit pharmacological differences dependent on receptor isoform and host cell environment. J Pharmacol Exp Ther 2000;294:61–72.
[136] Tomoda Y, Isumi Y, Katafuchi T, Minamino N. Regulation of adrenomedullin secretion from cultured cells. Peptides 2001;22:1783–94.
[137] Tomoda Y, Kikumoto K, Isumi Y, Katafuchi T, Tanaka A, Kangawa K, et al. Cardiac fibroblasts are major production and target cells of adrenomedullin in the heart in vitro. Cardiovasc Res 2001;49:721–30.
[138] Totsune K, Takahashi K, Mackenzie HS, Arihara Z, Satoh F, Sone M, et al. Adrenomedullin and its receptor complexes in remnant kidneys of rats with renal mass ablation: decreased expression of calcitonin receptor-like receptor and receptor-activity modifying protein-3. Peptides 2001;22:1933–7.
[139] Tsuruda T, Kato J, Kitamura K, Kuwasako K, Imamura T, Koiwaya Y, et al. Adrenomedullin: a possible autocrine or paracrine inhibitor of hypertrophy of cardiomyocytes. Hypertension 1998;31:505–10.
[140] Udawela M, Christopoulos G, Morfis M, Christopoulos A, Ye S, Tilakaratne N, et al. A critical role for the short intracellular C terminus in receptor activity-modifying protein function. Mol Pharmacol 2006;70:1750–60.
[141] Wimalawansa SJ. Amylin, calcitonin gene-related peptide, calcitonin, and adrenomedullin: a peptide superfamily. Crit Rev Neurobiol 1997;11:167–239.
[142] Wunder F, Rebmann A, Geerts A, Kalthof B. Pharmacological and kinetic characterization of adrenomedullin 1 and calcitonin gene-related peptide 1 receptor reporter cell lines. Mol Pharmacol 2008;73:1235–43.
[143] Yamaguchi T, Baba K, Doi Y, Yano K. Effect of adrenomedullin on aldosterone secretion by dispersed rat adrenal zona glomerulosa cells. Life Sci 1995;56:379–87.
[144] Yamaguchi T, Baba K, Doi Y, Yano K, Kitamura K, Eto T. Inhibition of aldosterone production by adrenomedullin, a hypotensive peptide, in the rat. Hypertension 1996;28:308–14.
[145] Zhao Y, Bell D, Smith LR, Zhao L, Devine AB, McHenry EM, et al. Differential expression of components of the cardiomyocyte adrenomedullin/intermedin receptor system following blood pressure reduction in nitric oxide-deficient hypertension. J Pharmacol Exp Ther 2006;316:1269–81.

Adrenomedullin: Roles for Structure and Function in Cardiac or Vascular Tissues

Toshihiro Tsuruda* and Kazuo Kitamura

Department of Internal Medicine, Circulatory and Body Fluid Regulation, Faculty of Medicine, University of Miyazaki, Japan

Abstract: Adrenomedullin (AM) is a unique bioactive molecule, originally isolated from human pheochromocytoma by monitoring cyclic adenosine monophosphate (cAMP) elevation in platelets. PreproAM mRNA and its translated peptide have been recognized to be widely distributed in the organs of rodents and humans, including heart and vasculature. AM exhibits vasorelaxant activity working on vascular endothelial cells and smooth muscle cells. In addition, AM modulates left ventricular contractility and remodeling in the hypertrophied/failing heart, and alters the structural integrity of the vascular wall. Furthermore, immunocompetent cells, such as macrophage-, and mast cell-derived AM might contribute to the pathogenesis of cardiovascular disorders. Most biological actions mediate cAMP-protein kinase A signaling, whereas cAMP-independent pathways, such as the nitric oxide/soluble guanylate cyclase/cGMP pathway, modulated in molecules/signaling associated with anti-oxidative stress and anti-apoptotic pathways, are also reported. Overall, the actions of AM are assumed to be beneficial against vasoconstrictive factors activated in the diseased heart and vascular wall, whereas some reports imply that the biological activity of AM might be dependent on circumstances. Specifically, inotrophic action, activation of adhesion molecules and smooth muscle proliferation by AM has been debated. In this review, we will present the recent advances in AM research, and discuss the controversy of AM actions in cardiac and vascular tissues.

Keywords: Atherosclerosis, contractility, fibrosis, heart failure, myocyte hypertrophy, remodeling.

INTRODUCTION

Adrenomedullin (AM) is a potent vasodilatory peptide that was originally isolated from human pheochromocytoma [1]; however, we now know that preproAM mRNA and its translated peptide are widely distributed in tissues and organs, including heart and vasculature [2]. Specifically, AM synthesis and peptide concentration are increased in the hypertrophied and failing heart, and injured vasculature [3-5]. In this review article, we introduce the recent advances in AM research and discuss controversies in cardiac or vascular tissues.

Is the AM Effect on LV Remodeling Beneficial or Detrimental?

It is fundamental for the heart to adapt the cellular response to mechanical load by hypertrophy of cardiomyocytes and hyperplasia of cardiac fibroblasts in order to maintain structural integrity and function; however, it becomes maladaptative to long-term, inadequate stimuli. It is of great interest that AM production is augmented by hypertrophic/hyperplastic stimuli, such as angiotensin II [6-8], endothelin-1 [9] and mechanical stretching [10]. AM is capable of inhibiting cardiomyocyte hypertrophy and fibroblast proliferation/collagen synthesis *in vitro* [6, 7, 11]. A number of animal studies have support the *in vitro* observation that AM exerts beneficial effects on cardiac hypertrophy without affecting systemic blood pressure in models of chronic nitric oxide deficiency combined with pressure overload [12], pressure overload or angiotensin II stimulation in genetically modified AM heterozygotes [13], and of ischemia/reperfusion [14]. However, the anti-remodeling property of AM might be dependent on the situation; overexpression of AM by adenovirus-mediated gene transfer resulted in beneficial effects on angiotensin II-induced cardiac hypertrophy in rats; in contrast, the authors found profound dilatation in post-infarcted LV following AM overexpression [15]. Potential mechanisms include a delayed healing process, probably due to anti-inflammatory action [16], decreased proliferation [7, 11] and activation of fibroblasts [17], and the stimulation of extracellular matrix enzymes [18]; therefore, the question remains to be resolved of "when" AM should be administered to the diseased heart. The "amount" of AM necessary to administer seems to be another concern for future clinical application.

Does AM have a Stronger or Weaker Effect on Myocardial Contractility?

Activation of the stimulatory G protein (Gαs)-adenylate cyclase-cyclic adenosine monophosphate (cAMP) system is one of the major pathways for the stimulation of cardiac

*Address correspondence to this author at the Department of Internal Medicine, Circulatory and Body Fluid Regulation, Faculty of Medicine, University of Miyazaki, 5200 Kihara Kiyotake, Miyazaki 889-1692, Japan; Tel: +81-985-85-0872; Fax: +81-985-85-6596;
E-mail: ttsuruda@med.miyazaki-u.ac.jp

1573-4021/11 $58.00+.00

contractility in the mammalian heart. The level of Gαs is inactivated and internalized in the failing heart, whereas the level of inhibitory G protein (Gαi) is increased [19]. Because AM exerts biological action by cAMP elevation, it is supposed to directly increase the contractility by Gαs-mediated adenylate cyclase-cAMP; however, the inotrophic effects of AM are inconclusive; a positive inotrophic effect of AM was reported in papillary muscles (trabecullae) of rats [20] and human atrium and ventricles [21], and in perfused whole rat heart [22]. On the other hand, negative inotrophic effects have been reported in the rabbit papillary muscle [23], isolated ventricular myocyte of adult rats [24], rabbits [25] and humans [26]. Lainchbury *et al.* [27] reported that AM did not influence contractility in dogs. Although the preparation of myocardial tissues from different species, experimental conditions, and concentration of AM used might have affected the inconsistent results, Mittra and Bourreau [24] reported that long-time exposure to AM by adult rat cardiomyocytes switched the sensitivity of receptor activity-modifying protein (RAMP) coupled with Gαs to Gαi, resulting in changing positive inotrophic effects to negative effects [24].

Are Non-myocytes (Fibroblasts) a Dominant Target for AM?

The effect of AM on inhibiting proliferation/collagen in non-myocytes (cardiac fibroblasts) was consistent in Horio *et al.*'s [11] and our results [7], whereas the biological activity of AM on cardiac myocyte hypertrophy was inconsistent between the two groups [6, 11]. It could be argued that AM preferentially acts on fibroblasts, but not on cardiomyocytes, and contaminated fibroblasts (~10%) by the isolation technique of cardiomyocytes would lead to the decreased incorporation of ^{14}C-labeled phenylalanine; however, we confirmed that cardiomyocyte size was decreased by AM under microscopic observation [28]. Harada *et al.* [29] reported a critical role for fibroblasts in cardiomyocyte hypertrophy, and we speculate that AM reduced cardiomyocyte hypertrophy in part though the modulation of fibroblast activation, and that undetermined substances secreted from fibroblasts might have influenced myocyte hypertrophy. Subsequently, we reported that a sub-depressor dose of AM administration to angiotensin II-induced hypertensive rats for two weeks preferentially inhibited collagen synthesis and deposition without affecting cardiocyte hypertrophy [17]. Taken together, AM exhibited the biological activity in cultured fibroblasts with a lower concentration ($\geq 10^{-8}$ mol/L) than in myocytes ($\geq 10^{-7}$ mol/L), and we support the hypothesis that the main cellular target for AM is fibroblasts in the heart [30].

Is AM Good, Bad or Ugly for Vascular Structural Integrity?

The arterial wall is composed of 3 layers (intima, media and adventitia). The intima consists of a monolayer of endothelial cells. The media is the main structural component supported by smooth muscle cells and extra-cellular matrix elements, such as elastin, collagen and fibronectin [31]. The adventitia is populated by terminal nerve fibers, vasa vasorum and surrounding connective tissue, which contains a few resident fibroblasts and inflammatory cells. Vascular protection of AM was previously reviewed [32], and we discuss the action of AM categorized into 3 layers in this review. **Intima:** Leukocyte adhesion to vascular endothelium is an essential event in the development of atherosclerosis, in which E-selectin, intracellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 play important roles in the process. The effect of AM on the activation of adhesion molecules is under debate. AM has been reported to be capable of inhibiting the expressions of E-selectin, VCAM-1 and ICAM-1 induced by vascular endothelial growth factor in human umbilical vein endothelial cells (HUVEC) [33]. On the other hand, Hagi-Pavli *et al.* [34] reported that AM increased these expressions in a dose-dependent manner in the cells. The stimulation period (4 hour) and concentration of AM (10^{-12} ~10^{-8} mol/L), and the cell source (HUVEC) were consistent in the two studies. The only difference was in the experimental protocol; whether HUVEC treated by AM was stimulated with a pharmacological agent such as vascular endothelial growth factor. **Media:** The effect of AM on smooth muscle proliferation also seems controversial. Kohno *et al.* [35, 36] showed that AM inhibited rat aortic smooth muscle cell proliferation in serum-containing medium or attenuated migration in angiotensin II-stimulated human coronary artery smooth muscle cells. On the other hand, Shichiri *et al.* [37] reported that AM is mitogenic in rat aortic vascular smooth muscle cells. They added synthetic AM directly to the culture medium in the serum-free medium without any growth-promoting agents. In an animal study, AM administration/gene delivery has been reported to inhibit neointimal hyperplasia by attenuating smooth muscle migration/proliferation and by inducing the apoptosis of myofibroblasts in a rat injured carotid artery [38, 39]. In addition, neointimal hyperplasia induced by a cuff on the femoral artery was attenuated in AM transgenic mice [40]. Moreover, overexpression of RAMP2 exhibited reduced aortic medial thickness without affecting systemic blood pressure induced by angiotensin II in mice [41]. The authors also demonstrated that cultured vascular smooth muscle cells obtained from RAMP2 transgenic mice showed a slower growth compared with wild types, and blocking the action of endogenous AM with an AM receptor antagonist, AM_{22-52}, promoted cell proliferation in the presence of angiotensin II in these cells. However, Shichiri's group extended their *in vitro* study, showing that the blockade of AM action with another type of AM receptor antagonist, calcitonin gene-related peptide $(CGRP)_{8-37}$, led to the inhibition of neointimal hyperplasia following a balloon injury in the rat carotid artery [42]. Although the majority of reports addressed the issue supporting the anti-proliferative action of AM in smooth muscle cells, a concern might be raised whether AM has a divergent action that is dependent on the circumstances. **Adventitia:** A few data are available for AM action on the adventitial layer. We and others have consistently suggested that AM might have anti-remodeling effects by inhibiting the proliferation and activation of

adventitial fibroblasts [17, 39, 43], and by stimulating matrix metalloproteinase-2 activity [18]. Considering the importance of extracellular matrix formation in the adventitial layer in determining the stiffness of the vascular wall [44], AM may exert a beneficial action alleviating vascular stiffness; however, extracellular matrix degraded by excessive activity of AM might cause the aortic structure to weaken, exaggerating outward remodeling.

Decreased uptake of AM to the arteries along with reduced RAMP2 expression was reported in a model of pulmonary hypertension induced by monocrotaline [45]. In addition, vasodilatory action of AM diminishes in the setting of heart failure [27], probably due to digestion of the amino-acid sequence of AM by vasopeptidase [46] and/or by matrix metalloproteinase-2 [47]. This suggests that significant roles of AM and its receptor system contribute to the pathogenesis of cardiovascular disorders, and that AM infusion would complement the relatively insufficient activity of this peptide under these situations.

Is Immunoregulatory Cell-Derived AM an Additional Player?

Atherosclerotic plaque contains a number of macrophages (foam cells) that contribute to inflammatory processes that promote thrombosis by stimulating the production of collagen-degrading proteinases [48]. Mast cell number is increased to distribute and activate in the interstitium of ischemic/dilated cardiomyopathy [49] and in the adventitia of the atherosclerotic aorta and aneurysm [50]. AM was reported to be produced in macrophages [51-53] and mast cells [54, 55]. There are few reports with regards to the biological activity of AM on macrophages (ex. M1, M2 phenotypes, matrix metalloproteinase). Mast cells release a number of substances such as histamine, trypsin and chymase as well as proinflammatory cytokines (interleukin-6 and interferon-γ) to possibly modulate the atherosclerotic process [56]. More interestingly, mast cells contain vasoactive peptides, such as atrial natriuretic peptide, B-type natriuretic peptide, AM, endothelin-1 and relaxin [54]. Degranulated mast cells are assumed to evoke myocardial and vascular injury by stimulating vasoconstriction, leukocyte recruitment and extracellular matrix alternation, whereas mast cell-derived natriuretic peptides, AM or relaxin might be protective against vasoconstrictive factors [57]. We have demonstrated that synthetic AM inhibited collagen synthesis in the co-culture of a mast cell line with adventitial fibroblasts, whereas neutralization of AM secreted from the cells stimulated collagen synthesis [55]. It remains to be elucidated to what extent AM derived from these immuno-competant cells contributes to the pathogenesis, because the concentration appears to be lower than in other cell types of the myocardium or vascular wall.

Does AM have Multiple Pathways for Cardiovascular Protection?

The underlying mechanisms by which AM protects against cellular damage remain unknown. AM was initially isolated from human pheochromocytoma by monitoring cAMP elevation in platelets [1]; however, there are several reports showing that the action of AM is cAMP-independent [22, 58], in which the nitric oxide/soluble guanylate cyclase/cGMP pathway is dominant [59]. The mechanisms also involve the decrease in oxidative stress [59], apoptosis determined by TUNEL-positive nuclei in myocytes as well as non-myocytes [14], accompanied by reduced levels of BAX, cleaved caspase-3 and phosphorylation of p38, and by increased phosphorylation of Akt and Bad [59], and by the opening of large conductance Ca^{2+}-activated K^+ channels in the mitochondrial inner membrane [60] and the induction of heat-shock protein 72 [61].

What is the New Era for AM2/Intermedin in Heart and Vasculature Research?

AM2/intermedin is a newly identified member of the CGRP superfamily [62, 63]. The putative mature bioactive AM2 peptide consists of 47 amino acids, which share 34% sequence homology with AM and <20% similarity with CGRP. Similarly to AM, AM2/intermedin expression was increased in the hypertensive left ventricle and aorta in rats

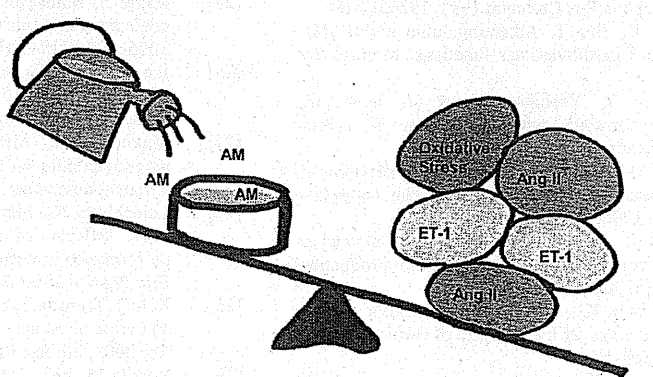


Fig. (1). AM supplementation for potential clinical application in cardiovascular diseases. AM, adrenomedullin; Ang II, angiotensin II; ET-1, endothelin-1

[64, 65], and was reported to stimulate its production under angiotensin II stimulation. It exerts an anti-hypertrophic response in cultured neonatal cardiomyocytes [66], and protects against myocardial ischemia-reperfusion injury [67]. Intravenous infusion of AM2/intermedin showed beneficial effects on hemodynamics (increased cardiac output, decreased vascular resistance) and endocrine profiles (decreased plasma renin activity and brain natriuretic peptide concentration) [68]. So far, AM2/intermedin seems to have similar biological properties to AM.

CONCLUSION

Considering with the published articles, we agree that AM works as a protective factor in the damaged heart and vasculature. AM appears to relieve the "scene of fire from a raging storm". On the other hand, we understand that the action of AM might be dependent on the circumstance. Fig. (1) illustrates the relatively insufficient activity of AM in cardiovascular diseases. The adequate amount and appropriate timing of AM supplementation could overcome maladaptative stimuli, such as oxidative stress, angiotensin II and endothelin-1, resulting in attenuating of the progression of the disorders.

CONFLICTS OF INTEREST

Declared none.

ACKNOWLEDGEMENTS

The authors wish to acknowledge Ms. Mariko Tokashiki for the illustration. This study was supported by grants-in-aid for Scientific Research (C) (23591052) from the Ministry of Education, Culture, Sport, Science and Technology, Japan, Mitsubishi Pharma Research Foundation and Takeda Science Foundation.

REFERENCES

[1] Kitamura K, Kangawa K, Kawamoto M, *et al.* Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. Biochem Biophys Res Commun 1993; 192: 553-60.

[2] Kitamura K, Kangawa K, Eto T. Adrenomedullin and PAMP: discovery, structures, and cardiovascular functions. Microsc Res Tech 2002; 57: 3-13.

[3] Morimoto A, Nishikimi T, Yoshihara F, *et al.* Ventricular adrenomedullin levels correlate with the extent of cardiac hypertrophy in rats. Hypertension 1999; 33: 1146-52.

[4] Jougasaki M, Wei CM, McKinley LJ, Burnett JC, Jr. Elevation of circulating and ventricular adrenomedullin in human congestive heart failure. Circulation 1995; 92: 286-89.

[5] Hojo Y, Ikeda U, Ohya K, *et al.* Interaction between monocytes and vascular endothelial cells induces adrenomedullin production. Atherosclerosis 2001; 155: 381-87.

[6] Tsuruda T, Kato J, Kitamura K, *et al.* Adrenomedullin: a possible autocrine or paracrine inhibitor of hypertrophy of cardiomyocytes. Hypertension 1998; 31: 505-10.

[7] Tsuruda T, Kato J, Kitamura K, *et al.* An autocrine or a paracrine role of adrenomedullin in modulating cardiac fibroblast growth. Cardiovasc Res 1999; 43: 958-67.

[8] Onitsuka H, Imamura T, Yamaga J, Kuwasako K, Kitamura K, Eto T. Angiotensin II stimulates cardiac adrenomedullin production and causes accumulation of mature adrenomedullin independently of hemodynamic stress *in vivo*. Horm Metab Res 2005; 37: 281-85.

[9] Mishima K, Kato J, Kuwasako K, *et al.* Effects of endothelin on adrenomedullin secretion and expression of adrenomedullin receptors in rat cardiomyocytes. Biochem Biophys Res Commun 2001; 287: 264-69.

[10] Tsuruda T, Kato J, Kitamura K, *et al.* Enhanced adrenomedullin production by mechanical stretching in cultured rat cardiomyocytes. Hypertension 2000; 35: 1210-14.

[11] Horio T, Nishikimi T, Yoshihara F, Matsuo H, Takishita S, Kangawa K. Effects of adrenomedullin on cultured rat cardiac myocytes and fibroblasts. Eur J Pharmacol 1999; 382: 1-9.

[12] Bell D, Campbell M, Wang X, Earle JA, Cosby SL, McDermott BJ. Adrenomedullin gene delivery is cardio-protective in a model of chronic nitric oxide deficiency combining pressure overload, oxidative stress and cardiomyocyte hypertrophy. Cell Physiol Biochem 2010; 26: 383-94.

[13] Niu P, Shindo T, Iwata H, *et al.* Protective effects of endogenous adrenomedullin on cardiac hypertrophy, fibrosis, and renal damage. Circulation 2004; 109: 1789-94.

[14] Okumura H, Nagaya N, Kangawa K. Adrenomedullin infusion during ischemia/reperfusion attenuates left ventricular remodeling and myocardial fibrosis in rats. Hypertens Res 2003; 26: S99-S104.

[15] Leskinen H, Rauma-Pinola T, Szokodi I, *et al.* Adaptive or maladaptive response to adenoviral adrenomedullin gene transfer is context-dependent in the heart. J Gene Med 2008; 10: 867-77.

[16] Miksa M, Wu R, Cui X, *et al.* Vasoactive hormone adrenomedullin and its binding protein: anti-inflammatory effects by up-regulating peroxisome proliferator-activated receptor-γ. J Immunol 2007; 179: 6263-72.

[17] Tsuruda T, Kato J, Hatakeyama K, *et al.* Antifibrotic effect of adrenomedullin on coronary adventitia in angiotensin II-induced hypertensive rats. Cardiovasc Res 2005; 65: 921-29.

[18] Tsuruda T, Kato J, Cao YN, *et al.* Adrenomedullin induces matrix metalloproteinase-2 activity in rat aortic adventitial fibroblasts. Biochem Biophys Res Commun 2004; 325: 80-84.

[19] Böhm M, Eschenhagen T, Gierschik P, *et al.* Radioimmunochemical quantification of Giα in right and left ventricles from patients with ischaemic and dilated cardiomyopathy and predominant left ventricular failure. J Mol Cell Cardiol 1994; 26: 133-49.

[20] Ihara T, Ikeda U, Tate Y, Ishibashi S, Shimada K. Positive inotropic effects of adrenomedullin on rat papillary muscle. Eur J Pharmacol 2000; 390: 167-72.

[21] Bisping E, Tenderich G, Barckhausen P, *et al.* Atrial myocardium is the predominant inotropic target of adrenomedullin in the human heart. Am J Physiol Heart Circ Physiol 2007; 293: H3001-H07.

[22] Szokodi I, Kinnunen P, Tavi P, Weckström M, Tóth M, Ruskoaho H. Evidence for cAMP-independent mechanisms mediating the effects of adrenomedullin, a new inotropic peptide. Circulation 1998; 97: 1062-70.

[23] Fontes-Sousa AP, Pires AL, Carneiro CS, Brás-Silva C, Leite-Moreira AF. Effects of adrenomedullin on systolic and diastolic myocardial function. Peptides 2009; 30: 796-802.

[24] Mittra S, Bourreau JP. Gs and Gi coupling of adrenomedullin in adult rat ventricular myocytes. Am J Physiol Heart Circ Physiol 2006 ;290: H1842-H47.

[25] Ikenouchi H, Kangawa K, Matsuo H, Hirata Y. Negative inotropic effect of adrenomedullin in isolated adult rabbit cardiac ventricular myocytes. Circulation 1997; 95: 2318-24.

[26] Mukherjee R, Multani MM, Sample JA, *et al.* Effects of adrenomedullin on human myocyte contractile function and beta-adrenergic response. J Cardiovasc Pharmacol Ther 2002; 7: 235-40.

[27] Lainchbury JG, Meyer DM, Jougasaki M, Burnett JC, Jr., Redfield MM. Effects of adrenomedullin on load and myocardial performance in normal and heart-failure dogs. Am J Physiol Heart Circ Physiol 2000; 279: H1000-H06.

[28] Kato J, Tsuruda T, Kitamura K, Eto T. Adrenomedullin: a possible autocrine or paracrine hormone in the cardiac ventricles. Hypertens Res 2003; 26: S113-S19.

[29] Harada M, Itoh H, Nakagawa O, *et al.* Significance of ventricular myocytes and nonmyocytes interaction during cardiocyte hypertrophy: evidence for endothelin-1 as a paracrine hypertrophic factor from cardiac nonmyocytes. Circulation 1997; 96: 3737-44.

[30] Tomoda Y, Kikumoto K, Isumi Y, *et al.* Cardiac fibroblasts are major production and target cells of adrenomedullin in the heart *in vitro*. Cardiovasc Res 2001; 49: 721-30.

[31] Bou-Gharios G, Ponticos M, Rajkumar V, Abraham D. Extra-cellular matrix in vascular networks. Cell Prolif 2004; 37: 207-20.

[32] Kato J, Tsuruda T, Kita T, Kitamura K, Eto T. Adrenomedullin: a protective factor for blood vessels. Arterioscler Thromb Vasc Biol 2005; 25: 2480-87.

[33] Kim W, Moon SO, Lee S, Sung MJ, Kim SH, Park SK. Adrenomedullin reduces VEGF-induced endothelial adhesion molecules and adhesiveness through a phosphatidylinositol 3'-kinase pathway. Arterioscler Thromb Vasc Biol 2003; 23: 1377-83.

[34] Hagi-Pavli E, Farthing PM, Kapas S. Stimulation of adhesion molecule expression in human endothelial cells (HUVEC) by adrenomedullin and corticotrophin. Am J Physiol Cell Physiol 2004; 286: C239-C46.

[35] Kano H, Kohno M, Yasunari K, *et al.* Adrenomedullin as a novel antiproliferative factor of vascular smooth muscle cells. J Hypertens 1996; 14: 209-13.

[36] Kohno M, Yokokawa K, Kano H, *et al.* Adrenomedullin is a potent inhibitor of angiotensin II-induced migration of human coronary artery smooth muscle cells. Hypertension 1997; 29: 1309-13.

[37] Iwasaki H, Eguchi S, Shichiri M, Marumo F, Hirata Y. Adrenomedullin as a novel growth-promoting factor for cultured vascular smooth muscle cells: role of tyrosine kinase-mediated mitogen-activated protein kinase activation. Endocrinology 1998; 139: 3432-41.

[38] Rauma-Pinola T, Pääkkö P, Ilves M, *et al.* Adrenomedullin gene transfer induces neointimal apoptosis and inhibits neointimal hyperplasia in injured rat artery. J Gene Med 2006; 8: 452-58.

[39] Tsuruda T, Kato J, Matsui E, *et al.* Adrenomedullin alleviates not only neointimal formation but also perivascular hyperplasia following arterial injury in rats. Eur J Pharmacol 2005; 508: 201-04.

[40] Imai Y, Shindo T, Maemura K, *et al.* Resistance to neointimal hyperplasia and fatty streak formation in mice with adrenomedullin overexpression. Arterioscler Thromb Vasc Biol 2002; 22: 1310-15.

[41] Liang L, Tam CW, Pozsgai G, *et al.* Protection of angiotensin II-induced vascular hypertrophy in vascular smooth muscle-targeted receptor activity-modifying protein 2 transgenic mice. Hypertension 2009; 54: 1254-61.

[42] Shimizu K, Tanaka H, Sunamori M, Marumo F, Shichiri M. Adrenomedullin receptor antagonism by calcitonin gene-related peptide(8-37) inhibits carotid artery neointimal hyperplasia after balloon injury. Circ Res 1999; 85: 1199-205.

[43] Jiang W, Yang JH, Pan CS, Qi YF, Pang YZ, Tang CS. Effects of adrenomedullin on cell proliferation in rat adventitia induced by aldosterone. J Hypertens 2004; 22: 1953-61.

[44] Intengan HD, Schiffrin EL. Vascular remodeling in hypertension: roles of apoptosis, inflammation, and fibrosis. Hypertension 2001; 38: 581-87.

[45] Dupuis J, Harel F, Fu Y, *et al.* Molecular imaging of monocrotaline-induced pulmonary vascular disease with radiolabeled linear adrenomedullin. J Nucl Med; 50: 1110-15.

[46] Cataliotti A, Boerrigter G, Chen HH, *et al.* Differential actions of vasopeptidase inhibition versus angiotensin-converting enzyme inhibition on diuretic therapy in experimental congestive heart failure. Circulation 2002; 105: 639-44.

[47] Martínez A, Oh HR, Unsworth EJ, *et al.* Matrix metalloproteinase-2 cleavage of adrenomedullin produces a vasoconstrictor out of a vasodilator. Biochem J 2004; 383: 413-18.

[48] Libby P, Okamoto Y, Rocha VZ, Folco E. Inflammation in atherosclerosis: transition from theory to practice. Circ J 2010; 74: 213-20.

[49] Patella V, Marinò I, Arbustini E, *et al.* Stem cell factor in mast cells and increased mast cell density in idiopathic and ischemic cardiomyopathy. Circulation 1998; 97.

[50] Tsuruda T, Kato J, Hatakeyama K, *et al.* Adventitial mast cells contribute to pathogenesis in the progression of abdominal aortic aneurysm. Circ Res 2008; 102: 1368-77.

[51] Øie E, Ahmed MS, Ueland T, *et al.* Adrenomedullin is increased in alveolar macrophages and released from the lungs into the circulation in severe heart failure. Basic Res Cardiol 2010; 105: 89-98.

[52] Wong LY, Cheung BM, Li YY, Tang F. 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 2005; 146: 1321-27.

[53] Kubo A, Minamino N, Isumi Y, *et al.* Production of adrenomedullin in macrophage cell line and peritoneal macrophage. J Biol Chem 1998; 273: 16730-38.

[54] Belloni AS, Guidolin D, Salmaso R, Bova S, Rossi GP, Nussdorfer GG. Adrenomedullin, ANP and BNP are colocalized in a subset of endocrine cells in the rat heart. Int J Mol Med 2005; 15: 567-71.

[55] Tsuruda T, Kato J, Hatakeyama K, *et al.* Adrenomedullin in mast cells of abdominal aortic aneurysm. Cardiovasc Res 2006; 70: 158-64.

[56] Sun J, Sukhova GK, Wolters PJ, *et al.* Mast cells promote atherosclerosis by releasing proinflammatory cytokines. Nat Med 2007; 13: 719-24.

[57] Walsh SK, Kane KA, Wainwright CL. Mast cells, peptides and cardioprotection - an unlikely marriage? Auton Autacoid Pharmacol 2009; 29: 73-84.

[58] Kato H, Shichiri M, Marumo F, Hirata Y. Adrenomedullin as an autocrine/paracrine apoptosis survival factor for rat endothelial cells. Endocrinology 1997;138: 2615-20.

[59] Kato K, Yin H, Agata J, Yoshida H, Chao L, Chao J. Adrenomedullin gene delivery attenuates myocardial infarction and apoptosis after ischemia and reperfusion. Am J Physiol Heart Circ Physiol 2003; 285: H1506-H14.

[60] Nishida H, Sato T, Miyazaki M, Nakaya H. Infarct size limitation by adrenomedullin: protein kinase A but not PI3-kinase is linked to mitochondrial KCa channels. Cardiovasc Res 2008; 77: 398-405.

[61] Torigoe Y, Takahashi N, Hara M, Yoshimatsu H, Saikawa T. Adrenomedullin improves cardiac expression of heat-shock protein 72 and tolerance against ischemia/reperfusion injury in insulin-resistant rats. Endocrinology 2009;150: 1450-55.

[62] Takei Y, Inoue K, Ogoshi M, Kawahara T, Bannai H, Miyano S. Identification of novel adrenomedullin in mammals: a potent cardiovascular and renal regulator. FEBS Lett 2004; 556: 53-58.

[63] Roh J, Chang CL, Bhalla A, *et al.* Intermedin is a calcitonin/calcitonin gene-related peptide family peptide acting through the calcitonin receptor-like receptor/receptor activity-modifying protein receptor complexes. J Biol Chem 2004; 279: 7264-74.

[64] Bell D, Zhao Y, McCoy FP, Devine A, McDermott BJ. Expression of the counter-regulatory peptide intermedin is augmented in the presence of oxidative stress in hypertrophied cardiomyocytes. Cell Physiol Biochem 2008; 21: 409-20.

[65] Zeng Q, Yuan Y, Wang X, *et al.* Upregulated expression of intermedin and its receptor in the myocardium and aorta in spontaneously hypertensive rats. Peptides 2009; 30: 391-99.

[66] Yang JH, Ma CG, Cai Y, *et al.* Effect of intermedin1-53 on angiotensin II-induced hypertrophy in neonatal rat ventricular myocytes. J Cardiovasc Pharmacol 2010; 56: 45-52.

[67] Yang JH, Qi YF, Jia YX, *et al.* Protective effects of intermedin/adrenomedullin2 on ischemia/reperfusion injury in isolated rat hearts. Peptides 2005; 26: 501-07.

[68] Rademaker MT, Charles CJ, Nicholls MG, Richards AM. Hemodynamic, hormonal, and renal actions of adrenomedullin 2 in experimental heart failure. Circ Heart Fail 2008; 1: 134-42.

Received: July 06, 2011 Revised: August 18, 2011 Accepted: August 30, 2011

8. アドレノメデュリンの内分泌・代謝系へのユニークな作用

北 俊弘，北村和雄

アドレノメデュリンは血管拡張性のペプチドで，血圧低下，体液量減少，細胞増殖抑制，抗酸化，炎症調節作用など多彩な作用を有している．内分泌関係では，アルドステロンの過剰分泌を抑制するとともに，アルドステロンの腎外作用を抑制し，内因性の抗アルドステロン因子となっている．代謝系ではインスリン分泌を抑制し，抗酸化作用によりインスリン抵抗性を改善する作用があるが，一方，インスリン抵抗性が亢進すると，アドレノメデュリンの作用は減弱してしまう．これらのユニークな作用は，今後，新しい治療ターゲットとなるかもしれない．

はじめに

アドレノメデュリン（AM）は血管拡張性の降圧ペプチドであり，全身の血管系に広く発現しており，加えて心臓，肺，腎臓，脳，副腎などの重要臓器にも分布している．血管拡張による血圧調整以外に，免疫・炎症調整作用，抗動脈硬化作用（抗酸化作用，細胞増殖抑制作用），内分泌調整作用など多彩な作用を有している[1]．本稿では，内分泌・代謝に関連したAMの作用，特にAMとアルドステロンの相互作用，AMとインスリン抵抗性について解説したい．

[キーワード&略語]
アドレノメデュリン，アルドステロン，インスリン抵抗性，アンジオテンシンII

A-Ⅱ：angiotensin II（アンジオテンシンII）
AM：adrenomedullin（アドレノメデュリン）
MS：metabolic syndrome（メタボリックシンドローム）
PA：primary aldosteronism（原発性アルドステロン症）
ZG：zona glomerulosa（副腎皮質球状層）

1 アドレノメデュリンの構造と主要な作用

AMはカルシトニン関連ペプチド（CGRP）やアミリンと同じスーパーファミリーに属するペプチドで，C末端のアミド構造と分子内リング構造が共通しており，これらは生理活性に必須である．AMはcAMPをセカンドメッセンジャーとして作用を発現するが，AMとCGRPには構造上の共通性があり，CRLR（calcitonin-receptor-like receptor）という7回膜貫通型受容体を共有している．加えてRAMP（receptor-activity-modifying protein）という1回膜貫通型のタンパクが，受容体の親和性を規定し，さらには細胞膜での発現を調整している．RAMPには相互に相同性を示す3種類のタンパクが存在し，CRLR＋RAMP1でCGRPの，CRLR＋RAMP2またはCRLR＋RAMP3でAMの受容体を形成する．さらに，RAMP1とcalcitonin receptorの組み合わせはアミリンの受容体を形成し，これらの相互関係は複雑である[2]．

Unique effects of adrenomedullin for endocrine and metabolic system
Toshihiro Kita/Kazuo Kitamura：Department of Internal Medicine, Division of Circulatory and Body Fluid Regulation, Faculty of Medicine, University of Miyazaki（宮崎大学医学部内科学講座循環体液制御学分野）

AMの主要な作用は血管拡張による血圧低下作用であり，血管平滑筋への直接作用が主体であるが，一部一酸化窒素（NO）分泌を介する作用もある．AMの発現は全身の血管系や心臓，腎臓，副腎，肺などの重要臓器に広く認められ，AM受容体も同様に広く分布している．このため，AMは非常に多様な作用を示すが，全体としては血圧を下げ，体液量を減少させる方向に作用する．

また，細胞増殖や炎症にも関与し，循環器疾患においては主に酸化ストレスに対抗して，臓器障害や動脈硬化を抑制し臓器保護的に作用する．ただし，腫瘍細胞に対しては細胞増殖促進作用を有しており，状況によっては細胞増殖因子ともなる．強い炎症が存在する場合は抗炎症因子として作用し，特に敗血症ではAMの著明な上昇が認められる．一方で，基礎状態のマクロファージに対しては炎症促進的にも作用する（IL-6等の産生亢進）．最近，われわれは炎症のないヒトにAMを長時間投与すると，軽度だがIL-6を介してCRP（C-reactive protein）上昇が起こることを確認している[3]．

2 アドレノメデュリンの内分泌系への作用

AMは血圧を下げ，体液量を減らす方向に働くため，下垂体からのACTH（副腎皮質刺激ホルモン）やAVP（アルギニンバソプレシン）分泌を抑制し，心臓からのANP（心房性ナトリウム利尿ペプチド）分泌を促進し，血管壁ではエンドセリンの産生や効果を抑制し，一酸化窒素（NO）産生を促進する．レニン・アンジオテンシン・アルドステロン系の作用にも拮抗し，血圧だけでなくアンジオテンシンII（A-II）による心臓，腎臓，血管（動脈硬化促進）などの臓器障害を抑制する[1]．しかし，AMを持続静注した場合，血漿レニン活性が抑制されるわけではなく，むしろ血圧低下に対する反作用としてレニン活性は上昇する．AMには腎交感神経抑制作用なども報告されているが，AMを持続静注した場合，血中カテコラミンも反応性に上昇する．レニンやカテコラミンの上昇反応は，AM投与時とCa拮抗薬のニカルジピン投与時で全く同等であり，AMがこれらの分泌を直接抑制するとは考えにくい[4]．

1）アドレノメデュリンとアルドステロンの相互関係

AMはもともと褐色細胞腫から分離精製され，その名前からも明らかなように副腎髄質に強く発現している．ところが，アルドステロンを産生する副腎皮質球状層（zona glomerulosa：ZG細胞）にも同程度の発現が認められる（**図1**）．さらに，原発性アルドステロン症（primary aldosteronism：PA）の腺腫細胞（Conn細胞）にも発現が確認されている[5]．副腎皮質球状層および腺腫の細胞にはAMの受容体も発現しており，AMがアルドステロン分泌調節に重要な役割を果たしていることが推察される．大まかにAMはアルドステロン分泌抑制的に働くとされているが，両者の関係は単純ではない（**表**）．ZG細胞だけでなくConn細胞においても，AMは細胞増殖促進とアポトーシス抑制によりアルドステロン分泌細胞の増殖・維持に重要な働きをしており[5][6]，細胞レベルでアルドステロンの基礎分泌は抑制しない[7][8]．ZG細胞に関しては，ごく軽度であるが，アルドステロン分泌を促進するとのデータもある[9]．一方，A-IIやK^+によるアルドステロン分泌亢進に対しては，AMは抑制的に作用する[7][8][10]．ACTH刺激によるアルドステロン分泌への抑制はないか，あっても軽度である[7][10]．以上をまとめると，AMはアルドステロンの基礎分泌を維持するとともに，過剰な分泌（オーバーシュート）を抑制することで，アルドステロンの適正な分泌を調整していると考えられる．

K^+刺激によるアルドステロン分泌亢進に対するAMの抑制効果は，AMやCGRPアナログにより阻害され

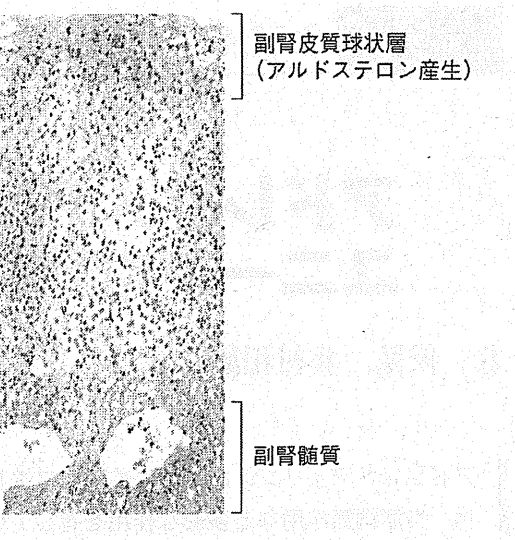


図1　副腎におけるアドレノメデュリンの局在
（巻頭カラー図1参照）

表　アドレノメデュリンのアルドステロン分泌への影響

		基礎分泌	刺激後の分泌			細胞増殖
			A-II	ACTH	K^+	
ZG細胞	細胞実験	→〜↗	↓	→〜↓	↓	↑
	動物実験	→〜↘	↓	↘	↓	ND
	ヒト	→〜↘	→〜↓	→	ND	ND
Conn細胞	細胞実験	→	↓	ND	ND	↑
	ヒト	↓	ND	ND	ND	ND

ND：検討なし

ることから，先に述べた受容体を介する作用と考えられる[11]．AMが受容体と結合した後，Caチャネルを介する細胞内へのCa^{2+}流入を阻害することで，アルドステロン分泌を抑制すると報告されている[11]．一部，NOを介する作用があるとの報告もあるが，機序が完全に解明されたわけではない．

実験動物やヒトにAMを静注した場合，実験条件によって，血中アルドステロン濃度は変化しないか，軽度低下する[12]．具体的には，食塩制限などで事前にアルドステロン濃度を高くしておくとAM投与によりアルドステロンは低下しやすく[13]，心不全患者などもともとアルドステロン濃度が高い患者でも低下しやすい．一方，アルドステロン濃度が正常の場合，十分な量のAMを長時間投与しないとアルドステロンは低下しない[4) 12) 13)]．これには，AM投与によりレニン活性が上昇し，アルドステロン分泌が刺激されることも関与していると考えられる．Conn細胞では，AMによるアルドステロン抑制作用が強く表れ，感受性が高いことが報告されている[8]．われわれの試験では，AM持続静注によりアルドステロン値が正常な対象者では中等度の，PA患者では強いアルドステロン分泌抑制効果が認められた[3]（**図2**）．本試験でAMはACTH-コルチゾール系には全く影響を与えておらず，PA患者におけるAMによる選択的アルドステロン抑制効果は，PAの診断に応用できる可能性がある．

血中AM濃度と血圧の相関は非常に弱く，臓器障害のない高血圧患者の血中AM濃度上昇はごく軽度である[1]．一方，PA患者ではAM濃度は明らかに上昇しており，手術で腺腫を取り除くとAMは低下する[3) 14)]．面白いことに，手術で摘出したPA患者の腺腫の直径と血中AM濃度が相関していた[14]．一方，ANPも正常

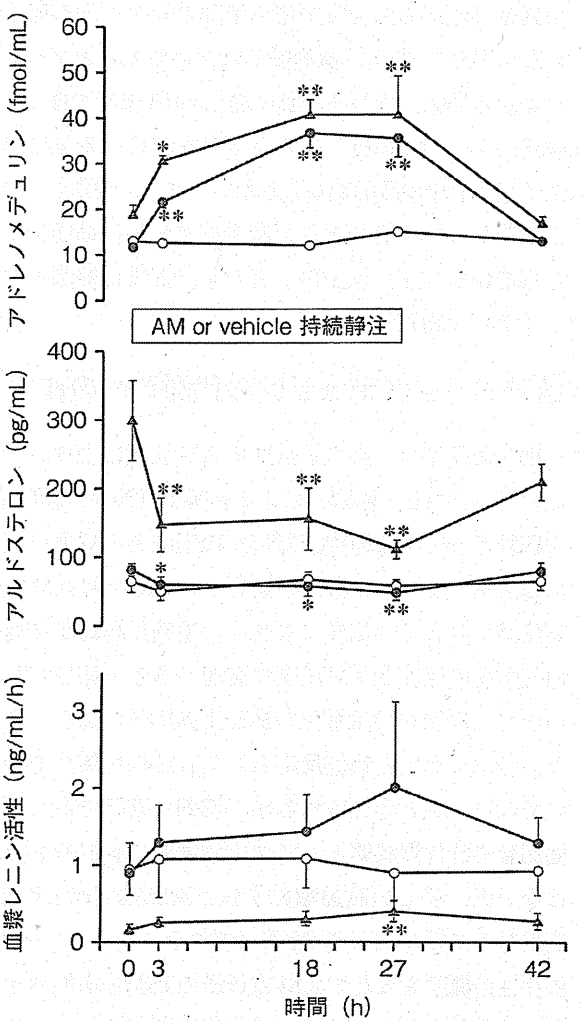


図2　アドレノメデュリンのアルドステロン抑制作用
原発性アルドステロン症5名（▲：AM投与）と本態性高血圧患者7名（●：AM投与，○：vehicle投与）にAMを持続静注（2.5pmol/min/kg, 27時間）したときの，アルドステロンとレニンの反応

副腎からのアルドステロン分泌を抑制するが，PAの腺腫にはANP受容体がなく，PA患者にANPを投与してもアルドステロンは抑制されない[15]．PA患者のアル

ドステロンを強力に抑制する内因性物質としてはAM以外に報告がなく，PAにおいてAMがアルドステロン分泌調整（たぶん抑制的調整）に重要な因子となっていることは疑いない．

分泌だけでなく，標的臓器においてもAMとアルドステロンは相互に関連を持っている．アルドステロンは心臓や血管において細胞増殖，線維化などのリモデリングを促進し，酸化ストレスを増加させる作用（アルドステロンの腎外作用※1）があるが，AMはこれらの作用を抑制する．一方で，アルドステロンは血管平滑筋や心筋からのAM分泌を増加させることが明らかとなっており[16)17)]，標的臓器においてAMとアルドステロンは拮抗的な平衡関係にある．PA患者で血中AM濃度が上昇するのは，アルドステロンによる末梢でのAM産生増加が原因かもしれない．また，増加したAMは副腎でのアルドステロン産生を部分的に抑制している可能性もある．以上のことから，AMは抑制的アルドステロン調整因子と考えられる．

③アドレノメデュリンの代謝系への作用

糖尿病患者で，必ずしも血中AM濃度は上昇していない[2)]．しかし，糖尿病による合併症（腎症，網膜症，神経障害—特に自律神経障害）の程度とAM血中濃度には関連があり，合併症が進行した患者ではAM血中濃度が上昇している[18)]．また，1型糖尿病患者では心血管障害の程度とAM濃度に関連があると報告されている[19)]．糖尿病では種々の血圧上昇因子が増加しており，これに対する代償反応としてAMが増加しているのではないかと考えられるが，詳細は不明である．断面調査で糖代謝状態とAM血中濃度間に直接的な関係はないが，著しい高血糖にするとAM濃度は上昇し，高血糖が血管におけるPKC（プロテインキナーゼC）依存性の機序を介してAM遺伝子の発現を増加させるとの報告がある[20)]．また，糖尿病患者で高インスリン血症状態にするとAM濃度が上昇することも確認されている[21)]．AMとその受容体は膵臓のランゲルハンス島β細胞にも発現しており，AMはインスリン分泌を抑制する[22)]．よって，AM投与下に経口糖負荷試験を行うと，コントロールと比較して，血中インスリン濃度は低下し，血糖値は高くなる．糖尿病で血中AM濃度が高い患者では，AMが糖代謝を悪くしている可能性があり，糖尿病患者でAMは良い作用だけをしているとは限らない．推測の域を出ないが，PA患者では糖代謝が障害されている場合が多く，低K血症によるインスリン分泌障害が原因とされているが，増加したAMによるインスリン分泌抑制も関係があるかもしれない．

インスリン抵抗性は，メタボリックシンドローム（MS）の重要な基礎病態であり，一連の病気進行の上流に位置している．肥満がインスリン感受性を低下させる主因であることは周知の事実であるが，レニン・アンジオテンシン系もインスリン感受性を低下させる重要な因子である．このことは，MSや糖尿病を伴う高血圧患者にARBが推奨される根拠ともなっている．マウスにA-IIを持続的に投与すると酸化ストレスが増加してインスリン抵抗性が出現するが，AMノックアウトマウス（ヘテロ体）ではより強いインスリン抵抗性が出現した[23)]．さらに，このマウスでは加齢により自然にインスリン抵抗性が出現し，酸化ストレスも増加していた[24)]．加えて，このマウスに抗酸化剤を投与すると，インスリン抵抗性が改善した[23)24)]．総合すると，AMは血中インスリン濃度を低下させるとともに，酸化ストレスを減らすことで，インスリン抵抗性を改善すると考えられる．

それでは，インスリン抵抗性がある状態でのAMの効果はどうなのだろうか．肥満ラット（fa/fa Zucker rat）では，外因性に投与したAMに対する感受性が低下していた[25)]．われわれは，糖尿病患者を含む対象者にAMを持続静注したところ，インスリン抵抗性の指標であるHOMA indexが高い対象者でのみ，AMによる動脈stiffness※2指標の改善効果が低いことを確認した[4)]（**図3**）．AMとインスリンは拮抗関係にあり，AMはインスリン抵抗性を改善する作用があるものの，す

※1　アルドステロンの腎外作用

アルドステロンは，ミネラルコルチコイド受容体（MR）を介した直接作用により，心血管系の炎症を惹起し，最終的に組織の線維化を起こす．MR阻害薬はこれを抑制し，心不全患者などの生命予後を改善する．

※2　動脈stiffness

大動脈壁の硬化は，大動脈による血圧緩衝作用を減弱させ，収縮期高血圧をきたす．独立した生命予後規定因子である．脈波伝播速度（PWV）やaugmentation indexが簡便な指標として普及している．

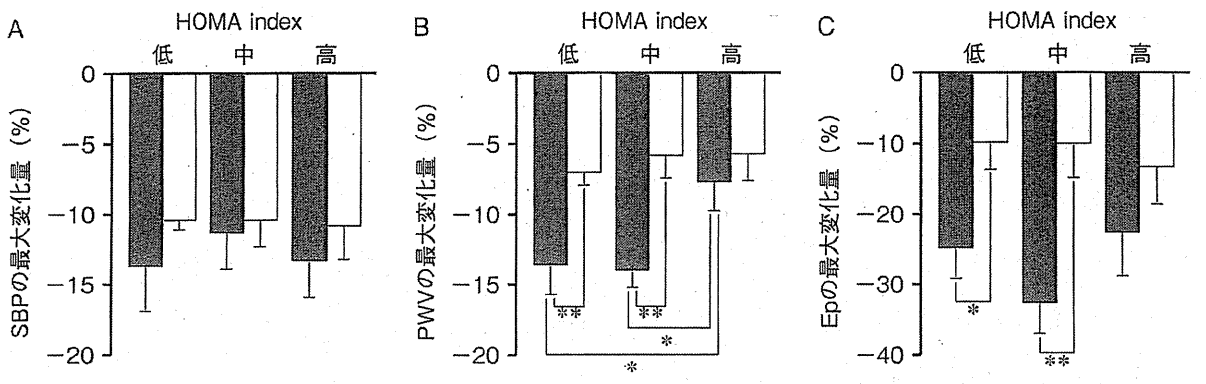


図3　インスリン抵抗性とアドレノメデュリンの作用
28名の対象者にAM（■：5 pmol/min/kg）またはニカルジピン（□：1～1.5 μg/min/kg）を1時間静注したときの，収縮期血圧（SBP），脈波伝播速度（PWV），頸動脈弾性特性（Ep）の最大変化量．降圧量を合わせると，AMの方がPWVなどの改善効果が強い．しかし，HOMA indexで3等分すると，インスリン抵抗性を有する群（高，HOMA index≧2.0）のみAMの効果が減弱し，ニカルジピンと差がなくなった

でにインスリン抵抗性が存在する状態では，AMによる抗動脈硬化作用は減弱していることになる．インスリン抵抗性を基礎とする病態では，AMの活性を十分増強してインスリン抵抗性を解消すれば，AM自体の有益な効果も向上し，全体的に病態を改善することが期待でき，新たな治療ターゲットとなるかもしれない．

おわりに

AMの臨床応用として，肺高血圧への吸入療法，心筋梗塞後の治療（リモデリングを抑制することで心機能維持を期待），炎症性腸疾患に対する治療（抗炎症作用を期待），閉塞性動脈硬化症に対する治療（血管新生促進作用を期待）などが試されている．しかし，本稿で記したように，AMには他にも多彩な作用があり，内分泌・代謝分野での活用も期待したい．

文献

1）北村和雄：Heart View, 13（増刊号）：69-75, 2009
2）Gibbons, C. et al.：Mol. Endocrinol., 21：783-796, 2007
3）Kita, T. et al.：Hypertens. Res., 33：374-379, 2010
4）Kita, T. et al.：Hypertens. Res., 33：314-319, 2010
5）Forneris, M. et al.：Int. J. Mol. Med., 8：675-679, 2001
6）Rossi, G. P. et al.：Hypertens. Res., 26：S85-S92, 2003
7）Yamaguchi, T. et al.：Life Sci., 56：379-387, 1995
8）Andreis, P. G. et al.：J. Clin. Endocrinol. Metab., 83：253-257, 1998
9）Kapas, A. et al.：J. Endocrinol., 156：477-484, 1998
10）Salemi, R. et al.：J. Endocrinol., 166：389-399, 2000
11）Mazzocchi, G. et al.：Peptides, 20：1479-1487, 1999
12）Charles, C. J. et al.：Regul. Pept., 112：41-49, 2003
13）Yamaguchi, T. et al.：Hypertension, 28：308-314, 1996
14）Letizia, C. et al.：Blood Press., 7：19-23, 1998
15）Rocco, S. et al.：Am. J. Hypertens., 3：668-673, 1990
16）Jiang, W. et al.：J. Hypertens., 22：1953-1961, 2004
17）Jiang, W. et al.：Biochim. Biophys. Acta, 1690：265-275, 2004
18）Nakamura, T. et al.：Endocr. J., 45：241-246, 1998
19）Garcia-Unzueta, M. T. et al.：Diabetes Care, 21：999-1003, 1998
20）Hayashi, M. et al.：Biochem. Biophys. Res. Commun., 258：453-456, 1999
21）Katsuki, A. et al.：Eur. J. Endocrinol., 147：71-75, 2002
22）Martinez, A. et al.：Endocrinology, 137：2626-2632, 1996
23）Xing, G. et al.：Endocrinology, 145：3647-3651, 2004
24）Shimosawa, T. et al.：Hypertension, 41：1080-1085, 2003
25）Chan, C. B. & Johnson, K. J.：Can. J. Physiol. Pharmacol., 75：1138-1141, 1997

<筆頭著者プロフィール>
北　俊弘：1985年宮崎医科大学（現宮崎大学医学部）卒業．宮崎医科大学附属病院，潤和会記念病院，三股町立病院などで内科研修を行う．'92～'95年に米国Monsanto社研究所に留学．'98年より宮崎大学医学部第一内科助手，2009年より同講師．ナトリウム利尿ペプチド，ウログアニリン，アドレノメデュリンなどの生理活性ペプチドの研究を継続し，最近はアドレノメデュリンのトランスレーショナル研究に力を入れている．

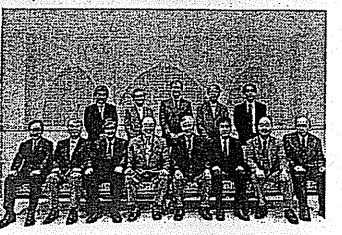
九州11大学循環器フォーラム会報

2011年 第7回

2011年6月25日(土)、福岡市のホテルニューオータニ博多にて、第7回九州11大学循環器フォーラムが開催されました。多数の先生方にご参加頂き、盛大に研究会が開催されました。

第7回 九州11大学循環器フォーラム
循環器医専門医のプロフェッショナルリズム
日時:平成23年6月25日(土)18:00〜
(循環器地方会終了後)
場所:ホテルニューオータニ博多

- 第一部　教育講演
- 第二部　特別講演
- 第三部　新臨床研修医制度によって何がどう変化したか?
  各大学がいかに取り組んでいるのか?



第一部　教育講演

「降圧因子アドレノメデュリンの新たな可能性」

北村　和雄 先生(宮崎大学医学部内科学講座循環体液制御学分野 教授)

1993年、ヒト副腎髄質由来の褐色細胞腫より発見されたアドレノメデュリンは、強力な降圧作用を有する生理活性ペプチドで、副腎の他、心房や肺などの組織で高濃度存在します。とりわけ血管内皮からはエンドセリンと同程度分泌され、強力な血管新生作用、血管拡張作用等を有する事が分かってきました。

閉塞性動脈硬化症実験モデル動物においては、アドレノメデュリンの投与と抹消血単核細胞移植を併用することによって血管新生効果の上がることが分かり、当大学では今年から抹消血単核球移植にアドレノメデュリン持続静注療法する併用する臨床研究を開始しました。炎症性疾患に対してもアドレノメデュリンを投与する事により、効果が上がる事も分かってきたので、難治性炎症性腸疾患の治療薬としての特許申請も行い、実用化をめざしています。

従来は、優れた業績を基に研究資金と人材の獲得・育成が可能となり、さらに業績をあげるという良い循環ができていました。しかし、新臨床研究制度が導入されてからは人材の確保が難しくなり、それに伴い業績も上がりにくくなり、研究資金の獲得ができなくなるという悪循環に陥っている所が増えてくるのではと危惧しています。

司会　鄭　忠和 先生
(鹿児島大学大学院
循環器・呼吸器・代謝内科学 教授)

第二部　特別講演

「臨床研修医制度の今後について」

田原　克志 先生(厚生労働省医政局医師臨床研修推進室 室長)

臨床研修医制度は平成16年から施行され、今年8年目です。医師不足など様々な問題が顕在化したため、臨床研修の質の向上と医師不足への対応を図ることを主眼として見直しを行い、平成22年度の研修から適用しています。

見直しの内容は研修プログラムの基準を弾力化すること、臨床研修病院の基準を強化すること、そして募集定員を見直し都会に研修医が集中するのを防ぐという3つの柱があります。プログラムの見直しについては必修科目を絞り、将来専門を希望する診療科を中心とした研修ができるようになりました。また臨床研修病院の基準を強化した事によって病院の数を絞り、研修希望者の数と合わせて定員を削減することができました。採用実績を見ると、臨床研修制度が始まる前よりも都市部の研修医は減り、その他の地方の方が増えています。

現在、全臨床研修修了者に対するアンケート調査のほか、制度導入前後の研修医の地域分布や移動状況について様々な調査を行っています。今後、こういったデータを題材にした入念な議論と積極的な情報発信が必要だと思います。

司会　今泉　勉 先生
(久留米大学医学部
心臓・血管内科部門 主任教授)

第三部　新臨床研修医制度によって何がどう変化したか？
各大学がいかに取り組んでいるのか？

司会　小川　久雄 先生
（熊本大学大学院
生命科学研究部循環器病態学 教授）

朔　啓二郎 先生（福岡大学医学部心臓・血管内科学 主任教授）

福岡大学病院は、地下鉄を降りると病院の受付に出られる新しい新診療棟ができ、非常に新しくなったというインパクトがあります。マッチングについては100%マッチしていますが、その後国家試験に落ちるところが1つの問題です。福大病院の研修医は、九州で一番給料が安かったので、ある程度是正していただいたのですが、医学部生の話を聞くと、少しでも高いところに行きたいという意見があります。やはり一定の給料は必要で、研修病院の質をきちんとキープする評価機関をもっとしっかり働かせるべきだと思います。

尾辻　豊 先生（産業医科大学第二内科学 教授）

研修制度に関して言えば、産業医科大学は破綻的な危機を迎えています。40人ぐらいの定数でスタートしたのですが現在10人、さらに減る傾向にあります。都会の研修医を減らし、地方の研修医を増やす政策や研修の質が高くない（人気がない）病院の定数を減らす政策は理解できますが、研修の質が高い病院の定数を増やせるシステムを考えていただければと思います。また現在の研修制度では優秀な研修医は医師として成長出来ますが、優秀でない研修医はどこでもお客さん扱いになり成長出来ないのではないかということを危惧しています。

野出　孝一 先生（佐賀大学医学部内科学 教授）

国立大学が法人化し経営タスクが多くなったところに相まって、指導医の業務が増えたという印象です。研修医は2、3ヶ月のローテートなので実際は指導医が主治医として働くこともあり、同時に循環器内科に興味を持たせるという役目もあるので、かなり責任が増えた感があります。この研修制度を評価するべきは、研修医とともに、税金の支払い者である国民、住民です。

地域の開業医や公的病院の医師などの現場の意見を吸い上げていくことが、研修医制度改革には必要です。オーバーロールで解析された地域格差、診療価格差など数字だけで表す事のできない現場の声を反映させてほしいところです。

前村　浩二 先生（長崎大学大学院循環病態制御内科学 教授）

長崎県では、医師を長崎大学からの派遣にかなり依存していますが、現在では大学の入局者が80数名からおよそ半数に減ったため派遣先を維持できず、各地から引き揚げざるを得ない状況です。研修医の数も一時マッチングが30人台になり、研修内容の充実、研修環境の向上、広報に取り組んできました。プログラムを充実させ、メンター制度のほか海外留学も行っています。またコメディカルチームに業務のかなりの部分を移し、給料の面や研修医室の環境も改善しました。webでの情報発信も積極的に行い、ようやくマッチングが50人台に回復しました。評価の際は、臨床研修制度は本当に国民が望む医師が育つ制度なのか、独立的に論議される必要があると思います。

大屋　祐輔 先生（琉球大学大学院 循環器・腎臓・神経内科学 教授）

琉球大学では当初から臨床研修制度の理念にのっとった研修プログラムを作り、指導医を育ててきました。プログラムは非常に自由度が高く幅々な研修ができ、メンター制度もあります。自慢できるプログラムなのに学生が入らない。その理由をずっと探ってきましたが、要因は複合的で難しい。強るは精神論だという結論に返し、いま「I LOVE琉大運動」に取り組んでいます。精神論とはつまりプロフェッショナリズムです。医師、循環器内科医としての使命、地域や国への貢献の尊さなどを学生に説き続けることが大切だと思っています。

岸川　哲典 先生（大分大学医学部臨床検査・診断学講座 教授）

大分大学では様々なプログラムのほかドクターヘリや救急の体験、ロボット導入の予定など様々なアピールをしてきた結果、定員64に対してマッチ者が44と7割弱になりました。平成16年当時に比べると少しよくなりましたが、旧制度には追いついていません。最近、大学への回帰傾向は感じられますが、根拠の乏しい選択によって一般の総合病院を希望する傾向が強く、予断を許さない状況です。細切れにいろんな科を回る状況では、2年終わっても十分な研修の成果をあげられているか気になっております。

砂川　賢二 先生（九州大学大学院循環器内科学 教授）

新研修医制度は何を目指したのか問われているのではないかと思います。これからの日本の医療制度、カルチャーまで包括する長いレンジで考えていなかったため、今ほつれが見え始めたのではないかと感じています。我々の大学では、初期研修も後期研修も以前と変わらない人数が入ってきています。ただ大きく違うのは、いわゆる臨床志向の人が明らかに増え、非常に重要な基礎研究に情熱を持つ若手が減少していることです。国の将来を考えた時、現在の医師不足についての議論だけではなく、将来の医療を担う有能な研究者をいかに育てていくのかという長い視点の制度設計が大切だと思います。

第7回　九州11大学循環器フォーラム（五十音順）

当番世話人
今泉　勉　久留米大学医学部内科学講座 心臓・血管内科部門　主任教授

代表世話人
小川　久雄　熊本大学大学院 生命科学研究部循環器病態学　教授
鄭　忠和　鹿児島大学大学院 循環器・呼吸器・代謝内科学　教授

顧問
瀧下　修一　琉球大学　名誉教授
矢野　捷介　長崎国際大学　学部長

世話人
今泉　勉　久留米大学医学部心臓・血管内科部門　主任教授
大屋　祐輔　琉球大学大学院 循環器・腎臓・神経内科学　教授
尾辻　豊　産業医科大学第二内科学　教授
北村　和雄　宮崎大学医学部循環体液制御学分野　教授
岸川　哲典　大分大学医学部臨床検査・診断学講座　教授
朔　啓二郎　福岡大学医学部心臓・血管内科学　主任教授
砂川　賢二　九州大学大学院循環器内科学　教授
野出　孝一　佐賀大学医学部内科学　教授
前村　浩二　長崎大学大学院循環病態制御内科学　教授