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REVIEW

The Cardiopulmonary Effects of the *Calcitonin* Gene-related Peptide Family

Kalsitonin-Geni İle İlişkili Peptit Ailesinin Kardiyopulmoner Etkileri

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ABSTRACT

Cardiopulmonary diseases are very common among the population. They are high-cost diseases and there are still no definitive treatments. The roles of members of the calcitonin-gene related-peptide (CGRP) family in treating cardiopulmonary diseases have been studied for many years and promising results obtained. Especially in recent years, two important members of the family, adrenomedullin and adrenomedullin2/intermedin, have been considered new treatment targets in cardiopulmonary diseases. In this review, the roles of CGRP family members in cardiopulmonary diseases are investigated based on the studies performed to date.

Key words: CGRP family, cardiopulmonary diseases, adrenomedullin, adrenomedullin2/intermedin, pulmonary hypertension

ÖZ

Kardiyopulmoner hastalıklar toplumda sık görülen, tedavi maliyeti oldukça yüksek ve halen kesin bir tedavisi bulunmayan hastalıklardır. Kalsitonin-geni ile ilişkili peptit (CGRP) ailesinin üyelerinin bir çok kardiyopulmoner hastalıktaki rolleri uzun yıllardır çalışılmakta ve umut vadeden sonuçlar elde edilmektedir. Özellikle son yıllarda CGRP ailesine ait peptitlerden adrenomedullin ve intermedin kardiyopulmoner hastalıklarda yeni tedavi hedefleri olarak değerlendirilmektedir. Bu derleme ile CGRP ailesi peptitlerinin kardiyopulmoner hastalıklardaki rolleri günümüze kadar yapılan çalışmalar doğrultusunda incelenmiştir.

Anahtar kelimeler: CGRP ailesi, kardiyopulmoner hastalıklar, adrenomedullin, adrenomedullin2/intermedin, pulmoner hipertansiyon

INTRODUCTION

The calcitonin gene-related peptide (CGRP) family consists of calcitonin, amylin (AMY), CGRP, adrenomedullin (ADM), calcitonin receptor (CTR) stimulating peptides 1-3, and the latest member of the family, ADM2/intermedin (IMD).[1,2] These peptides are included in the same family because of their similar chemical structures and they have important roles in the homeostasis of the body.[3-6] The effects of these peptides on the cardiovascular and pulmonary systems, especially ADM and ADM2/IMD, sparked interest as many studies were presented for the new targets of cardiovascular diseases.[7-9] In this review, we aim to summarize the cardiopulmonary effects of the CGRP family.

DISTRIBUTION OF MEMBERS OF THE CGRP FAMILY

Peptides of the CGRP family are widely expressed in the body. The first peptide of this family, calcitonin, was synthesized by a calcium-dependent mechanism and released from thyroid C-cells.[10,11] Another peptide, AMY, was isolated from amyloid plaques in β-cells found in pancreatic islets of Langerhans.[12] The rest of the family, CGRP, ADM, and ADM2/IMD, have more effect on the cardiovascular and pulmonary system. CGRP is expressed in both central and peripheral nerves associated with blood vessels. Perivascular nerves were suggested as important sources of plasma CGRP. Although CGRP is mainly expressed in nerves, it is also located in endothelial cells, adipocytes, keratinocytes, and immune cells.[13]

ADM was isolated for the first time from human pheochromocytoma cells; however, in following years it has been shown to be expressed in many tissues in the body.[14] It is found in the adrenal medulla, kidneys, lungs, ventricles, and especially endothelial cells in high amounts.[15,16]

The distribution of ADM2/IMD is largely similar to that of ADM. The expression of ADM2/IMD was demonstrated in the brain, liver, intestines, heart, kidneys, plasma, hypothalamus, and

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like ADM widely in endothelial cells.[17-22] In addition to being expressed widely in physiological conditions, their levels change under pathological conditions.[13,23-26]

RECEPTORS OF THE CGRP FAMILY

The peptides of the CGRP family interact with CTRs or calcitonin receptor-like receptors (CLRs). CTRs were first identified in pigs in 1991 and two different variants were found in humans, named hCT_aR and hCT_bR. These receptors are located on the cell surface. hCT_aR is widely distributed in the body, while hCT_bR was found in the placenta, ovaries, lungs, and bone marrow.[27] CLRs were first demonstrated in rats in 1993 and 2 years later were shown in different tissues of humans.[28,29] CLRs were found in the central nervous system, kidneys and spleen, endothelial cells, vascular smooth muscle cells, and the heart. CTRs and CLRs are G protein-dependent receptors and contain 7 transmembrane regions.[30,31] The receptors must also interact with the related receptor-activating modified protein (RAMP), depending on the type of peptide. These proteins facilitate the transfer of receptors from the plasma membrane and translocations of them into the cells.[32,33] RAMPs are composed of 148 to 189 amino acids and although they exhibit a homology less than 30%, they are structurally similar to each other. These proteins are named RAMP1, RAMP2, and RAMP3.[13] AMY shows high affinity when CTRs are activated by RAMPs.[33,34] RAMPs that bind to CTRs allow the receptor to show affinity to AMY instead of calcitonin. When the CTRs are connected with RAMP1, RAMP2, and RAMP3 they are called AMY1, AMY2, and AMY3, respectively. CGRP and ADM are activated by binding to CLRs. CLRs must interact with RAMP1 in order to function as CGRP receptors. CLRs must be bound to RAMP2 and -3 to act as ADM receptors (AM1 and AM2, respectively) (Table 1).

RAMP1 is commonly found in the uterus, bladder, brain, pancreas, and gastrointestinal tract.[35-37] It has been also shown in the veins, perivascular nerves, arteries, and endothelial cells of arterioles and smooth muscle cells and cardiomyocytes.[38] RAMP2 is found in the lungs, spleen, immune system, and kidneys, and widely distributed in the cardiovascular system, especially in vascular endothelium and smooth muscle cells.[39] RAMP3 is found in high levels in the kidneys, lungs, and spleen, similar to RAMP2.[35,36]

Table 1. The receptors and receptor components that interact with the CGRP family

Receptor	Receptor component	Agonist
CGRP	CLR/RAMP1	CGRP, ADM2/IMD
AM1	CLR/RAMP2	ADM, ADM2/IMD
AM2	CLR/RAMP3	ADM, CGRP, ADM2/IMD
Calcitonin	CTR	CT, CRSP
AMY1	CTR/RAMP1	AMY, CGRP
AMY3	CTR/RAMP3	AMY

CGRP: Calcitonin-gene related-peptide, AMY: Amylin, CLR: Calcitonin receptor-like receptor, RAMP: Related receptor-activating modified protein, CTR: Calcitonin receptor, ADM: Adrenomedullin, IMD: intermedin

Other than RAMPs, CLRs need another adapter protein to show optimum activity. This protein is called receptor component protein (RCP) and provides more effective binding with stimulator G protein and thus increases the activity of peptides[32,40] (Figure 1).

CARDIOPULMONARY EFFECTS OF THE CGRP FAMILY

Peptides of the CGRP family show widespread biological activity in the body, and in the cardiopulmonary system especially CGRP, ADM, and ADM2/IMD have remarkable effects.

Amylin

AMY acts on the cardiovascular system via CGRP receptors.[41] However, AMY has to reach a high plasma concentration to show activity. Intravenous (i.v.) AMY application provided potent vasodilatation and decreased arterial blood pressure in rats.[42] However, human studies showed no significant effect after AMY application.[43] In studies on rat cardiomyocytes and isolated heart, AMY showed a direct inotropic effect that was mediated by CGRP receptors. However, because of the side effects on the heart of high doses of AMY, it was stated that it could not be applied clinically.[44,45]

Calcitonin gene-related peptide

CGRP is one of the most potent and effective vasodilators and it has a longer duration of action.[46,47] Its relaxing effects on coronary, cerebral, pulmonary, and renal arteries were shown in both *in vitro* and *in vivo* experiments. CGRP has also regulatory effects on the vascular system; it was shown to reduce the vascular resistance and to increase the blood supply to organs in both normotensive and hypertensive animals.[48,49] In hypertensive rats, systemically administrated CGRP decreased blood pressure and had positive inotropic and chronotropic effects. After ischemic injury CGRP released in rats and also CGRP infusion reduced ischemia-reperfusion-induced arrhythmias. In addition, many studies have shown that CGRP is also protective against ischemic damage. These

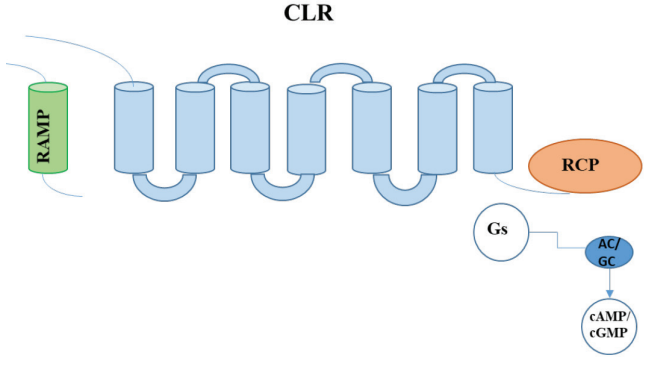

Figure 1. CLRs are G protein-dependent receptors and contain 7 transmembrane domains. CLRs require RAMPs and RCP for activation. The activated CLRs stimulate the G protein complex and provide activity
RCP: Receptor component protein, CLRs: Calcitonin receptor-like receptors, RAMPs: Related receptor-activating modified proteins, cAMP: Cyclic adenosine monophosphate, cGMP: Cyclic guanosine monophosphate

effects of CGRP are generally thought to be the result of its vasodilatory effect.[50-52] Furthermore, CGRP also suppressed the release of potent vasoconstrictor agents such as endothelin and angiotensin.[53]

CGRP provided important relaxation in the pulmonary vascular system and was found in high amounts in lung tissue.[54] In pulmonary hypertension (PH), plasma CGRP levels were decreased and CGRP infusion has been shown to be effective in treatment.[13,23-25] Adenovirus-mediated CGRP transfection before chronic hypoxia exposure in mice lungs provided cyclic adenosine monophosphate (cAMP)-mediated protection against pulmonary vascular resistance and decreased vascular remodeling.[53] CGRP has been shown to provide protection against hypoxia-induced remodeling in human tissue studies[55] and it was shown that in rat hypoxic lung the expression levels of the CGRP receptor adapter protein RAMP1 were increased.[26]

CGRP shows all these effects through CGRP receptor and the effects of CGRP on the cardiovascular system are inhibited in the presence of selective CGRP antagonist $CGRP_{8-37}$.[46,56-58] It is suggested that both endothelium-dependent and endothelium-independent mechanisms have roles in CGRP-mediated vasodilatation.[5,59,60] In many tissues, such as cat cerebral artery, rat mesenteric artery, and pig coronary artery, the increase in cAMP was measured after CGRP administration and in the endothelium-damaged vessels vasodilation was also observed. However, even high doses of CGRP did not stimulate the cyclic guanosine monophosphate (cGMP) levels directly.[59,60] Therefore, it may indicate that CGRP directly activates cAMP-dependent vasodilation.[61-63] In the studies that were performed in the pig coronary artery and guinea pig ureter, CGRP-mediated vasodilation was inhibited by the K_{ATP} channel inhibitor glibenclamide. Therefore, it was stated that the increase in cAMP activates protein kinase A and subsequently K_{ATP} channels.[61,63-67] Basal and nitric oxide (NO)-stimulated CGRP release were increased in the human right atrium in patients that underwent cardiopulmonary bypass.[68,69] However, there are also contradictory studies that indicated the role of endothelium in CGRP-mediated vasodilation. CGRP provided NO- and cGMP-dependent vasodilation in the rat aorta.[70]

On the other hand, in the perivascular nerves of the rat mesentery artery, CGRP was found more sensitive to endothelin-1 mediated constructions and this effect was not associated with NO or cyclic nucleotides.[71]

Adrenomedullin

For many years, the effects of ADM on the cardiovascular system have attracted attention. Potent, NO-mediated hypotension was observed after the infusion of ADM both in animals and in humans.[72-74] After acute and chronic administration of ADM in rats, total peripheral vascular resistance and blood pressure were decreased significantly. The heart rate and cardiac output were increased simultaneously. Similar effects were also observed in hypertensive rats.[75,76] ADM is an important vasorelaxant agent, especially in the mesentery, renal, pulmonary, and cerebral arteries and aorta, but the mechanism of this effect varies according to species and the vascular bed.[77-80] The vasorelaxing effects act through CGRP and ADM receptors. In the rat mesenteric artery and dog renal arteries, the relaxing effect of ADM was inhibited in the presence of CGRP receptor antagonist, whereas in some studies that were performed in the cerebral arteries of cat and rat hind limb, inhibition of CGRP receptors did not alter the relaxation response.[78,81,82] Similarly, the role of endothelium and NO in the relaxation effect of ADM also varies between different studies. Numerous studies have shown that endothelium-mediated vasorelaxation occurred in different vessels such as the rat renal, pulmonary, and mesenteric arteries and vasorelaxation was inhibited in the presence of NO synthase (NOS) inhibitors.[72,83,84] However, in contrast to these studies, no changes were observed in the presence of NOS inhibitor in studies that were performed in isolated rat lung, cat hind limb arteries, and the cat penile artery.[85-87] Studies in human and dog coronary arteries and rat cerebral arteries showed inhibited ADM response with high potassium.[78,88,89] Although there are contradictory results in the literature, it has been shown in many studies that ADM provides relaxation through the cAMP, NO, or K^+ channels in vascular systems.[90]

According to its potent and long-lasting vasodilatory activity in the peripheral microcirculation, ADM also could be effective in PH.[91] In hypoxia-induced PH, ADM reduced pulmonary arterial pressure.[92] Systemic i.v. administration of ADM reduced pulmonary vascular resistance and increased arterial oxygen levels with no effect on systemic blood pressure.[93] In the studies performed in PH patients, the plasma level of ADM increased along with the severity of the disease. In contrast to the increase in the endogenous production of ADM, i.v. ADM administration reduced pulmonary artery pressure and pulmonary vascular resistance in PH patients.[94,95] In another study performed with a small number of PH patients, acute inhaled ADM was shown to improve selectively the hemodynamic parameters in the pulmonary system and increase exercise capacity.[96] Multicenter, randomized, controlled clinical trials should be conducted to evaluate the long-term safety and efficacy of ADM, to be able to consider it as a future treatment target in PH.[9]

Adrenomedullin2/intermedin

ADM2/IMD has quite a similar structure and function to CGRP and ADM. Therefore, it is also expected that ADM2/IMD can be effective in the vascular system. In many studies, blood pressure and vascular resistance were decreased and the heart rate was increased with the application of ADM2/IMD.[17,30,97,98] After cardiac ischemia/reperfusion injury, the administration of ADM2/IMD increased the coronary perfusion and contractile strength of the left ventricle and reduced myocardial infarct size, hypertrophy, and cardiac fibrosis.[99-101] In normotensive and hypertensive rats, i.v. infusion of ADM2/IMD increased cardiac output by reducing total peripheral vascular resistance.[102] ADM2/IMD has been shown to be a potent vasodilator in many vessel beds such as pulmonary, renal, and abdominal arteries.[103-106]

$CGRP_{8-37}$ and ADM receptor antagonist AM_{22-52} inhibited the effects of ADM2/IMD on the cardiovascular system under both

physiological and pathophysiological conditions. The CLR/RAMP receptors are responsible for the actions of ADM2/IMD in the cardiovascular system.[17,20,103] Although the effects of ADM2/IMD on the cardiovascular system frequently act through the CGRP receptors, in different vascular beds ADM2/IMD can interact with the both CGRP and ADM receptors.[5,57] The ADM2/IMD-mediated response acts through CGRP receptor in the hypotension of rat systemic pressure and the vasodilation of rat coronary, carotid, supramesenteric, and pulmonary arteries. However, the ADM2/IMD responses were AM1 and AM2 receptor-mediated in pig coronary and rat renal arteries.[17,20,103,105,107,108] Several studies have shown that the cardiovascular effects of ADM2/IMD are endothelium-mediated and NO-dependent. In the pulmonary vascular system and aorta, the relaxation responses were inhibited by the presence of NOS inhibitor Nω-Nitro-L-arginine methyl ester hydrochloride and in the damaged endothelium.[99,103,109] The NO production increased dose-dependently with ADM2/IMD administration in cerebral endothelial cells and pulmonary smooth muscle cells.[110,111]

The positive inotropic effects of ADM2/IMD and the role in cell proliferation, apoptosis, and cell migration were related to the increase in cAMP production.[112-114] The mRNA and protein levels of ADM2/IMD increased in the right ventricles, lung tissues, and plasma of hypoxia-induced pulmonary hypertensive rats.[115-117] The symptoms of PH were alleviated by ADM2/IMD treatment in rats, right ventricular hypertrophy was prevented, and hypoxic pulmonary vascular remodeling was inhibited.[111] According to studies that were performed in pulmonary hypertensive rats, ADM2/IMD is thought to be effective in PH.[118] In chronic hypoxia-induced PH ADM2/IMD provided potent vasodilation in the pulmonary arteries of rats and intraarterial administration reduced the perfusion pressure of hypoxic lungs. This reduction indicates the possible application of ADM2/IMD administration in humans with PH.[119,120]

CONCLUSION

Peptides of the CGRP family exhibit cardiopulmonary effects and have been investigated for many years. Especially CGRP and ADM were proposed as new vasodilator agents in the treatment of many cardiovascular disease, such as hypertension and PH. ADM2/IMD is also a potent vasodilator in the cardiopulmonary system and in recent years it has been shown as a new drug candidate for cardiometabolic disease. However, further investigations should be performed for understanding these possible effects of ADM2/IMD before clinical investigations.

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