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The Cardiopulmonary Effects of Calcitonin-Gene Related Peptide Family
Kalsitonin-Geni İle İlişkili Peptit Ailesinin Kardiyopulmoner Etkileri

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The cardiopulmonary diseases are very common in 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 the cardiopulmonary diseases have been studied for many years and promising results are obtained. Especially in recent years, two important members of the family, adrenomedullin and adrenomedullin2/intermedin, are considered as new treatment targets in cardiopulmonary diseases. In this review, the roles of CGRP family peptides in cardiopulmonary diseases has been investigated according to the studies that were performed to the present day.
Keywords: CGRP family, cardiopulmonary diseases, adrenomedullin, adrenomedullin2/intermedin, pulmonary hypertension

Kardiyopulmoner hastalıklar toplumda sık görülen, tedavi maliyeti oldukça yüksek ve halen kesin bir tedavisi bulunmayan hastalıklardır. Kalsitonin-geni ile ilişkili peptit 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 roller 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 stimulating peptide 1-3 and the latest member of the family, adrenomedullin2/intermedin (ADM2/IMD)[1,2]. These peptides are included in the same family because of the similar chemical structures and have important roles in the homeostasis of the body[3-6]. The effects of these peptides on cardiovascular and pulmonary system, especially ADM and ADM2/IMD, sparked an 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 CGRP family.

The distribution of the Calcitonin-Gene Related Peptide family peptides

The peptides of the CGRP family are widely expressed in the body. The first peptide of this family, calcitonin, is synthesized by a calcium-dependent mechanism and released from thyroid C-cells [10,11]. Another peptide, amylin, was isolated from amyloid plaques in β-cells where found in the pancreatic islets of the Langerhans[12]. The rest of the family; CGRP, ADM, ADM2/IMD; is more effective on the cardiovascular and pulmonary system. The CGRP expressed in the both central and peripheral nerves that 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, immune cells[13].

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

The distribution of ADM2/IMD is largely similar with ADM. The expression of ADM2/IMD was demonstrated in brain, liver, intestine, heart, kidney, plasma, hypothalamus and 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].

The Receptors of Calcitonin-Gene Related Peptide Family

The peptides of CGRP family interact with calcitonin receptors (CTR) or calcitonin receptor-like receptors (CLR). CTR first identified in pigs in 1991 and two different variants was found in human which was called hCT_aR ve hCT_bR. These receptors are located on cell surface. The hCTaR is widely distributed in the body, however the hCTbR was found in placenta, ovary, lung and bone marrow[27]. The CLRs were first demonstrated in rats in 1993 and 2 years later were showed in different tissues of human[28,29]. CLRs were found in the central nervous system, kidney 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 aminoacids and although they exhibit a homology less than 30%, they are structurally similar to each other. These proteins are named RAMP-1, RAMP-2 and RAMP-3[13]. AMY shows high affinity when CTRs are activated by RAMPs[33,34]. RAMPs that bind to CTR allow the receptor to show affinity to AMY instead of calcitonin. When the CTRs are connected with RAMP1, RAMP2 and RAMP3 is called as 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 an ADM receptor (AM1 and AM2, respectively) (Table1.).

Table 1. The receptors and receptor components that interacts with 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

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

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

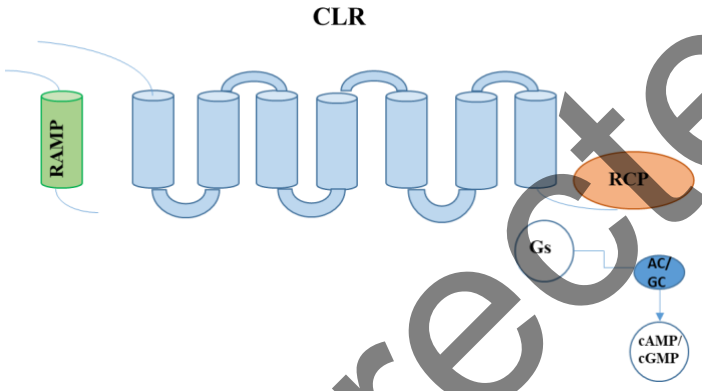

Figure1. CLRs are G protein-dependent receptors and contain 7 transmembrane domain.CLRs requires RAMPs and RCP for activation. The activated CLRs stimulate the G protein complex and provide activity.

Cardiopulmonary effects of the Calcitonin-Gene Related Peptide Family

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

1. Amylin (AMY)

AMY acts on the cardiovascular system via CGRP receptors[41]. However, AMY has to reach high plasma concentration to show activity. The 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]. Studies on rat cardiomyocytes and isolated heart, AMY showed a direct inotropic effect that mediated by CGRP receptors. However, because of the high doses AMY application side-effects on the heart, it was discussed that AMY could not be applied to the clinic[44,45].

2. Calcitonin Gene Related Peptide (CGRP)

CGRP is one of the most potent and effective vasodilator and it has a longer duration of action[46,47]. The relaxing effect of 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, the systemically administrated CGRP decreased the 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 also protective against ischemic damage. These effects of CGRP are generally thought to be the result of vasodilatory effect[50-52]. Furthermore CGRP also suppressed the releasing of potent vasoconstrictor agents such as endothelin and angiotensin[53].

CGRP provided important relaxation in the pulmonary vascular system and was found high amount in lung tissue[54]. In pulmonary hypertension (PH) the plasma CGRP levels were decreased and CGRP infusion has been shown to be effective in the treatment[13,23-25]. The adenovirus-mediated CGRP transfection before chronic hypoxia exposure in mice lungs provided cyclic adenosine monophosphate (cAMP)-mediated protection against pulmonary vascular resistance and decreased the vascular remodelling[53]. CGRP has been shown to provide protection against hypoxia-induced remodeling in human tissue studies[55] and it was showed 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 has 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 the vasodilation was also observed. However, even high doses CGRP did not stimulate the cylic guanosine monophosphate (cGMP) levels directly[59,60]. Therefore, it may indicate that CGRP directly activates the cAMP-dependent vasodilation[61-63]. In the studies that were performed in pig coronary artery and guinea pig uretery, CGRP-mediated vasodilation was inhibited with K_{ATP} channel inhibitor glibenclamide. Therefore, it was discussed that the increase in cAMP activates protein kinase A and subsequently K_{ATP} channels[61,63-67]. Basal and NO-stimulated CGRP release was increased in the human right atrium in the patients that was performed cardiopulmonary bypass [68,69]. However, there are also contradictory studies indicated the role of endothelium in CGRP-mediated vasodilation. CGRP provided nitric oxide (NO) and cGMP -dependent vasodilation in rat aorta[70].

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

3. Adrenomedullin (ADM)

In many years, ADM was drawn attention to the effects on cardiovascular system. A 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 was 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, 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 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 was also varies between different studies. Numerous studies have shown that endothelium-mediated vasorelaxation was occurred in different vessels such as rat renal, pulmonary, mesenteric artery and the vasorelaxation was inhibited in the presence of nitric oxide synthase (NOS) inhibitors[72,83,84]. However, in contrary to these studies, no changes were observed in the presence of NOS inhibitor in the studies that were performed in isolated rat lung, cat hind limb arteries, cat penile artery studies[85-87]. Studies in human and dog coronary arteries and rat cerebral arteries have 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 the 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 peripheric microcirculation, ADM also could be effective in PH[91]. In hypoxia-induced PH, ADM reduced the pulmonary arterial pressure[92]. Systemic i.v. administration of ADM reduced pulmonary vascular resistance and increased the arterial oxygen levels without no effect in the systemic blood pressure[93]. In the studies that was performed on PH patients; the plasma level of ADM was increased along with the severity of the disease. In contrast to the increase of 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 has been shown to improve selectively the hemodynamic parameters in the pulmonary system and increased exercise capacity [96]. Multi-center, randomized, controlled clinical trials should be conducted to evaluate the long-term safety and efficacy of ADM, to be considered as a future treatment target in the PH [9].

4. Adrenomedullin2/Intermedin (ADM2/IMD)

ADM2/IMD has quite 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, the 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; reduced myocardial infarct size, hypertrophy and cardiac fibrosis[99-101]. In normotensive and hypertensive rats, the 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} were inhibited the effects of ADM2/IMD on cardiovascular system under both physiological and pathophysiological conditions. The CLR/RAMP receptors are responsible from the actions of ADM2/IMD in the cardiovascular system[17,20,103]. Although the effects of ADM2/IMD on the cardiovascular system are frequently act through the CGRP receptors, in different vascular bed, ADM2/IMD can interacts 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 found AM_1 and AM_2 receptors-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 pulmonary vascular system and in aorta, the relaxation responses were inhibited by the presence of NOS inhibitor Nω-Nitro-L-arginine methyl ester hydrochloride (L-NAME) and in the damaged-endothelium[99,103,109]. The NO production was 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 the cell proliferation, apoptosis and cell migration were related with the increase of cAMP production[112-114]. The mRNA and protein levels of ADM2/IMD increased in right ventricles, lung tissues and plasma of hypoxia-induced pulmonary hypertensive rats[115-117]. The symptoms of PH were alleviated with ADM2/IMD treatment in rats, the right ventricular hypertrophy was prevented and the hypoxic pulmonary vascular remodeling was inhibited[111]. According to these 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 pulmonary arteries of rats and intraarterial administration was reduced the perfusion pressure of hypoxic lungs. This reduction indicates the possible application of ADM2/IMD administration in the human with PH[119,120].

Conclusion

Peptides of the CGRP family exhibit cardiopulmonary effects and have been investigated for long years. Especially CGRP and ADM was 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 cardiopulmonary system and in recent years it is shown as a new drug candidate for cardiometabolic disease[7]. However, further investigations should be performed for understanding these possible effects of ADM2/IMD before clinical investigations.

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