Annals of Medical Research
DOI: 10.5455/annalsmedres.2019.02.073 2019;26(3):443-6

Original Article

Apelin has inhibitory effect of endothelium-independent relaxation in the human internal mammary artery

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Abstract

Aim: Apelin has important effects on the circulatory system and heart. The main aim of this study was to investigate the effects of apelin-13 on the contraction induced by norepinephrine (NE), and the endothelium-independent relaxation induced by sodium nitroprusside (SNP) in human internal mammary artery (IMA) obtained from patients undergoing coronary artery bypass grafting (CABG) surgery.

Material and Methods: IMA rings, obtained from patients undergoing CABG surgery, were suspended in isolated tissue baths containing Krebs-Henseleit solution, which were continuously gassed with 95% O2 and 5% CO2 at 37°C.

Results: The IMA rings were pre-contracted with increasing concentrations of norepinephrine (NE 10-9–10-4 mol/l) and the endothelium-independent relaxation responses to sodium nitroprusside (SNP) were studied. Apelin-13 (10 μM) caused a dose-dependent relaxation in NE pre-contracted IMA rings. Apelin also facilitated the endothelium-independent relaxation induced by SNP.

Conclusion: According to the results, apelin facilitated the endothelium-independent relaxation and inhibited the contractile activity of IMA. These results suggest that apelin may be a physiological agent against the deterioration of vascular elasticity caused by endothelial damage especially in atherosclerotic cardiac patients and hypertensive patients.

Keywords: Apelin; Nitroprusside; Internal Mammary Artery; Coronary Artery Bypass.

INTRODUCTION

Apelin was isolated from the bovine stomach extracts as an endogenous ligand of the APJ receptor, which is a member of the G-protein-bound receptor family, in 1998 (1). Apelin is a peptide having a strong homology with the angiotensin II type 1 receptor (AT1R) in terms of the amino acid sequence (54% in transmembrane domains and 30% for the entire sequence) (2) and it is synthesized as pre-apelin containing 77 amino acids(3). Pre-apelin is transformed into different apelin peptides including apelin-12, apelin-13, apelin-17 and apelin-36. Apelin-13 and apelin-36 are biologically most active forms among these peptides (4). Apelin and its receptor APJ are located in many organs and systems of the body such as heart, lung, brain, kidney, liver, blood vessels, gastrointestinal system and adipose tissue (5). This broad distribution suggests that the apelin can be involved in many physiological and pathological processes (6). Studies showed that the apelin / APJ system has important functions in the cardiovascular homeostasis, the regulation of the central and peripheral cardiovascular system (7,8). It was found that apelin and APJ are expressed in cardiomyocytes and cardiac endothelium (9). It is known that apelin / APJ system are involved in blood pressure regulation and regulation of myocardial contractility (10). It was shown that apelin also increases the cardiac inotropic effect (11-15) in addition to increasing the vasodilator effect (16). It was detected that apelin applied to normal and hypertensive animals caused a strong antihypertensive effect (17,18). Since this antihypertensive effect is inhibited when administered concurrently with the NO synthase inhibitor, it suggests that apelin causes vasodilation via a NO-dependent mechanism (19). Also in vitro studies, it was shown that apelin leads to NO-dependent vasodilation in human mesenteric arteries (20). In the light of this information,

Received: 05.02.2019 **Accepted:** 12.02.2019 **Available online:** 13.02.2019
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it is seen that apelin has important effects on the heart and circulatory system. However, the number of in vitro studies examining its effects on vascular smooth muscle contraction is quite small.

Internal mammary artery (IMA) is used frequently as a coronary artery bypass graft, because the development of atherosclerosis is difficult in IMA, its diameter and flow characteristic are proper and rate of obstruction is very low compared to the saphenous vein used as a coronary bypass graft (21,22).

In the light of the above information, it was stated that apelin is vasodilator activity and this activity occurs via NO (19). However, there is no study describing the endothelium-independent activity of IMA on the contraction-relaxation mechanism. The main purpose of this study is; The aim of this study was to investigate the endothelium independent activity of Apelin-13 on the IMA's contraction-relaxation mechanism.

MATERIAL and METHODS

Ethical approval for present study was taken from Firat University Faculty of Medicine Clinical Research Ethics Committee (Elazig, Turkey), and the informed consents were taken from all patients. In the study, the use of human IMA segments, which were unused and discarded after coronary artery bypass grafting surgery was approved. Left IMA segments of 13 patients (2 females and 11 males) operated at the Cardiovascular Surgery Clinic, were used. Demographic characteristics of the patients are shown in Table 1.

Table 1. Some clinical features of 13 patients undergoing CABG

Clinical features	Mean ± SD, n (%)
Age	64.2 ± 7.0
Weight	70.2 ± 8.0
Body mass index	26.2 ± 2.3
Gender	
Male	11(85)
Female	2(15)
Smoking	7(54)
Diseases	
Hypertension	7 (55)
Heart failure	2 (15)
Diabetes	4 (30)
Medication	
Organic nitrates	0(0)
Aspirin	8(61)
Beta-blockers	7(54)
Calcium channel blockers	3(23)
Hypolipidaemics	6(46)
Angiotensin inhibitors	5(38)

The operation material was immediately placed in the krebs solution so as not to lose its viability after the removal and the experiment was performed by placing it in an isolated organ bath in about 20 minutes. The loose connective tissues were carefully removed from IMA segments and these segments were cut into rings so that their length was about 2-3 mm. These prepared tissue pieces were placed in an isolated organ bath containing Krebs-Henseleit solution (composition in mM: NaCl 118, KCl 4.7, MgSO4 1.2, CaCl2 1.25, KH2PO4 1.2, NaHCO3 25, glucose 11, EDTA 0.03) and the temperature of this solution was set to 37° C and the pH was set to 7.4.The vessels in the organ bath were continuously ventilated with 95% oxygen and 5% CO2. The contractile activities of the vessels were transferred to the computer via MP150WS for Windows (Biopac Systems Inc, CA, ABD) and recorded using a physiological force transducer (FDT05, Commat Ltd., Ankara, Turkey).

At the beginning of the experiment, the resting tensions of the IMA vessels were adjusted to 1 gram, and the vessels were kept under this resting tension for 180 minutes to adapt to the isolated medium.During this time, the Krebs-Henseleit solution in the isolated organ bath container was changed every 10 minutes.Following the stabilization period, cumulative NE (10-9-10-4M) and SNP (10-9-10-4M) concentrations were applied to the organ bath to determine the concentration for the maximum response.

Apelin-13 was obtained from Cayman-Chemical (Cat No: 13523, Ann Arbor, MI, USA) and NE, SNP and acetylcholine chloride from Sigma (St Louis, MO, USA). Each stock solution was diluted to the desired concentration just prior to administration to the organ bath.

Statistical analysis

Data were expressed as mean ± standard deviation. The effect of apelin-13 (10 μM) on contractile activity was evaluated using unpaired Student's T-test. In all statistical analyzes, p value < 0.05 was considered to be statistically significant.

RESULTS

In present study, the effects of apelin-13 at 10 μM dose on the maximum contraction induced by NE (10-9–10-4M) and the maximum relaxation induced by SNP (10-9–10-4M) were investigated in human IMA rings.At the beginning of the study, we first investigated the effect of Apelin-13 at 10 μM dose on basal tension, and in this practice, apelin-13 showed no effect on baseline tension (data not shown).The dose-dependent contractile activity of IMA rings was determined by applying cumulative concentrations of NE. After the administration of Apelin-13 at a dose of 10 μM, the same protocol was reapplied. According to the results, Apelin-13 caused significant inhibition on the contractions induced by NE administration (10μM, Figure 1, $p < 0.05$, $n = 13$). Apelin-13 at dose of 10 μM caused statistically significant increase in the cumulatively added SNP-induced endothelium-independent relaxation (10μM, Figure 2, $P < 0.05$, $n = 13$).

According to the results, apelin-13 inhibited NE-induced IMA contractile activity whereas it facilitated the SNP-induced endothelium-independent relaxation.

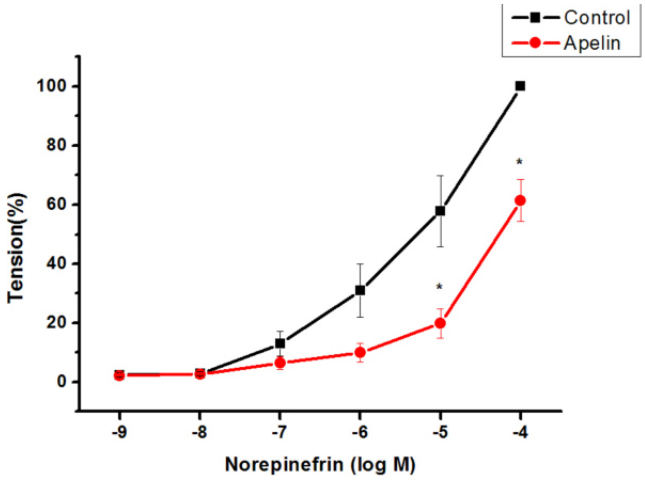

Figure 1. Effects of apelin on norepinephrine induced responses (contraction) in human internal mammary artery rings

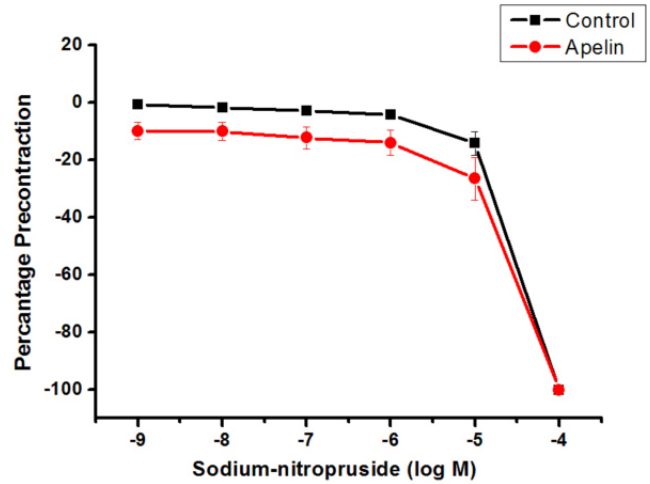

Figure 2. Effects of apelin on sodium nitroprusside induced responses (relaxation) in human internal mammary artery rings

DISCUSSION

In our study, we investigated the effect of Apelin-13 on NE-induced contraction and SNP-induced endothelium-independent relaxation in IMA rings.According to our results, apelin-13 inhibited NE-induced IMA contractile activity whereas it facilitated the SNP-induced endothelium-independent relaxation.Our study is the first study showing that Apelin-13 provides a relaxing effect on NE-induced contraction of IMA segments isolated from human.

IMA rings used in the present study were obtained from patients who underwent coronary bypass grafting (CABG) surgery due to various cardiovascular diseases as shown in Table-1.The SNP-induced endothelium-independent relaxation protocol was used as the relaxation protocol by taking the possibility of vascular endothelium being damaged during the operation into consideration.The most commonly used vessels as graft for coronary arteries are IMA and saphenous vein. The use of these vessels as grafts instead of the heart-feeding blood vessels makes the contractile activity in these vessels quite important and therefore this topic has become an important research area (23).

In previous studies, it was shown that apelin can lead to vasoconstriction and vasodilatation on the vasomotor system according to the conditions. This double effect of apelin is associated to the presence of APJ receptors in both endothelium and smooth muscle cell layers of the blood vessel wall. Vasoactive agents are said to be capable of acting on secretory endothelial cells that cause vasoconstriction (eg, endothelin) as well as substances that mediate vasodilation on vascular smooth muscle cells that cause relaxation (eg, NO, prostacyclin). Vasoactive agents can be effective on the substances (e.g. NO, prostacyclin) mediating the vasodilatation on vascular smooth muscle cells, as well as endothelium cells producing secretions that cause vasoconstriction (e.g. endothelin) (24). In a previous study, it was shown that Apelin-13 has vasodilator activity on IMA (25). However, the vasodilator effect in this study was based on endothelium-dependent NO-induced mechanisms. It was said that vasodilator effect was lost in groups with endothelial damage. However, practice with SNP showing endothelium-independent relaxation was not performed. The degree of endothelium damage and whether all endothelium was affected were unclear. In addition, there was no data about the additional diseases and the drugs of the individuals included in the experimental group. It is unclear whether the results in this study were due to the effects of the drugs used by the patient or if it is due to apelin. However, in our study, the IMA strips were washed for 180 minutes instead of the standard 120 minutes and the effects of the drugs and anesthesia used by the individuals were eliminated. SNP having endothelium-independent effect was used to put forward the endothelium-independent effect clearly.

As a result, the findings obtained from our study showed that apelin-13 has the inhibitory activity on NE-induced contractions and the facilitative activity on SNP-induced endothelium-independent relaxation in human IMA. In the light of these information, Apelin-13 shows its relaxation effect on IMA contractile activity with not only endothelium-dependent NO mechanisms but also by facilitating SNP-induced endothelium-independent relaxation. Because of this, not only endothelium-dependent but also endothelium independent mechanisms plays a role in the occurrence of apelin-13 vasodilator effect. Similarly, in most of the studies showing the vasodilator activity of apelin, vasodilation mechanisms were based on cGMP-mediated NO pathways (20,26,27). For the first time with our study, it was shown that endothelium-independent pathways were also effective under the vasodilator mechanism except for endothelium-dependent NO.

Coronary bypass surgery is an important treatment option in patients with coronary artery disease. The use of IMA as

a graft instead of the coronary artery increased the number of the studies about the contractile activity in the IMA and hormonal or pharmacological agents that may affect this. Apelin is a hormone that has effects in many systems but has significant effects in the cardiovascular system. Previous studies have demonstrated its efficacy on many vessel contractile activities in both experimental animals and humans. However, previous studies suggested that the vasodilator activity of apelin depend on cGMP-associated endothelium-dependent NO mechanisms. In addition, these previous studies showed that apelin does not have an endothelium-independent activity on the vessel or cause vasoconstriction in the case of endothelial damage. However, our study is important since it is the first study showing that apelin-13 leads to endothelium-independent vasodilation on human IMA. Our study, which demonstrates the presence of endothelium-independent activity of Apelin-13, is a pioneering study in order to clarify the endothelium-independent mechanisms of apelin-13. Further studies are needed to reveal the molecular mechanisms underlying endothelium-independent effect.

CONCLUSION

In conclusion, while apelin-13 facilitates SNP-induced endothelium-independent relaxation in human IMA, it causes inhibition on NE-induced contractions.

Competing interests: The authors declare that they have no competing interest.
Financial Disclosure: There are no financial supports
Ethical approval: Ethical approval for present study was taken from Firat University Faculty of Medicine Clinical Research Ethics Committee

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REFERENCES

1. Tatemoto K, Hosoya M, Habata Y, et al. Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. Biochem Biophys Res Commun 1998;251:471-6.
2. Lu L, Wu D, Li L, et al. Apelin/APJ system: A bifunctional target for cardiac hypertrophy. Int J Cardiol 2017;230:164-70.
3. Medhurst AD, Jennings CA, Robbins MJ, et al. Pharmacological and immunohistochemical characterization of the APJ receptor and its endogenous ligand apelin. J Neurochem 2003;84:1162-72.
4. Huang Z, Wu L, Chen L. Apelin/APJ system: A novel potential therapy target for kidney disease. J Cell Physiol 2018;233:3892-900.
5. Kawamata Y, Habata Y, Fukusumi S, et al. Molecular properties of apelin: tissue distribution and receptor binding. Biochim Biophys Acta 2001;1538:162-71.
6. Liu J, Liu M, Chen L. Novel pathogenesis: regulation of apoptosis by Apelin/APJ system. Acta Biochimt Biophys Sin (Shanghai) 2017;49:471-8.
7. Lee DK, Cheng R, Nguyen T, et al. Characterization of apelin, the ligand for the APJ receptor. J Neurochem 2000;74:34-41.
8. Masri B, Knibiehler B, Audigier Y. Apelin signalling: a promising pathway from cloning to pharmacology. Cell Signal 2005;17:415-26.
9. Yamaleyeva LM, Shaltout HA, Varagic J. Apelin-13 in blood pressure regulation and cardiovascular disease. Curr Opin Nephrol Hypertens 2016;25:396-403.
10. Rostamzadeh F, Najafipour H, Yeganeh-Hajahmadi M, et al. Heterodimerization of apelin and opioid receptors and cardiac inotropic and lusitropic effects of apelin in 2K1C hypertension: Role of pERK1/2 and PKC. Life Sci 2017;191:24-33.
11. Dai T, Ramirez-Correa G, Gao WD. Apelin increases contractility in failing cardiac muscle. Eur J Pharmacol 2006;553:222-8.
12. Chun HJ, Ali ZA, Kojima Y, et al. Apelin signaling antagonizes Ang II effects in mouse models of atherosclerosis. J Clin Invest 2008;118:3343-54.
13. Hashimoto T, Kihara M, Imai N, et al. Requirement of apelin-apelin receptor system for oxidative stress-linked atherosclerosis. Am J Pathol 2007;171:1705-12.
14. Li F, Li L, Qin X, et al. Apelin-induced vascular smooth muscle cell proliferation: the regulation of cyclin D1. Front Biosci 2008;13:3786-92.
15. Yu XH, Tang ZB, Liu LJ, et al. Apelin and its receptor APJ in cardiovascular diseases. Clin Chim Acta 2014;428:1-8.
16. Reaux A, De Mota N, Skultetyova I, et al. Physiological role of a novel neuropeptide, apelin, and its receptor in the rat brain. J Neurochem 2001;77:1085-96.
17. Katugampola SD, MacGuire JJ, Mathewson SR, et al. (125I)-(Pyr1)Apelin-13 is a novel radioligand for localizing the APJ orphan receptor in human and rat tissues with evidence for a vasoconstrictor role in man. Br J Pharmacol 2001;132:1255-60.
18. Napoli C, Ignarro LJ. Nitric oxide and atherosclerosis. Nitric Oxide 2001;5:88-97.
19. Szokodi I, Tavi P, Földes G, et al. Apelin, the novel endogenous ligand of the orphan receptor APJ, regulates cardiac contractility. Circ Res 2002;91:434-40.
20. Jia YX, Lu ZF, Zhang J, et al. Apelin activates L-arginine/nitric oxide synthase/nitric oxide pathway in rat aortas. Peptides 2007;28:2023-9.
21. Koike R, Suma H, Kondo K, et al. Pharmacological response of internal mammary artery and gastroepiploic artery. Ann Thorac Surg 1990;50:384-6.
22. Burma O, Ozcan M, Kacar E, et al. In vitro effects of sodium nitroprusside and leptin on norepinephrine-induced vasoconstriction in human internal mammary artery. Cardiovascular J Afr 2015;26:4-7.
23. Mendonça L, Mendes-Ferreira P, Bento-Leite A, et al. Angiotensin-(1–7) modulates angiotensin II-induced vasoconstriction in human mammary artery. Cardiovasc Drugs Ther 2014;28:513-22.
24. O'Rourke ST, Vanhoutte PM, Miller VM. Vascular Pharmacology. In: Creager MA, Dzau VJ, Loscalzo J, eds. Vascular Medicine: A Companion to Braunwald's Heart Disease. 3rd edition. Philadelphia: Saunders/Elsevier; 2006. p. 71-100.
25. Maguire JJ, Kleinz MJ, Pitkin SL, et al. (Pyr1)apelin-13 identified as the predominant apelin isoform in the human heart: vasoactive mechanisms and inotropic action in disease. Hypertension 2009;54:598-604.
26. Mughal A, Sun C, O'Rourke ST. Activation of large conductance, calcium-activated potassium channels by nitric oxide mediates apelin-induced relaxation of isolated rat coronary arteries. J Pharmacol Exp Ther 2018;366:265-73.
27. Busch R, Strohbach A, Pennewitz M, et al. Regulation of the endothelial apelin/APJ system by hemodynamic fluid flow. Cell Signal 2015;27:1286-96.