ORIGINAL ARTICLE

The differential effects of low and high doses of apelin through opioid receptors on the blood pressure of rats with renovascular hypertension

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The apelin/APJ system has an important role in the regulation of vascular tone and blood pressure. Opioid receptors (OPRs) are also important cardiovascular regulators and exert many of their effects by modulating the function of other G-protein-coupled receptors. The aim of this study was to analyze the interaction of apelin and the opioid system with respect to vascular responses to apelin in rats with renovascular hypertension (two-kidney, one clip (2K1C)). Homodynamic studies were carried out in 2K1C rats. Naloxone (a nonselective OPR inhibitor) or nor-binaltorphimine dihydrochloride (norBNI, a kappa OPR inhibitor) and signaling pathway inhibitors PTX (a Gi path inhibitor) and chelerythrine (a protein kinase C (PKC) inhibitor) were administered before apelin at 20 and 40 $\mu g\,kg^{-1}$. Apelin at 20 and 40 $\mu g\,kg^{-1}$ decreased the systolic blood pressure by 15% and 20%, respectively ($P < 0.05$). The pressure drop caused by apelin 20 was inhibited by naloxone, norBNI and PTX, but it was not affected by chelerythrine. The pressure drop caused by apelin 40 was augmented by naloxone and chelerythrine, and it was not affected by norBNI or PTX. The lowering effect of apelin 20 on blood pressure is exerted through OPRs and stimulation of Gi and PKC pathways. However, apelin 40 functions independently of OPRs, Gi and PKC. This dose-dependent differential effect of apelin may have potential clinical applications as opioids are currently used, and apelin has been introduced as a potential therapeutic agent in cardiovascular complications.

INTRODUCTION

Hypertension is one of the most important risk factors of coronary artery disease.[1] One of the aims of Healthy People 2020 is to reduce the hypertensive population.[2] Between 2000 and 2013 in the United States, the hypertension death rate increased by 23.1%, whereas deaths caused by other causes decreased by 21%.[3]

Kidney disease is a very important cause of secondary hypertension, which can damage other organs, such as the heart.[4] One of the experimental models used to assess hypertension, which is comparable to similar situations in humans, is the two-kidney, one clip (2K1C) hypertension model.[5] In this model, a clamp is placed on one of the renal arteries, whereas the other renal artery remains intact. This leads to a sustained increase in blood pressure, which is dependent on plasma renin–angiotensin system activity[6] and nitric oxide (NO) availability.[7,8]

G-protein-coupled receptors (GPCRs) are a main target for drugs. About one-third of manufactured drugs act through this pathway.[9] This is very striking, especially in the treatment of cardiovascular diseases. Modulation of GPCR function is useful for controlling blood pressure, stimulating cardiac contractility, preventing thrombosis and multiple other conditions.[9]

Apelin and its receptor (APJ) are an autocrine or paracrine system that potentially regulates heart and vascular function.[10] In human blood vessels, apelin and its receptor are found in endothelial cells, vascular smooth muscle cells of conductive vessels, and pulmonary, adrenal and renal vessels.[10] Vasodilation and the hypotensive effects of apelin are endothelium and NO-dependent.[11]

Opioid peptides and their GPCR receptors are also important regulators of the cardiovascular system and have an important role in regulating heart electrophysiology, heart rate and vascular function.[12] Vasodilator/hypotensive impacts of opioids are the best-known non-analgesic effects of opioids. The opioid system functions either directly or indirectly by modulating the function of other cardiovascular regulators.[12]

Apelin and opioids receptors are both class A GPCRs. They transmit signals through the activation of the protein Gi/o, which leads to the

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activation of ERK1/2 and the inhibition of adenylate cyclase activity.[13] The distribution of opioid and apelin receptors is similar in the cardiovascular and central nervous systems.[14] In addition, they have similar signal transmission pathways in cardiovascular, neurological protection and pain regulation systems.[15] It has been shown that APJ and kappa opioid receptors (KORs) can form a heterodimer. During the formation of the heterodimer, transmission of the message induced by apelin or dynorphin A changes such that with the activation of Gi, the activity of the protein kinase C (PKC) pathway increases, and the activity of the PKA pathway decreases.[14]

Our previous studies, which investigated cardiovascular responses to apelin and the expression of apelin receptors in acute renovascular hypertension, revealed that apelin decreases blood pressure in hypertensive rats.[16,17] The expression of apelin receptors is reduced in the heart, aorta and kidneys of 2K1C animals.[16,18] Renovascular hypertension is associated with changes in the endogenous opioid system,[19] and opioids are currently used in the management of conditions such as myocardial infarction and stroke, which are complications of hypertension. Apelin has been introduced as a potential therapeutic goal in cardiovascular complications. There is a close relationship between the apelin and opioid systems, and similarities include (1) the type of receptors, (2) signaling pathways, (3) the ability of their receptors to create a heterodimer and (4) the ability of opioids to modulate the action of other systems on the heart and blood vessels. Therefore, the purpose of this study was to evaluate the interaction between apelin/APJ and opioid/OPR systems regarding vascular responses to apelin in an acute renovascular hypertension model and to investigate some related downstream pathways.

METHODS
Materials
Male Wistar rats (180–200 g) were obtained from Kerman Physiology Research Center. The animals were housed on a 12-h light/dark cycle with standard rat chow and water *ad libitum*. Apelin-13 and its antagonist (apelin F13A) were obtained from Phoenix Pharmaceuticals (Strasbourg, France). The amino-acid sequence of apelin-13 and apelin F13A are Gln–Arg–Pro–Arg–Leu–Ser–His–Lys–Gly–Pro–Met–Pro–Phe and Gln–Arg–Pro–Arg–Leu–Ser–His–Lys–Gly–Pro–Met–Pro–Ala, respectively. Naloxone hydrochloride dihydrate (a nonselective opioid receptor antagonist) and nor-binaltorphimine dihydrochloride (norBNI, a selective KOR antagonist) were purchased from Sigma (St Louis, MO, USA). PTX (a Gi protein inhibitor) and chelerythrine (a PKC inhibitor) were purchased from Santa Cruz Biotechnology (Beverly, MA, USA). Apelin, F13A, naloxone, norBNI, PTX and chelerythrine were dissolved in saline.

Methods
Study groups. All tests were performed according to the national guidelines for performing animal studies. The study was approved by the ethics committee of the Kerman University of Medical Sciences (Kerman, Iran; permission code: 93/189KA). The study included 152 animals, which were randomly divided into 19 groups of eight animals each. Three groups did not receive apelin, including the sham, sham-vehicle and 2K1C-vehicle groups. The remaining 16 groups were divided into two subgroups: apelin 20 (receiving 20 μg kg^{-1} apelin) and apelin 40 (receiving 40 μg kg^{-1} apelin). These doses were based on our previous studies showing that apelin 20 induced positive inotropic effects, whereas apelin 40 induced negative inotropic effects.[16,17] Furthermore, pilot experiments showed that that apelin at 60 μg kg^{-1} did not lower blood pressure significantly more than apelin at 40 μg kg^{-1}. Each subgroup consisted of (1) saline+apelin, (2) F13A+apelin, (3) naloxone+apelin, (4) F13A+naloxone +apelin, (5) norBNI+apelin, (6) F13A+norBNI+apelin, (7) PTX+apelin and (8) chelerythrine+apelin.

Induction of hypertension. Animals were anesthetized with an intraperitoneal injection of ketamine (80 mg kg^{-1}) and xylazine (10 mg kg^{-1}). A 4-cm

Table 1 Effect of clamping the left renal artery on the weight of left kidney, and the ratios of left ventricle and lung weights to body weight and on the systolic and diastolic arterial pressures in sham and 2K1C +vehicle groups

Variables	Sham (n = 8)	2K1C+vehicle (n = 8)
	Groups	
LKW/RKW (mg mg^{-1})	0.94 ± 0.06	0.62 ± 0.22[a]
LVW/BW (mg g^{-1})	2.35 ± 0.17	2.47 ± 0.23
LW/BW (mg g^{-1})	6.88 ± 0.95	7.52 ± 0.79
SBP (mm Hg^{-1})	114.6 ± 8.3	169.5 ± 4.7[a]
DBP (mm Hg^{-1})	80.3 ± 6.2	121.7 ± 4.1[a]

Abbreviations: BW, body weight; DBP, diastolic blood pressure; LVW, left ventricle weight; LKW, left kidney weight; LW, lung weight; RKW, right kidney weight; SBP, systolic blood pressure.
[a]$P<0.005$ *vs.* Sham.

longitudinal incision was made in the abdominal wall of the left flank. The left renal artery was exposed and separated from the renal vein, and a solid Plexiglas clip with a 0.2-mm cleft diameter was placed around the artery. The sham groups were subjected to the same procedure, but no clip was applied. Procaine penicillin-G powder (30 000 U) was applied to the incision. Then, the abdominal muscular and skin layers were sutured using 5-0 chromic catgut and 4-0 silk threads, respectively. Once recovered from anesthesia, animals were kept in individual cages in standard conditions. The induction of acute renovascular hypertension took 4 weeks.[20]

Measurement of blood pressure
After 4 weeks,[20] animals were anesthetized with sodium thiopental (50 mg kg^{-1}, intraperitoneally). The right femoral artery was cannulated, and the catheter was connected to a pressure transducer for recording arterial blood pressure via a Powerlab Physiograph system (AD Instruments, Bella Vista, NSW, Australia). The left jugular vein was cannulated for intravenous injection of apelin and antagonists. The sham group received an intravenous injection with an equal volume of saline. Only rats with a systolic arterial pressure of 150 mm Hg or higher were included in the study.

After cannulation, at least 10 min of recovery allowed pressure to stabilize before the first injection of saline or antagonists (except PTX). Ten minutes after the first injection, apelin was injected. Immediately after the administration of each drug, 0.2 ml of saline was injected to wash the drug from the cannula. The antagonists included F13A (30 μg kg^{-1}),[16] naloxone (3 mg kg^{-1}),[21] norBNI (3 mg kg^{-1})[21] and chelerythrine (5 mg kg^{-1}).[22] PTX (10 μg kg^{-1}) was injected intraperitoneally 48 h before the hemodynamic experiments.[23]

While animals were in deep anesthesia at the end of the experiment, they were killed by withdrawing the femoral artery cannula. Immediately, the heart, lungs and kidneys were removed, washed in saline and dehumidified using gauze. The right ventricle and atria of the heart were removed using scissors, and the left ventricle, lungs and kidneys were weighed. The weight ratio of the left kidney to the right kidney is the kidney ischemia index. The ratio of the lung weight to body weight is the pulmonary edema index. The ratio of the left ventricular weight to body weight is the left ventricular hypertrophy index.

Statistical analysis
Data in the table and figures are presented as the means ± s.e.m. Repeated analysis of variance was used to compare the effect of apelin on blood pressure in the presence and absence of antagonists over time. For additional variables, differences among groups were analyzed by analysis of variance, followed by Tukey's *post hoc* test. $P<0.05$ was considered significant. The percentage of change for each variable compared with the basal value was calculated by:

$$\%\text{change} = [(B - A)/A] * 100$$

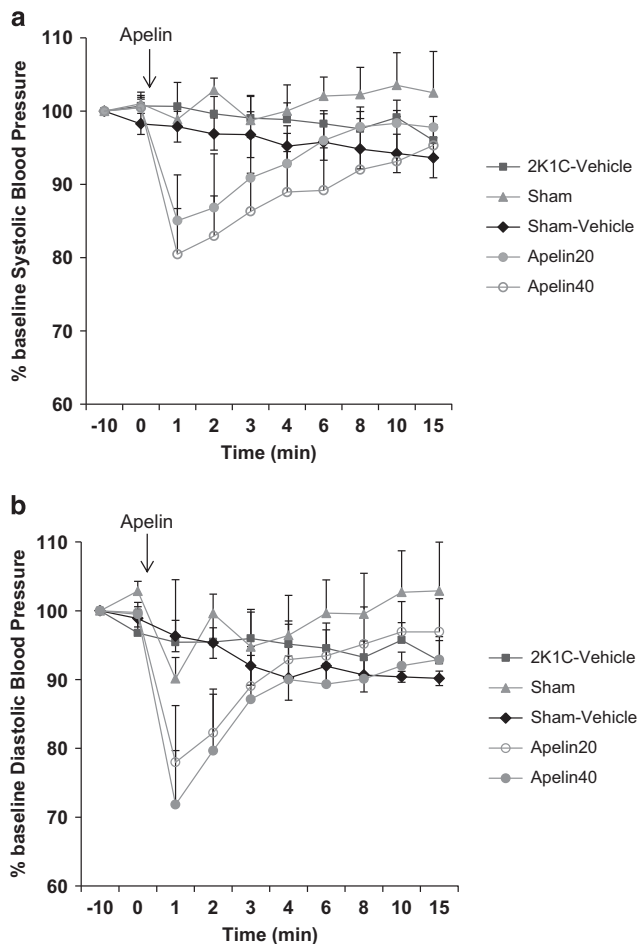where *B* is the value after intervention and *A* was the value before intervention.

Figure 1 (a) Systolic and (b) diastolic blood pressure changes in response to injection of vehicle or apelin at doses of 20 and 40 $\mu g\,kg^{-1}$ in the study groups. The values are expressed as the mean $\pm$ s.e.m. $N=8$ in all groups.

RESULTS

In the sham group, systolic and diastolic blood pressures (SBP and DBP) were 114.8 ± 8.2 and 75.8 ± 8.3 mm Hg, respectively, compared with 169.4 ± 4.7 and 121 ± 4.1 mm Hg, respectively, in the 2K1C +vehicle group. Both SBP and DBP were significantly higher in the 2K1C+vehicle group compared with the sham group ($P<0.01$). Clamping the left renal artery significantly decreased the weight of the left kidney compared with the right kidney (Table 1). There was no significant increase in the ratio of left ventricle and lung weights to body weight.

Injection of apelin at 20 and 40 $\mu g\,kg^{-1}$ decreased the SBP by 15% and 20%, respectively (Figure 1a), and the DBP by 22% and 28%, respectively (Figure 1b) ($P<0.05$). The maximum change in BP occurred 1 min after injection and returned to approximately preinjection levels in 2–4 min. The basal (preinjection) SBP was 169 ± 4.8 mm Hg, and DBP was 117.8 ± 4.2 mm Hg in the apelin 20 group compared with a SBP of 168.8 ± 2.5 mm Hg and a DBP of 119.4 ± 2.5 mm Hg in the apelin 40 group.

There were no significant changes in the SBP and DBP in the sham, sham−vehicle and 2K1C−vehicle groups during the recording period. Therefore, the sham and sham−vehicle groups have been removed to simplify the following graphs.

To ensure that the effects of apelin 20 are mediated via its receptor, APJ, 30 $\mu g\,kg^{-1}$ F13A (an apelin receptor antagonist) was introduced

before the administration of apelin. F13A completely inhibited the drop in blood pressure mediated by apelin 20 (Figure 2a). To investigate the role of opioid receptors in mediating the effects of apelin, 3 $mg\,kg^{-1}$ naloxone (a nonselective opioid receptor antagonist) was administered 10 min before the injection of apelin. The drop in SBP caused by apelin was completely prevented by naloxone. A combination of F13A and naloxone also completely inhibited the pressure drop mediated by apelin 20. To investigate which opioid receptors are involved in this effect, norBNI (a selective KOR antagonist) was administered at 0.3 $mg\,kg^{-1}$. The injection of norBNI significantly inhibited the BP drop caused by apelin (from 15 to 4%) ($P<0.005$). This pressure drop remained stable for 8 min. Similarly, when norBNI and F13A were used together, the pressure drop was 4% ($P<0.05$). However, pressure recovered rapidly and returned to its previous level within 2 min (Figure 2a). Apelin 20 decreased DBP by 22%, and it returned to preinjection levels within 3–4 minutes. The effects of F13A, naloxone and norBNI on DBP drops were similar to their effects on SBP (Figure 2b).

In the apelin 40 group, SBP decreased by 20%, and the drop in pressure was maintained longer than the apelin 20 group. F13A did not inhibit the effect of apelin 40 on SBP. In contrast to the effect of apelin 20, naloxone intensified the SBP drop caused by apelin 40, which was sustained for 30 min. Naloxone combined with F13A significantly inhibited the pressure drop caused by apelin 40. NorBNI failed to inhibit the effects of apelin 40 on SBP; however, BP recovered faster (Figure 3a). NorBNI in combination with F13A did not significantly inhibit the pressure drop caused by apelin 40.

The effects of 40 $\mu g\,kg^{-1}$ apelin on DBP (maximum reduction to 28% basal levels) and on SBP were very similar. F13A did not inhibit the apelin-induced diastolic pressure drop, and naloxone intensified this pressure drop with no recovery for 30 min. Naloxone in combination with F13A significantly inhibited the pressure drop caused by apelin 40. NorBNI failed to inhibit the effects of apelin 40 on DBP, but recovery to normal pressures was faster (Figure 3b). NorBNI in combination with F13A did not significantly inhibit the DBP drop caused by apelin 40.

Our data show that inhibiting opioid receptors inhibits the hypotensive effect of apelin 20. However, it enhances the effect of apelin 40 on BP. Therefore, we hypothesized that different doses of apelin activate different pathways. To investigate downstream signaling pathways, we inhibited Gi and PKC signaling by administering PTX and chelerythrine, respectively.

Gi inhibition suppressed the decrease in SBP induced by apelin 20 (Figure 4a). PKC inhibition suppressed part of the decrease in SBP caused by apelin 20 (8% vs. 15%). Both inhibitors had a similar effect on DBP (Figure 4b).

Gi inhibition did not significantly suppress the inhibition of SBP in response to apelin 40 (Figure 5a). PKC inhibition did not decrease the response to apelin 40; it actually intensified the BP drop (44% vs. 20%) (Figure 5a). These inhibitors had a similar effect on DBP (Figure 5b).

DISCUSSION

We investigated the effects of two doses of apelin on the blood pressure of renovascular hypertensive rats and the interaction of apelin and opioid receptors in this conditions. Our main finding was that naloxone and norBNI inhibited the antihypertensive effect of 20 $\mu g\,kg^{-1}$ apelin. However, when the dose was increased to 40 $\mu g\,kg$, naloxone intensified the effect of apelin, and norBNI failed to exert any inhibitory effect.
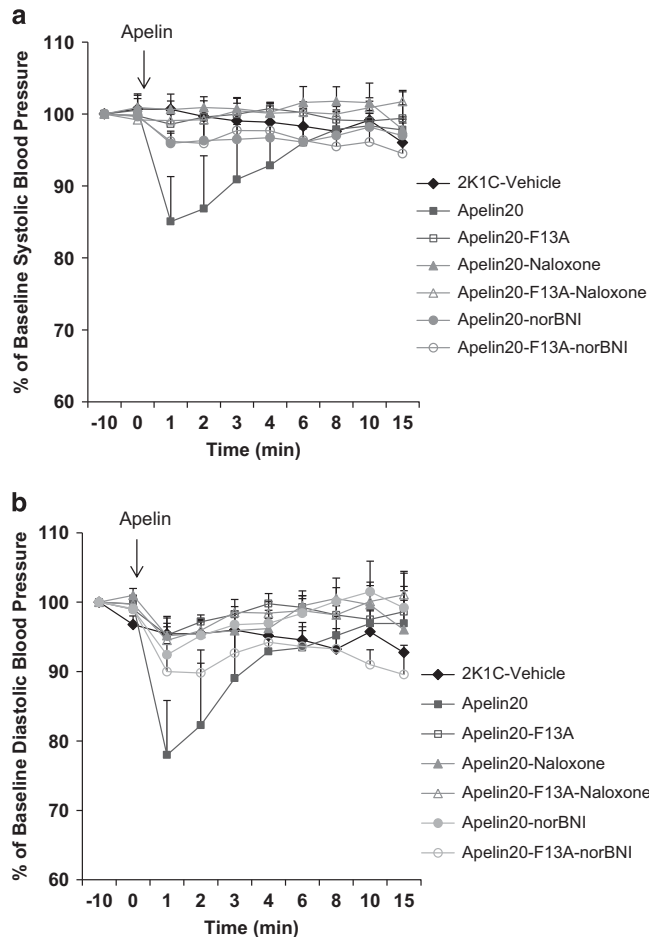
Figure 2 (a) Systolic and (b) diastolic blood pressure changes in response to apelin injection at 20 µg kg^{-1} in the presence of antagonists in the study groups. The values are expressed as the mean ± s.e.m. $N=8$ in all groups.

Figure 3 (a) Systolic and (b) diastolic blood pressure changes in response to apelin injection at 40 µg kg^{-1} in the presence of antagonists in the study groups. The values are expressed as the mean ± s.e.m. $N=8$ in all groups.

Apelin (both doses) decreased the SBP and DBP, with a larger decrease in the latter (Figures 1–3). Apelin acts on the heart and blood vessels to affect blood pressure. These effects are in opposite directions, with increased myocardial contractility leading to increase in blood pressure[24] and vasodilation of blood vessels leading to lower blood pressure.[25–27] Diastolic pressure is more affected by vascular resistance compared with systolic pressure, which is influenced more by cardiac output.[28] Therefore, the vascular effects of apelin seem dominant.

F13A inhibited the apelin 20-mediated suppression of systolic and diastolic blood pressures. This indicates that the effects of apelin 20 are mediated through its receptor, APJ, which is consistent with a previous study on the APJ-mediated hypotensive effect of apelin.[29] The use of naloxone alone and in combination with F13A completely inhibited the blood pressure drop in response to apelin 20, indicating that its effects are mediated via opioid receptors. The use of norBNI alone inhibited a large part of the pressure drop caused by apelin 20 (11% of 15%). Thus, it seems that the effects of apelin are mediated primarily by KORs, and delta and mu receptors have only minor roles. Consistent with this hypothesis, KORs are more dominant in blood vessels compared with other types of opioid receptors.[30] When norBNI was used in combination with F13A, the impact was similar to using norBNI alone (Figure 2a). Although we expected that F13A would completely block the effects of apelin 20 in
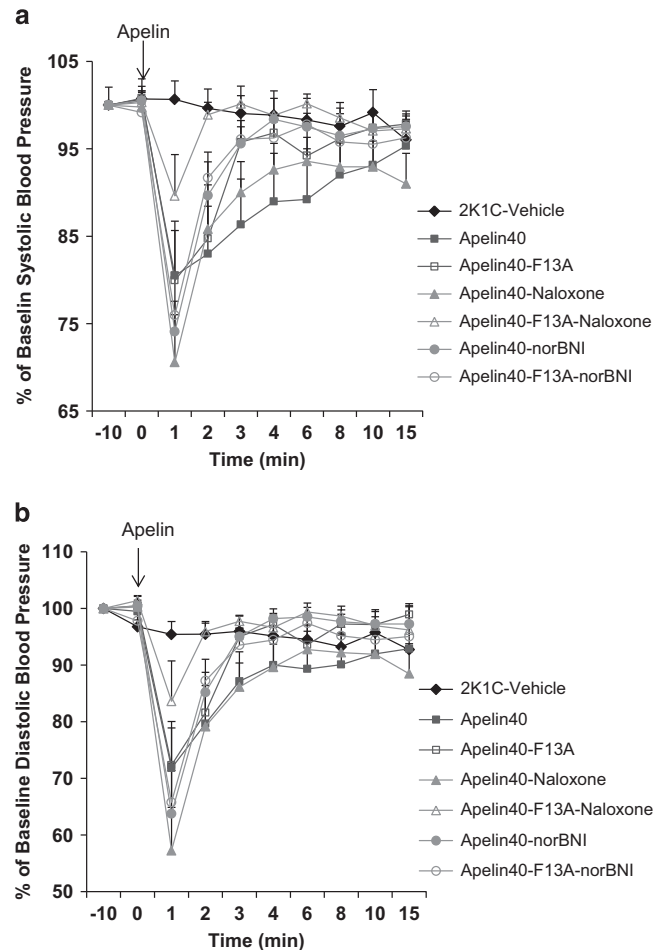
the presence of norBNI, this was not the case. There are two possibilities: either norBNI counteracts the inhibitory effects of F13A or apelin causes the remaining 4% drop in BP through a receptor-independent route. Kappa, mu, delta opioid and APJ are all class A GPCRs,[31] which can form heterodimers[32] and alter the signaling pathway. In addition, the presence of agonists and antagonists change the heterodimers and their affinity for their respective ligands.[32] Therefore, F13A may not fully exert its inhibitory effect in the presence of KOR inhibitor due to changes in the homo- and heterodimers, making it more likely that norBNI counteracts the inhibitory effects of F13A.

Apelin 40 caused a 20% drop in systolic pressure. Combined F13A and opioid antagonists had unexpected effects, which were largely the opposite results from combining F13A with apelin 20 µg kg^{-1}. Using 30 µg kg^{-1} of F13A did not inhibit the pressure drop caused by apelin 40, and the addition of norBNI increased the hypotensive effect. It may be argued that the dose of F13A and norBNI was insufficient to block the effect of a higher dose of apelin. Although we cannot exclude this possibility, it was expected that these two antagonists block, at least partially, the hypotensive effects of apelin 40, as they completely inhibited the effects of apelin 20 (see Figure 2). Therefore, at a high dose, apelin may exert effects that are not dependent on APJ. Moreover, the inhibition of all types of opioid receptors intensifies the effects of apelin 40 on lowering blood
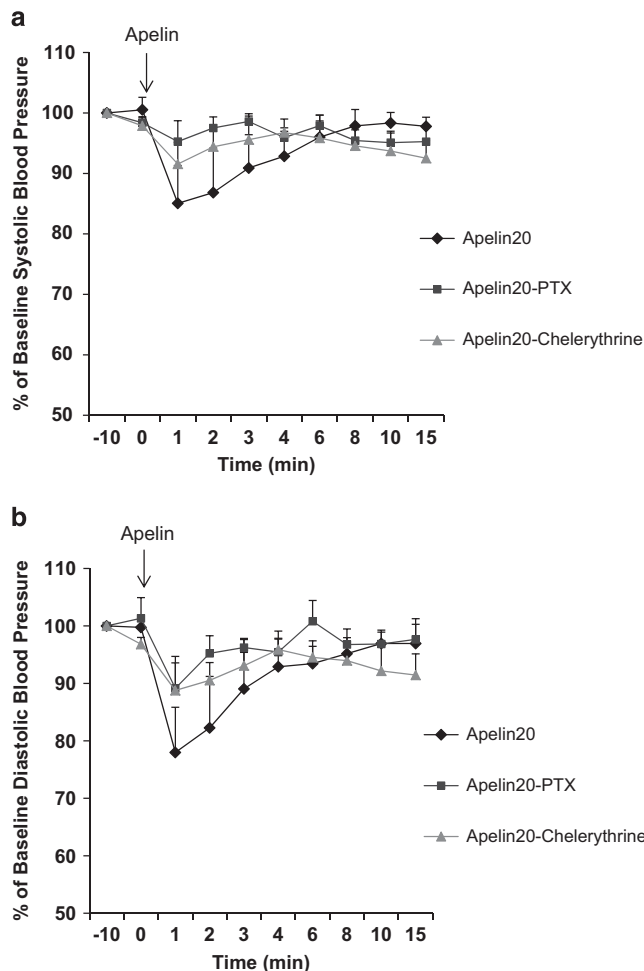
Figure 4 (a) Systolic and (b) diastolic blood pressure changes in response to apelin injection at 20 µg kg^{-1} in the presence of inhibitors of the signaling pathway. The values are expressed as the mean ± s.e.m. $N=8$ in all groups.

Figure 5 (a) Systolic and (b) diastolic blood pressure changes in response to apelin injection at 40 µg kg^{-1} in the presence of signaling pathway inhibitors. The values are expressed as the mean ± s.e.m. $N=8$ in all groups.

pressure, as was observed when naloxone alone increased the pressure drop in response to apelin 40 from 20 to 29%. When F13A was used in combination with naloxone, this response was significantly blocked. The use of norBNI in combination with F13A did not exert any inhibitory effect; it actually increased the effect of apelin 40 (see Figure 3). Therefore, apelin at high doses may exert its effects through APJ-dimerized mu and delta OPRs. As a result, it is possible that different doses of apelin exert their effects via different mechanisms.

The effect of apelin 40 was only blocked when all mu, kappa, delta and APJ receptors were simultaneously inhibited (Figure 3, apelin +F13A+naloxone curve). Inhibition of APJ or kappa receptors alone and inhibition of all opioid receptors (by naloxone) did not inhibit the effects of apelin 40.

Gi inhibition by PTX blocked the apelin 20-mediated decreases in systolic and diastolic pressures. The vasodilatory effect of apelin is NO-dependent,[25] and Gi can stimulate NOS via Akt and PKC.[31] Although the inhibition of PKC by chelerythrine suppressed some of the pressure drop caused by apelin 20 (15–8%), it was not significant. Therefore, apelin 20 causes vasodilatation through the Gi pathway and at least a part of this vasodilatation is via pathways other than PKC, probably Akt and/or phosphoinositide 3-kinase. In renovascular hypertensive rats, the inhibition of plasma renin activity using an
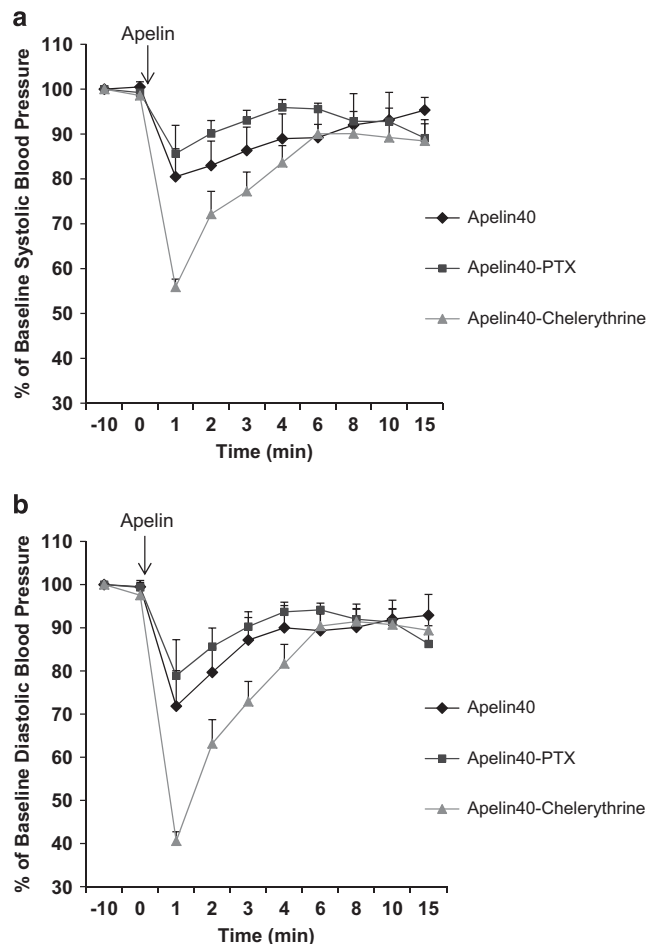
NO donor (L-arginine) normalized blood pressure and recovered impaired baroreflex sensitivity,[7,8] suggesting that the effects of apelin may be mediated via an increase in endothelial nitric oxide synthase activity/NO bioavailability, an improvement in baroreflex sensitivity and a decrease in oxidative stress. We propose to assess more detailed mechanisms including ERK/Akt downstream pathways to further clarify the underlying mechanisms.

Gi inhibition by PTX did not inhibit the systolic and diastolic pressure drop in response to apelin 40. Thus, it seems that high-dose apelin activates pathways other than Gi. PKC can be activated via the Gq pathway in addition to the Gi pathway. PKC inhibition by chelerythrine unexpectedly intensified the pressure drop in response to apelin 40 (44% vs. 20%, Figure 5a). These data suggest that apelin 40 causes vasodilatation through pathways other than Gi and PKC. The enhanced response to apelin 40 in the presence of chelerythrine may be due to the ability of PKC to function as a myosin light-chain phosphatase inhibitor,[32] which prevents relaxation. It is possible that apelin 40, in addition to activating of vasodilatory factors, activates the PKC pathway, which reduces the drop in BP. However, when PKC is disabled, this balance is disturbed, and apelin 40 causes a more severe drop in BP.

The results of this study, which show that low and high doses of apelin function via different pathways, are consistent with

findings from the study by Duparc showing that different doses of apelin activate different pathways in the hypothalamus.[33] Low doses of apelin increased the production of NO, whereas a high dose led to the production of hydrogen peroxide, which is an EDHF (endothelium-derived hyperpolarizing factor)[34] that hyperpolarizes and relaxes vascular smooth muscle. Therefore, apelin 40 may also act through NO-independent pathways.

It has been shown that GPCR ligands with different concentrations have different effects. For example, CGP12177A, which is a β1 and β2 receptor antagonist, has antagonistic and agonistic effects on β1-adrenergic receptors at low and high concentrations, respectively.[35] Information from human β1-adrenergic receptors supports the hypothesis that there are binding sites other than the classic ortho steric sites, which can stimulate functional responses. These sites could function cooperatively and independently.[36] In addition to different signaling, responses to antagonists may change. The stimulation of β1-adrenergic receptors by CGP12177A is more resistant to inhibition by antagonists compared with the stimulation of these receptors by catecholamines.[37] This may also be true for APJ, which has both classical and nonclassical binding sites.[14,38,39] These sites may be activated by a high dose of apelin, activating different signaling pathways. This is more complicated in the case of APJ, which can cause dimerization with other GPCRs, including KORs.[14]

CONCLUSION

This study shows that in rats with renovascular hypertension, opioid receptors mediate vasodilator responses to apelin; yet, these effects are dose-dependent. The antihypertensive effect of apelin in low doses is exerted via opioid receptors, but in high doses, this effect is probably mediated by dimerized receptors of apelin and opioids. Furthermore, apelin at low doses appears to exert its effect via stimulation of the Gi pathway and PKC. However, apelin in high doses activates alternate pathways. The dose-dependent differential effects of apelin may have potential clinical applications, as opioids are currently used in the management of MI and stroke, and apelin has been introduced as a potential therapeutic agent in cardiovascular complications.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

1 Ohira T, Iso H. Cardiovascular disease epidemiology in Asia. Circ J 2013; 77: 1646–1652.
2 US Department of Health and Human Services (HHS). Healthy People 2020. Available at: http://www.healthypeople.gov (last accessed 20 October 2016).
3 Kung H-C, Xu J. Hypertension-related mortality in the United States, 2000-2013. NCHS Data Brief 2015; 193: 1–8.
4 Levy D, Larson MG, Vasan RS, Kannel WB, Ho KK. The progression from hypertension to congestive heart failure. JAMA 1996; 275: 1557–1562.
5 Kagiyama S, Varela A, PhillipsMI, Galli SM. Antisense inhibition of brain renin-angiotensin system decreased blood pressure in chronic 2-kidney 1 clip hypertensive rats. Hypertension 2001; 37: 371–375.
6 Hall JE. Guyton and Hall Textbook of Medical Physiology, 13th edn. Elsevier Health Sciences: Philadelphia, PA, USA, 2015, pp 237–238.
7 Tiradentes RV, Santuzzi CH, Clandio FRG, Mengal V, Silva NF, Neto HAF, Bissoli N, Abren GR, Gonvea SA. Combined aliskiren and L-arginine treatment reverses renovascular hypertension in an animal model. Hypertens Res 2015; 38: 471–477.
8 Mengal V, Silva PH, Tiradentes RV, Santuzzi CH, de Almeida SA, Sena GC, Bissoli NS, Abreu GR, Gouvea SA. Aliskiren and L-arginine treatments restore depressed baroreflex

9 sensitivity and decrease oxidative stress in renovascular hypertensive rats. Hypertens Res 2016; 39: 769–776.
9 DeWire SM, Violin JD. Biased ligands for better cardiovascular drugs dissecting G-protein-coupled receptor pharmacology. Circ Res 2011; 109: 205–216.
10 Kleinz MJ, Skepper JN, Davenport AP. Immunocytochemical localisation of the apelin receptor, APJ, to human cardiomyocytes, vascular smooth muscle and endothelial cells. RegulPept 2005; 126: 233–240.
11 Kalea AZ, Batlle D. Apelin and ACE2 in cardiovascular disease. Curr Opin Investig Drugs 2010; 11: 273–282.
12 Headrick JP, Pepe S, Peart JN. Non-analgesic effects of opioids: cardiovascular effects of opioids and their receptor systems. Curr Pharm Des 2012; 18: 6090–6100.
13 Masri B, Lahlou H, Mazarguil H, Knibiehler B, Audigier Y. Apelin (65–77) activates extracellular signal-regulated kinases via a PTX-sensitive G protein. Biochem Biophys Res Commun 2002; 290: 539–545.
14 Li Y, Chen J, Bai B, Du H, Liu Y, Liu H. Heterodimerization of human apelin and kappa opioid receptors: roles in signal transduction. Cell Signal 2012; 24: 991–1001.
15 Xu N, Wang H, Fan L, Chen Q. Supraspinal administration of apelin-13 induces antinociception via the opioid receptor in mice. Peptides 2009; 30: 1153–1157.
16 Soltani Hekmat A, Najafipour H, Nekooian AA, Esmaeli-Mahani S, Javanmardi K. Cardiovascular responses to apelin in two-kidney–one-clip hypertensive rats andits receptor expression in ischemic and non-ischemic kidneys. Regul Pept 2011; 172: 62–68.
17 Najafipour H, Hekmat AS, Nekooian AA, Esmaeili-Mahani S. Apelin receptor expression in ischemic and non-ischemic kidneys and cardiovascular responseto apelin in chronic two-kidney–one-clip hypertension in rats. Regul Pept 2012; 178: 43–50.
18 Najafipour H, Vakili A, Shahouzehi B, Hekmat AS, Masoomi Y, Yeganeh Hajahmadi M, Esmaeli-Mahani S. Investigation of changes in apelin receptor mRNA and protein expression in the myocardium and aorta of rats with two-kidney, one-clip (2K1C) Goldblatt hypertension. J Physiol Biochem 2015; 71: 165–175.
19 Zamir N, Simantov R, Segal M. Pain sensitivity and opioid activity in genetically and experimentally hypertensive rats. Brain Res 1980; 184: 299–310.
20 Martinez-Maldonado M. Pathophysiology of renovascular hypertension. Hypertension 1991; 17: 707–719.
21 Gross GJ, Baker JE, Hsu A, Wu H-e, Falck JR, Nithipatikom K. Evidence for a role of opioids in epoxyeicosatrienoic acid-induced cardioprotection in rat hearts. Am J Physiol 2010; 298: H2201–H2207.
22 Fryer RM, Schultz JEJ, Hsu AK, Gross GJ. Importance of PKC and tyrosine kinase in single or multiple cycles of preconditioning in rat hearts. Am J Physiol 1999; 276: H1229–H1235.
23 Schultz JEJ, Hsu AK, Barbieri JT, Li P-L, Gross GJ. Pertussis toxin abolishes the cardioprotective effect of ischemic preconditioning in intact rat heart. Am J Physiol 1998; 275: H495–H500.
24 Szokodi I, Tavi P, Földes G, Voutilainen-Myllylä S, Ilves M, Tokola H, Pikkarainen S, Piuhola J, Rysä J, Tóth M. Apelin, the novel endogenous ligand of the orphan receptor APJ, regulates cardiac contractility. Circ Res 2002; 91: 434–440.
25 Tatemoto K, Takayama K, Zou M-X, Kumaki I, Zhang W, Kumano K, Fujimiya M. The novel peptide apelin lowers blood pressure via a nitric oxide-dependent mechanism. Regul Pept 2001; 99: 87–92.
26 Ishida J, Hashimoto T, Hashimoto Y, Nishiwaki S, Iguchi T, Harada S, Sugaya T, Matsuzaki H, Yamamoto R, Shiota N. Regulatory roles for APJ, a seven-transmembrane receptor related to angiotensin-type 1 receptor in blood pressure in vivo. J BiolChem 2004; 279: 26274–26279.
27 Zhong J-C, Yu X-Y, Huang Y, Yung L-M, Lau C-W, Lin S-G. Apelin modulates aortic vascular tone via endothelial nitric oxide synthase phosphorylation pathway in diabetic mice. Cardiovasc Res 2007; 74: 388–395.
28 Pappano AJ, Wier G. Cardiovascular Physiology, 10th edn, Chapter 7. "The arterial system". Mosby: St Louis, CO, Philadelphia, PA, USA, 2013, pp 135–151.
29 Lee DK, Saldivia VR, Nguyen T, Cheng R, George SR, O'Dowd BF. Modification of the terminal residue of apelin-13 antagonizes its hypotensive action. Endocrinology 2005; 146: 231–236.
30 Sun F-y, Zhang A-z. Dynorphin receptor in the blood vessel. Neuropeptides 1985; 5: 595–598.
31 Joost P, Methner A. Phylogenetic analysis of 277 human G-protein-coupled receptors as a tool for the prediction of orphan receptor ligands. Genome Biol 2002; 3: 1–16.
32 Zhang R, Xie X. Tools for GPCR drug discovery. Acta Pharmacol Sin 2012; 33: 372–384.
33 Duparc T, Colom A, Cani PD, Massaly N, Rastrelli S, Drougard A, Le Gonidec S, Moulédous L, Frances B, Leclercq I. Central apelin controls glucose homeostasis via a nitric oxide-dependent pathway in mice. Antioxid Redox Signaling 2011; 15: 1477–1496.
34 Shimokawa H, Morikawa K. Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in animals and humans. J Mol Cell Cardiol 2005; 39: 725–732.
35 Pak MD, Fishman PH. Anomalous behavior of CGP 12177A ON β2-adrenergic receptors. J Recept Signal Transd 1996; 16: 1–23.
36 Baker JG, Hill SJ. Multiple GPCR conformations and signalling pathways: implications for antagonist affinity estimates. Trends Pharmacol Sci 2007; 28: 374–381.
37 Konkar AA, Zhu Z, Granneman JG. Aryloxypropanolamine and catecholamine ligand interactions with the β1-adrenergic receptor: evidence for interaction with distinct conformations of β1-adrenergic receptors. J Pharmacol ExpTher 2000; 294: 923–932.
38 Jordan BA, Devi LA. G-protein-coupled receptor heterodimerization modulates receptor function. Nature 1999; 399: 697–700.
39 Jordan B, Trapaidze N, Gomes I, Nivarthi R, Devi L. Oligomerization of opioid receptors with β2-adrenergic receptors: a role in trafficking and mitogen-activated protein kinase activation. Proc Natl Acad Sci USA 2001; 98: 343–348.