

Angiotensin-(1-7) potentiates the coronary vasodilatory effect of bradykinin in the isolated rat heart

A.P. Almeida,
B.C. Frábregas,
M.M. Madureira,
R.J.S. Santos,
M.J. Campagnole-Santos
and R.A.S. Santos

Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas,
Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil

Abstract

It has been shown that angiotensin-(1-7) (Ang-(1-7)) infusion potentiates the bradykinin (BK)-induced hypotensive response in conscious rats. The present study was conducted to identify Ang-(1-7)-BK interactions in the isolated rat heart perfused according to the Langendorff technique. Hearts were excised and perfused through the aortic stump under a constant flow with Krebs-Ringer solution and the changes in perfusion pressure and heart contractile force were recorded. Bolus injections of BK (2.5, 5, 10 and 20 ng) produced a dose-dependent hypotensive effect. Ang-(1-7) added to the perfusion solution (2 ng/ml) did not change the perfusion pressure or the contractile force but doubled the hypotensive effect of the lower doses of BK. The BK-potentiating Ang-(1-7) activity was blocked by pretreatment with indomethacin (5 mg/kg, *ip*) or L-NAME (30 mg/kg, *ip*). The Ang-(1-7) antagonist A-779 (50 ng/ml in Krebs-Ringer) completely blocked the effect of Ang-(1-7) on BK-induced vasodilation. These data suggest that the potentiation of the BK-induced vasodilation by Ang-(1-7) can be attributed to the release of nitric oxide and vasodilator prostaglandins through an Ang-(1-7) receptor-mediated mechanism.

Key words

- Angiotensin-(1-7)
- Bradykinin
- Coronary artery
- Rat heart
- L-NAME
- Prostaglandins
- Bradykinin potentiation

Correspondence

R.A.S. Santos
Departamento de Fisiologia e
Biofísica, ICB, UFMG
Av. Antonio Carlos, 6627
31270-901 Belo Horizonte, MG
Brasil
Fax: +55-31-499-2924
E-mail: santos@icb.ufmg.br

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Introduction

The renin-angiotensin system plays an important role in the pathogenesis of arterial hypertension, cardiac hypertrophy and chronic heart failure. Since all of its components can be generated or activated at the tissue level, the structural and functional consequences of its activation in organs appear to be due to endocrine as well as

paracrine or autocrine mechanisms (1-3).

Inhibitors of angiotensin-converting enzyme (ACE) have been widely used for the treatment of hypertension and other cardiovascular diseases (4-6). The mechanism of action of ACE inhibitors has been thought to be due to interference with circulating or tissue angiotensin II (Ang II) formation and bradykinin (BK) degradation (4-7). However, ACE inhibition also markedly increases

angiotensin-(1-7) (Ang-(1-7)) concentration in plasma and tissues (3,8,9).

Several reports have described a BK-potentiating activity for Ang-(1-7) (10-15) that could contribute to the cardiovascular effects of ACE inhibition and AT₁ receptor blockers (16). The Ang-(1-7)-BK interaction has been studied *in vivo* (10,11), in cell culture (12) or in isolated vessels (14). However, there are no data available about the interaction between Ang-(1-7) and BK in the heart. Thus, the present study was conducted to determine the Ang-(1-7) and BK interaction in isolated rat heart perfused by the Langendorff technique.

Material and Methods

General procedures

Experiments were performed on male Wistar rats (250-280 g) bred at the Centro de Bioterismo (CEBIO), ICB-UFMG. Ten to fifteen min after an intraperitoneal injection of 200 IU heparin the rats were decapitated. The thorax was opened and the heart was carefully dissected and perfused with Krebs-Ringer solution through a 1.0 ± 0.3 cm aortic stump. The perfusion fluid was maintained at $37 \pm 0.5^\circ\text{C}$ with constant flow (Watson-Marlow 501 U pump) and oxygenation (5% CO₂ and 95% O₂). A force transducer (3 g, model FT03; Grass, West Warwick, RI, USA) was attached through a heart clip to the apex of the ventricles to record the contractile force (tension, g) and perfusion pressure (mmHg) was monitored with a solid-state strain-gauge transducer (Gold, P23XL). All variables were recorded continuously on a computer through a data-acquisition system (Coda, Dataq Instruments, Inc., Akron, OH, USA). Bradykinin was purchased from Sigma (St. Louis, MO, USA) and Ang-(1-7) and A-779 were purchased from Bachem (Torrance, CA, USA).

Effect of Ang-(1-7) on the cardiac actions of bradykinin. The hearts were perfused for an

initial 20-30-min period with 1) Krebs-Ringer solution (control, N = 4) or 2) Krebs-Ringer solution containing Ang-(1-7) (2.2 nM, N = 4). After the equilibration period, bolus injections of BK (2.5, 5.0, 10 and 20 ng) were made at intervals of at least 2-4 min.

Role of cyclo-oxygenase products in the BK-Ang-(1-7) interaction. In order to evaluate the role of cyclo-oxygenase products in the BK-Ang-(1-7) interaction, rats received indomethacin (5 mg/kg, *ip*) plus heparin (200 IU, *ip*). After 1 h the rats were decapitated, the thorax was opened and the heart was dissected and perfused with 1) Krebs-Ringer followed by 2) Krebs-Ringer solution containing Ang-(1-7) (2.2 nM, N = 4). The hypotensive effect of bradykinin (2.5 ng) was determined under both perfusion conditions.

Role of NO in the BK-Ang-(1-7) interaction. In order to evaluate the role of nitric oxide (NO) in the BK-Ang-(1-7) interaction, rats received L-NAME (30 mg/kg, *ip*) plus heparin (200 IU, *ip*). After 1 h the rats were decapitated, the thorax was opened and the heart was dissected and perfused with 1) Krebs-Ringer solution followed by 2) Krebs-Ringer solution containing Ang-(1-7) (2.2 nM, N = 4). The hypotensive effect of bradykinin (2.5 ng) was determined under both conditions of perfusion.

Effect of blockade of cyclo-oxygenase and NO-synthase on the BK-Ang-(1-7) interaction. Rats received indomethacin (5 mg/kg, *ip*) plus L-NAME (30 mg/kg, *ip*) plus heparin (200 IU, *ip*) and were decapitated 1 h later, the thorax was opened and the heart was dissected and perfused with 1) Krebs-Ringer solution followed by 2) Krebs-Ringer solution containing Ang-(1-7) (2.2 nM, N = 4). The hypotensive effect of bradykinin (2.5 ng) was determined under both perfusion conditions.

Effect of A-779 on the BK-Ang-(1-7) interaction. The hearts were perfused for an initial 20-30-min period with 1) Krebs-Ringer solution containing the selective Ang-(1-7)

antagonist (17), A-779, (50 ng/ml, N = 4) followed by 2) Krebs-Ringer solution containing A-779 (50 ng/ml) plus Ang-(1-7) (2.2 nM, N = 4). The hypotensive effect of bradykinin (2.5 ng) was determined under both perfusion conditions.

Statistical analysis

Data are reported as means \pm SEM. Statistical analysis was performed by the Student *t*-test or ANOVA followed by the least significant difference test or the Newman-Keuls test, when appropriate. The level of significance was set at $P < 0.05$.

Results

Ang-(1-7) at 2.2 nM concentration did not change inotropism or pressure perfusion. However, the hypotensive effect produced by bradykinin injected in bolus was potentiated by Ang-(1-7). The magnitude of the potentiation was dependent on the dose of bradykinin, being present only for the lower doses: Ang-(1-7) doubled the effect of the lower doses of BK while no significant change in the BK effect was observed when doses of BK higher than 5 ng were used (Figure 1).

To determine whether the effect of Ang-(1-7) was due to the release of cyclo-oxygenase products and/or NO release, the effect of the heptapeptide was evaluated in hearts removed from rats pre-treated with indomethacin and/or L-NAME. As shown in Figure 2, the potentiation of the hypotensive effect of BK by Ang-(1-7) was significantly blocked in indomethacin- or L-NAME-treated rats. Treatment with both drugs produced a similar effect.

The possibility that the BK-potentiating activity of Ang-(1-7) is a receptor-mediated event was evaluated using the selective Ang-(1-7) antagonist, A-779. Figure 3 shows that A-779 at 55 nM completely blocked the effect of Ang-(1-7) on the BK-induced vasodilation.

Discussion

In the present study we have found that: first, Ang-(1-7) potentiates the coronary vasodilatory effect of BK in the isolated rat hearts; second, the Ang-(1-7)/BK-potentiating activity was blocked by pre-treatment with indomethacin, L-NAME or both drugs. Finally, the Ang-(1-7) antagonist A-779 blocked the effect of Ang-(1-7) on BK-in-

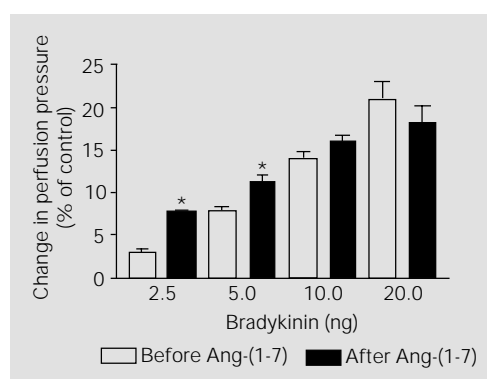


Figure 1 - Effect of bradykinin on isolated rat hearts perfused with Krebs-Ringer solution and Krebs-Ringer containing Ang-(1-7) (2.2 nM). Values are reported as percent change in basal perfusion pressure. * $P < 0.05$ compared to bradykinin alone (ANOVA followed by the least significant test).

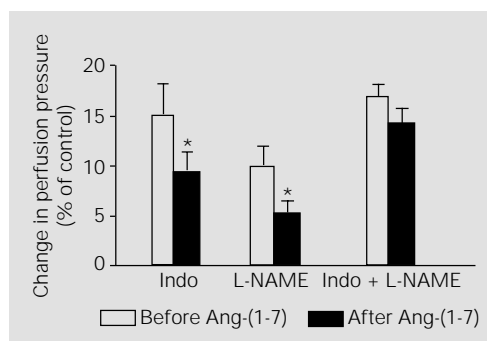


Figure 2 - Effect of indomethacin (Indo) and L-NAME on the bradykinin-potentiating activity of Ang-(1-7) in isolated rat hearts. Indomethacin (5 mg/kg, ip), L-NAME (30 mg/kg, ip) or indomethacin combined with L-NAME at the same doses as indicated above were administered 1 h before animal sacrifice. The effect of bradykinin (2.5 ng) was determined before and after perfusion with Ang-(1-7) (2.2 nM). * $P < 0.05$ compared to values obtained before perfusion with Ang-(1-7) (Student *t*-test).

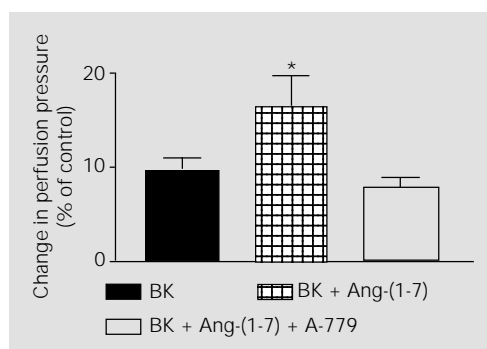


Figure 3 - Effect of A-779 on the bradykinin (BK)-potentiating activity of Ang-(1-7) in isolated rat hearts. The effect of BK (2.5 ng) was determined before and after perfusion with Ang-(1-7) (2.2 nM) or Ang-(1-7) combined with A-779 (55 nM). * $P < 0.05$ compared to BK alone (ANOVA followed by the Newman-Keuls test).

duced vasodilation.

In a previous study we have shown that Ang-(1-7) at a concentration of 27 nM decreases coronary flow (18). In contrast, at a concentration of 2.2 nM, as used in the present study, this heptapeptide had no detectable direct myotropic effect on coronary vessels. Although no direct effect of Ang-(1-7) on the isolated heart could be detected at this lower concentration, a clear potentiation of BK was observed. Thus, it appears that at concentrations close to the physiological range Ang-(1-7) would act as a kinin modulator on the coronary vessels. This observation is in accordance with a recent study showing a significant BK-potentiating activity of Ang-(1-7) in mesenteric vessels (13).

The mechanism of the interaction between Ang-(1-7) and BK is very complex (12,15). Deddish and colleagues (12) have provided evidence for an interaction of Ang-(1-7) with ACE that, independently of the enzymatic inhibition, would facilitate a crosstalk between ACE and the BK B₂ receptor leading to BK potentiation. On the other hand, in dog coronary artery the BK-potentiating activity of Ang-(1-7) was ascribed to ACE inhibition and NO release (14). In rats, we (11) and others (13) have blocked the BK-potentiating activity of Ang-(1-7) with its selective antagonist, A-779, indicating that in this species a receptor-mediated mechanism is involved in BK potentiation. This appears to be true at least for the mesenteric and coronary circulation. One may argue that A-779 could interfere with the binding of Ang-(1-7) to ACE. However, we have recently shown that A-779 does not inhibit ACE and does not prevent Ang-(1-7) from inhibiting this enzyme (15).

As observed in the present study, pretreatment with indomethacin completely blocked BK potentiation *in vivo* (10) or in mesenteric vessels (13). These observations contrast with findings in dog coronary vessels (14). However, as observed in dogs (14) and in the rat mesenteric vascular bed (13),

the BK-potentiating activity of Ang-(1-7) may be prevented by L-NAME treatment, suggesting a role for NO release in this action. The related observation that Ang-(1-7) did not change the vasodilator action of the NO donor sodium nitroprusside *in vivo* or *in vitro* (10,13) indicates that the NO-related mechanism is dependent on the formation rather than on the action and/or inactivation of NO. Furthermore, these observations tend to exclude a role for interference with superoxide production in the BK-potentiating mechanism.

We have observed that the BK-potentiating activity of Ang-(1-7) could be demonstrated only when low doses of BK were used. This finding further suggests that BK potentiation by this heptapeptide is not related to interference with ACE activity or with other BK-inactivating enzymes. On the other hand, this observation suggests that Ang-(1-7) triggers a membrane and/or intracellular mechanism(s) that usually is fully activated only with higher doses of BK. In other words, in the presence of Ang-(1-7), lower doses of BK became able to fully activate this mechanism. A similar bell-shaped BK-potentiating activity for Ang-(1-7) was recently described by Oliveira et al. (13) in rat mesenteric vessels. These findings are consistent with the related observation in cultured rabbit vascular smooth muscle cells that the release of arachidonic acid products by Ang-(1-7) follows a bell-shaped curve (19). Further experiments are needed to clarify these observations.

We have previously shown that Ang-(1-7) at 27 nM concentration increased the incidence and duration of reperfusion arrhythmias in isolated rat hearts (18). Our current data showing a significant BK-potentiating activity of Ang-(1-7) at a lower concentration and the proposed anti-arrhythmic action of BK in this preparation (4) indicate the importance of evaluating the effect of low concentrations of Ang-(1-7) on reperfusion arrhythmias.

In summary, we have shown that in isolated rat hearts Ang-(1-7) induced an increase in the vasodilator effect of BK by NO and prostaglandin release-related mechanisms and that this action may be receptor mediated.

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