Cardiovascular Effects of Angiotensin-(1–7) in Conscious Spontaneously Hypertensive Rats

Robert E. Widdop, Donella B. Sampey, Bevyn Jarrott

Abstract—In the present study, we reassessed whether angiotensin (Ang)-(1–7) can exert short- and long-term cardiovascular effects because there has been a resurgence of interest in this N-terminal heptapeptide fragment of Ang II. In particular, we studied 3 aspects relating to the reported cardiovascular effects of Ang-(1–7): does this peptide (1) potentiate the hypertensive effect of bradykinin in normotensive Wistar-Kyoto rats and spontaneously hypertensive rats (SHR), (2) cause a depressor effect after long-term treatment in SHR, and (3) contribute to the antihypertensive effects of angiotensin-converting enzyme inhibitors? In the first series of experiments, Ang-(1–7) failed to enhance the dose-related hypertensive responses evoked by bradykinin in SHR (n=11) and Wistar-Kyoto (n=5) rats. In the second series of experiments, a 7-day intravenous infusion of Ang-(1–7) (24 μg · kg⁻¹ · h⁻¹) decreased blood pressure in SHR (n=12) on days 4 and 5, although this effect waned despite continual Ang-(1–7) infusion. However, a new finding was that the Ang-(1–7) antagonist A-779 (24 μg · kg⁻¹ · h⁻¹ for 7 days) attenuated the depressor effect of Ang-(1–7) when given concurrently in a separate group of SHR (n=8). In the third series of novel experiments, the angiotensin-converting enzyme inhibitor perindopril was given in drinking water for 7 days (0.3 mg · kg⁻¹ · day⁻¹), either alone (n=6) or combined with an intravenous infusion of A-779 (24 μg · kg⁻¹ · h⁻¹ for 7 days, n=8). Although this dose of A-779 attenuated the depressor effect of Ang-(1–7), it did not alter the antihypertensive effect caused by perindopril. Thus, the present results contrast with a number of previous studies and argue against Ang-(1–7) playing a major role in blood pressure regulation. (Hypertension. 1999;34[part 2]:964-968.)

Key Words: angiotensin ■ bradykinin ■ angiotensin-converting enzyme inhibitors ■ rats, inbred SHR ■ blood pressure

Angiotensin (Ang)-(1–7), a heptapeptide lacking the phenylalanine at position 8 of the Ang II peptide, is now thought to be an active component of the renin-angiotensin system, and it may be generated from Ang I via angiotensin-converting enzyme (ACE)–independent, tissue-specific endopeptidases.1,2 Ang-(1–7) was initially considered an inactive fragment, primarily because of its lack of “Ang II-like” dipogenic, vasoconstrictor, and aldosterone secreting activity, although evidence now suggests that Ang-(1–7) has distinct and opposite effects from those of Ang II, such as vasorelaxation.3 However, the majority of such studies have been short-term experiments performed in vitro,4–6 and such studies have not always been reproducible.7 Long-term infusion of Ang-(1–7) was reported in 1 study8 in which a decrease in blood pressure occurred in spontaneously hypertensive rats (SHR); however, this effect was only seen on day 2 of a 7-day treatment protocol. Furthermore, Ang-(1–7) was reported to potentiate bradykinin-induced vasorelaxation in isolated canine coronary arteries,5,6 as well as in conscious normotensive rats8 and SHR.10 Moreover, the bradykinin-potentiating effects of Ang-(1–7) were attenuated by the Ang-(1–7) antagonist D-Ala7 –Ang-(1–7) (A-779).10

The well-documented antihypertensive and cardioprotective effects of ACE inhibitors are thought to occur through the inhibition of circulating or tissue Ang II formation and the prevention of bradykinin degradation.11 Interestingly, ACE inhibitors elicit 5- to 50-fold increases in plasma and tissue levels of Ang-(1–7) in both SHR and humans.12,13 Consequently, it has been postulated that Ang-(1–7) may contribute to the blood-pressure–lowering effect of ACE inhibition.3 Therefore, it is important for other researchers to confirm some of these interesting effects of Ang-(1–7). An interaction between Ang-(1–7) and bradykinin may contribute to the antihypertensive effect of ACE inhibitors, and a direct vasodilator effect of Ang-(1–7) may exist in vivo. Therefore, in this study, we attempted to confirm whether Ang-(1–7) (1) potentiates the hypertensive effect of bradykinin in both normotensive and hypertensive rats and (2) causes direct depressor activity after long-term treatment in SHR. In so doing, we also used a dose of A-779 that attenuated the depressor effect of Ang-(1–7) (see Results). Consequently, as our third aim, we determined if Ang-(1–7) contributes to the blood-pressure–lowering effects of ACE inhibitors by determining whether long-term treatment with A-779 interferes with the antihypertensive effects of ACE inhibitors.
with the antihypertensive effect of the ACE inhibitor perindopril.

**Methods**

Experiments were conducted in 16- to 18-week-old male Wistar Kyoto (WKY) rats and SHR obtained from the Austin Hospital Biological Research Laboratories (Melbourne, Australia). All experimental procedures were approved by the Monash University Animal Ethics Committee and performed according to the guidelines of the National Health and Medical Research Council of Australia for animal experimentation. SHR and WKY rats were anesthetized with methohexitone (60 mg/kg IP, supplemented as required), and 2 catheters were inserted into the right jugular vein for intravenous administration of drugs. A catheter was also implanted into the right carotid artery for recording arterial blood pressure with a pressure transducer via a Maclab data acquisition system (ADInstruments). After a 24- to 48-hour recovery period, experiments were commenced in conscious, unrestrained rats.

**Short-Term Studies**

In WKY rats (n=5) and SHR (n=11), a control dose-response curve to bolus injections of bradykinin (0.125, 0.25, 0.5, 1, and 2 µg IV) was constructed. Thereafter, a 2-hour intravenous infusion of either saline (0.35 mL/hr) or Ang-(1–7) (5 pmol/min) was begun, and the bradykinin dose-response curves were repeated at 30 and 90 minutes during infusion. Analogous experiments were performed in SHR during the infusion of saline or 5, 50, or 500 pmol/min Ang-(1–7) on 4 separate days. At least 5 minutes was allowed between each dose of bradykinin because that was the time required for mean arterial pressure (MAP) and heart rate (HR) to return to baseline levels.

**Long-Term Ang-(1–7) Infusions**

In 4 separate groups of SHR, MAP and HR were recorded at least 2 days before starting a 7-day intravenous infusion of (1) saline (5 μL/hr; n=11), (2) Ang-(1–7) (24 µg · kg⁻¹ · h⁻¹; n=12), (3) the putative Ang-(1–7) antagonist A-779 (24 µg · kg⁻¹ · h⁻¹; n=4), or (4) Ang-(1–7) and A-779 (both at 24 µg · kg⁻¹ · h⁻¹; n=8). The dose of Ang-(1–7) was chosen on the basis of a previous study.8 Dual infusions were carried out using a double-channel swivel system (Intech Laboratories). All rats received fresh drug daily, and MAP and HR were recorded for at least 1 hour per day throughout the 7-day treatment periods. Ang-(1–7) and A-779 were purchased from Bachem.

**Perindopril Treatment**

To determine the potential contribution of Ang-(1–7) to the antihypertensive effect of an ACE inhibitor in SHR, both MAP and HR were recorded at least 2 days before starting treatment with perindopril (0.3 mg · kg⁻¹ · day⁻¹ for 7 days; given in drinking water), either alone (n=6) or at the same time as receiving an intravenous infusion of A-779 (24 µg · kg⁻¹ · h⁻¹; n=8). MAP and HR were recorded for at least 1 hour each day throughout the 7-day treatment period, and rats received fresh drug daily. Perindopril was a gift from Servier (Melbourne, Australia).
Statistical Analysis

A 1-way ANOVA with repeated measures was used for comparisons within treatment groups for MAP and HR responses evoked by bradykinin in the short-term studies and the changes in basal MAP and HR values over time in the long-term studies. Comparisons of basal MAP and HR values between treatment groups in the long-term studies were analyzed using a 2-way ANOVA with repeated measures. Values are expressed as mean±SEM. Statistical significance was \( P < 0.05 \).

Results

Short-Term Studies

Bradykinin (0.125 to 2 \( \mu \)g IV) caused dose-dependent hypotension (range, \(-5 \) to \(-30 \) mm Hg) and tachycardia (range, 30 to 60 beats/min; data not shown) in both SHR and WKY rats. These MAP and HR effects were unchanged when bradykinin was retested 30 and 90 minutes after the commencement of saline (data not shown) or Ang-(1–7) (5 pmol/min) infusions in WKY rats (n=5) (Figure 1). Similarly, the bradykinin-induced hypotension observed in SHR was unaltered at 30 and 90 minutes during infusion of saline or Ang-(1–7) (at 5, 50, and 500 pmol/min). The effects of the 2 lower infusion rates of Ang-(1–7) are shown in Figures 2 and 3.

Long-Term Studies

In SHR, saline did not alter MAP or HR over the 7-day infusion period (Figure 4). Similarly, the selective Ang-(1–7) receptor antagonist A-779 did not alter MAP significantly over the infusion period; it did tend to decrease HR, although this effect was only significant compared with the saline-treated group on day 6 (\( P < 0.05 \)) (Figure 4). When Ang-(1–7) was infused long-term in SHR, a progressive decrease in MAP occurred; this was significant compared with baseline values on days 4 and 5 (\(-21±8 \) and \(-21±7 \) mm Hg, respectively; \( P < 0.05 \); Figure 5). This depressor response also differed from the change in MAP in the saline-treated group at this time (days 4 and 5, \(-6±6 \) and \(-1±5 \) mm Hg, respectively). In contrast, when Ang-(1–7) was coinfused with A-779, resting MAP was not significantly different from its own baseline or to that observed during saline infusion, although this Ang-(1–7)/A-779 combination was on the borderline of significance (\( P = 0.07 \)) when compared with Ang-(1–7) treatment alone (Figure 5). Ang-(1–7) alone caused minimal changes in HR, except for a small bradycardia, when compared with saline-treated SHR on day 5 (\( P < 0.05 \); Figure 5). Gradual tachycardia developed with the Ang-(1–7)/A-779 infusion compared with either the saline-treated (\( P < 0.05 \), day 7) or Ang-(1–7) alone groups (\( P < 0.05 \), days 5 to 7) (Figure 5).

In separate SHR, treatment with perindopril alone caused an immediate decrease in MAP of \(-35 \) mm Hg that was sustained throughout the 7-day treatment period; HR tended to decrease over this time. However, neither the MAP nor HR effects observed during perindopril treatment were significantly altered by cotreatment with A-779 (Figure 6).

Discussion

The main findings of the present study were that short-term infusion of Ang-(1–7) failed to potentiate bradykinin-induced hypotension in either SHR or WKY rats, although it did cause a gradual decrease in blood pressure when infused long-term in SHR. This latter effect of Ang-(1–7) was attenuated by the
putative Ang-(1–7)-selective antagonist A-779, although this compound did not unmask any contribution of Ang-(1–7) to the antihypertensive effect of long-term ACE inhibition in SHR.

Short-term infusion of Ang-(1–7) did not alter baseline MAP or HR at any of the doses used in either SHR or WKY rats. This is consistent with previous studies showing that Ang-(1–7) itself is a weak vasoactive peptide.\(^3\) Short-term infusion of Ang-(1–7) also failed to potentiate bradykinin-induced hypotension in WKY rats, although this dose was previously reported to augment bradykinin responses in conscious rats.\(^9\) Similarly, no significant difference existed in the dose-related hypotension produced by bradykinin after the infusion of Ang-(1–7) at the same or 10- to 100-fold higher doses in SHR. No obvious reasons exist regarding our negative findings, which contrast with those of 1 other group.\(^3,9,10\) Strain differences in normotensive rats may be involved, although this effect does not explain the opposite findings using SHR. Ang-(1–7) was obtained from a commercial supplier (Bachem), whereas this peptide was synthesized in-house in previous studies.\(^9,10\) However, we also used Ang-(1–7) from another source (American Peptide Co) and found identical results.

When infused over a 7-day period, Ang-(1–7) significantly reduced blood pressure in SHR on days 4 and 5 of the infusion, although this effect was not sustained. This blood pressure–lowering effect of Ang-(1–7) is consistent with the results of Benter et al.,\(^8\) who reported a more rapid decline in blood pressure (by day 2) and a gradual return to pretreatment levels by day 7. In this earlier study, osmotic minipumps were used to deliver the peptide. In the present study, we deliberately chose to infuse a fresh solution daily to avoid any confounding problems such as pump failure and/or degradation of peptide. However, given that both studies noted a depressor response that was not maintained, it is likely that other compensatory vasoconstrictor mechanisms were activated to offset the antihypertensive action of Ang-(1–7).

Ang-(1–7) is thought to act via an Ang (AT) receptor that is distinct from either AT\(_1\) or AT\(_2\) receptors.\(^3,5\) Moreover, the mechanisms by which Ang-(1–7) lowers MAP are likely to involve prostaglandins and/or nitric oxide.\(^4,5\) Importantly, we have shown for the first time that Ang-(1–7)-induced hypotension was attenuated with respect to predrug baseline values when coinfused with the selective Ang-(1–7) receptor antagonist A-779. The attenuation of the hypotensive effect of Ang-(1–7) was slightly less evident when compared with Ang-(1–7) alone, although the same analysis did reveal a significant difference in the HR effects. Moreover, the fact that the combination was similar to saline treatment would also argue for the lack of effect of Ang-(1–7) when combined with the antagonist. Indeed, several in vitro and in vivo studies have demonstrated an inhibitory effect of this compound when tested against Ang-(1–7),\(^10,14,15\) and thus our results are consistent with those studies.

The fact that reports have shown that plasma and tissue levels of Ang-(1–7) are elevated after ACE inhibition in both SHR and humans\(^2,13\) and the finding that similarities exist between the antihypertensive response produced by long-term Ang-(1–7) treatment and that of ACE inhibitors\(^8\) have lead some to think that this heptapeptide may contribute to the blood pressure–lowering effect of ACE inhibitors.\(^3\) Consequently, we determined whether Ang-(1–7) actually contributes to ACE inhibition by infusing the Ang-(1–7) selective antagonist A-779 during treatment with the ACE inhibitor perindopril. Importantly, at the dose used, A-779 attenuated the depressor effect of Ang-(1–7), although it did not significantly attenuate the antihypertensive effect of perindopril. Thus, these novel results indicate a lack of involvement of Ang-(1–7) in the effect of perindopril. It is possible that a higher dose of A-779, which could abolish the effects of Ang-(1–7), may attenuate the effects of ACE inhibition; however, due to cost considerations, this experiment could not be undertaken. Nevertheless, several points would argue against this possibility. First, the dose of A-779 used in the present study was effective against Ang-(1–7), and second, the depressor effect of Ang-(1–7) per se waned, despite continual infusion of the peptide (current study and reference 8).

However, our results are in direct contrast to recent studies which showed that both monoclonal antibodies against Ang-(1–7) and neutral endopeptidase inhibitors, given short-term, could partially reverse the antihypertensive effect of 8-day treatment with combined ACE inhibition and AT\(_1\) receptor blockade.\(^16,17\) Interestingly, the Ang-(1–7) antibody evoked a small pressor response in the treated SHR; however, this effect had waned by at least one-third during the 15-minute infusion of neutralizing antibody.\(^16\) In contrast, the pharmacodynamics of the pressor responses to the NEP inhibitors were quite different: maximum (albeit partial) reversal of blood pressure was achieved at the end of the infusion period (10 to 15 minutes), which suggests that differences exist between the mechanisms of action of these inhibitors of Ang-(1–7).\(^17\) Moreover, the short-term effects of blockade of Ang-(1–7) do not necessarily predict the long-term effects of such inhibition. Indeed, our results using long-term administration of A-779 to interfere with Ang-(1–7) imply a negligible role for this peptide in the antihypertensive effect of ACE inhibitors when tested under long-term conditions.

In summary, we have shown that Ang-(1–7) does not possess bradykinin-potentiating activity in either SHR or WKY rats short-term, but it does cause a slowly developing depressor response, which was not sustained, when infused long-term in SHR. However, at a dose that inhibited Ang-(1–7), A-779 failed to alter the cardiovascular effects of perindopril. Therefore, although a number of previous studies have implicated Ang-(1–7) as being involved in cardiovascular regulation, our results do not support the premise that Ang-(1–7) plays a key role in blood pressure regulation or as a component of the antihypertensive effect of ACE inhibitors.

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**References**


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