Angiotensin II attenuates myocardial interstitial acetylcholine release in response to vagal stimulation

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Angiotensin II inhibits the stimulation-induced ACh release (8.0 ng/g kg) by the administration of losartan (10 mg/kg, 5.8 ng/g kg) through AT1 receptor losartan (10 mg/kg, 6), ANG II was unable to inhibit the stimulation-induced ACh release (8.6 ± 1.5 vs. 8.4 ± 1.7 nM). In contrast, in a group with losartan administration of losartan (10 mg/kg, n = 6), ANG II inhibition was significantly increased in the stimulation-induced ACh release (8.0 ± 0.8 vs. 5.8 ± 1.0 nM, P < 0.05). In conclusion, losartan significantly inhibited the parasympathetic neurotransmission through AT1 receptors. The failure of local losartan administration to nullify the inhibitory effect of ANG II on the stimulation-induced ACh release indicates that the site of this inhibitory action is likely at parasympathetic ganglia rather than at postganglionic vagal nerve terminals.

captopril also enhanced the bradycardic response to vagal stimulation (5). In pithed rats, an angiotensin-converting enzyme (ACE) inhibitor captopril also enhanced the bradycardic response to vagal stimulation (25, 26). In all of these studies, changes in the heart rate were used as a functional measurement of peripheral vagal function because of the difficulty in measuring the ACh release in the in vivo heart. Accordingly, whether ANG II affects the vagal control over the ventricle remains unknown. The aim of the present study was to examine the effect of ANG II on the vagal stimulation-induced ACh release in dogs. In that study, administration of ACh reduced the heart rate to an identical degree in the presence or absence of ANG II, suggesting that the inhibition of bradycardia by ANG II was attributable to the inhibition of the ACh release from the vagal nerve terminals. In contrast, Andrews et al. (3) reported that ANG II (500 ng/kg iv) did not inhibit bradycardia induced by vagal stimulation in ferrets. In a rat heart failure model, ANG II receptor subtype 1 (AT1 receptor) antagonist losartan enhanced the bradycardic response to vagal stimulation (5). In pithed rats, an angiotensin-converting enzyme (ACE) inhibitor captopril also enhanced the bradycardic response to vagal stimulation (25, 26). In all of these studies, changes in the heart rate were used as a functional measurement of peripheral vagal function because of the difficulty in measuring the ACh release in the in vivo heart. Accordingly, whether ANG II affects the vagal control over the ventricle remains unknown. The aim of the present study was to examine the effect of ANG II on the vagal stimulation-induced ACh release in the left ventricular myocardium by measuring the interstitial ACh levels directly using a cardiac microdialysis technique (1, 13–15). We also explored the possible sites of action for the effect of ANG II on the stimulation-induced ACh release by administering losartan systemically from the femoral vein or locally through the dialysis fiber. Because ACh has a protective effect on the ischemic myocardium (12, 24, 29), elucidating the effect of ANG II on the ACh release in the ventricle would be helpful to understand the mechanism of ACE inhibitor or AT1 receptor antagonist for the treatment of heart diseases (16, 17).

MATERIALS AND METHODS

Surgical Preparation

Animal care was provided in strict accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences approved by the Physiological Society of Japan. All protocols were approved by the Animal Subject Committee of the National Cardiovascular Center. Twenty eight adult cats weighing from 1.9 to 4.9 kg were anesthetized using an intraperitoneal injection of pentobarbital sodium (30–35 mg/kg) and were then ventilated mechanically with room air mixed with oxygen. The depth of anesthesia was maintained by a continuous intravenous infusion of pentobarbital sodium (1–2 mg·kg⁻¹·h⁻¹) through a catheter inserted in the right femoral vein. Systemic AP was monitored by a catheter inserted in the right femoral artery. Heart rate was determined from an
electrocardiogram using a cardiotachometer. Esophageal temperature of the animal, measured using a thermometer (CTM-303; TERUMO), was maintained at ~37°C using a heating pad and a lamp. Both vagal nerves were exposed and sectioned bilaterally through a midline cervical incision. With the animal in the lateral position, we resected the left fifth and sixth ribs to approach the heart. After the incision of the pericardium, the heart was suspended in a pericardial cradle. Stainless steel wires were attached to the apex and the anterior wall of the left ventricle to pace the heart. Using a fine guiding needle, we implanted a dialysis probe transversely through the anterolateral free wall of the left ventricle. Next, we attached a pair of bipolar platinum electrodes to the cardiac end of each sectioned vagal nerve. The nerves and electrodes were covered in warmed mineral oil for insulation. We gave heparin sodium (100 U/kg) intravenously to prevent blood coagulation. At the end of the experiment, postmortem examination confirmed that the semipermeable membrane of the dialysis probe had been implanted in the left ventricular myocardium.

Dialysis Technique

The materials and properties of the dialysis probe have been described previously (1). Briefly, we designed a transverse dialysis probe in which a dialysis fiber of semipermeable membrane (13 mm length, 310 μm outer diameter, 200 μm inner diameter; PAN-1200, 50,000 mol wt cutoff; Asahi Chemical) was attached at both ends to polyethylene tubes (25 cm length, 500 μm outer diameter, 200 μm inner diameter). The dialysis probe was perfused at a rate of 2 μl/min with Ringer solution containing the cholinesterase inhibitor physostigmine (100 μM). Experimental protocols were started 2 h after implanting the dialysis probe when the ACh concentration in the dialysate reached a steady state. ACh concentrations in the dialysate were measured by an HPLC system with electrochemical detection (Eicom, Kyoto, Japan).

Figure 1 schematizes the three original protocols and two supplemental protocols utilized in the present study. The hatched rectangle indicates the baseline sampling, whereas the solid rectangles indicate the sampling during the 10-min vagal stimulation period (1 ms, 10 V, 20 Hz) in each protocol. The stimulus was set supramaximal to most easily delineate the possible effect of ANG II on myocardial interstitial ACh release. In all of the vagal stimulation periods, we paced the heart at 200 beats/min to avoid the difference in heart rate affecting the vagal stimulation-induced ACh release (14). For baseline sampling periods, we paced the heart at 200 beats/min when spontaneous heart rate was <200 beats/min.

Protocol 1 (n = 6). We examined the effects of intravenous administration of ANG II on vagal stimulation-induced myocardial ACh release. We collected a dialysate sample under baseline conditions. Then we stimulated the vagal nerve and paced the heart for 10 min and collected a dialysate sample during the stimulation period...
(VS1). After an intervening interval of 15 min, we repeated the 10-min vagal stimulation with fixed-rate pacing and collected another dialysate sample (VS2). After performing these two control trials, we began intravenous administration of ANG II at 10 μg·kg⁻¹·h⁻¹. Approximately 15 min after the onset of the ANG II administration, we collected a dialysate sample (A10-VS) during 10-min vagal stimulation with fixed-rate pacing. We then increased the dose of ANG II to 100 μg·kg⁻¹·h⁻¹. Approximately 15 min after the onset of the higher-dose ANG II administration, we collected a final dialysate sample (A100-VS) during 10-min vagal stimulation with fixed-rate pacing.

**Protocol 2 (n = 6).** We examined whether the intravenous AT1 receptor antagonist losartan would block the effects of ANG II on the vagal stimulation-induced myocardial ACh release. We infused losartan potassium intravenously at 10 mg/kg and waited for ~15 min. We then collected baseline, VS1, VS2, A10-VS, and A100-VS samples with an intervening interval of 15 min, as described in **protocol 1**. Next, after an additional bolus injection of losartan potassium at 10 mg/kg, we began intravenous infusion of ANG II at 10 μg·kg⁻¹·h⁻¹. After ~15 min, we obtained a dialysate sample of A10-VS. Finally, after another bolus injection of losartan potassium at 10 mg/kg, we began intravenous infusion of ANG II at 100 μg·kg⁻¹·h⁻¹. After an additional 15 min, we obtained a dialysate sample of A100-VS.

**Protocol 3 (n = 6).** We examined whether local administration of losartan would block the effects of ANG II on the vagal stimulation-induced myocardial ACh release. We perfused the dialysis probe with Ringer solution containing 10 mM of losartan potassium. Taking into account the distribution across the semipermeable membrane, we administered losartan at a concentration >400 times higher than that for intravenous administration in **protocol 2**. Because local administrations of larger molecules such as α-conotoxin GVIA (molecular weight 3037) and α-conotoxin MVIIIC (mol wt 2,749) were able to suppress vagal stimulation-induced ACh release in our previous study (15), it would be reasonable to assume that losartan potassium (mol wt 461) should have spread in the vicinity of the dialysis fiber, from which the dialysate was collected. Using the same procedures as described in **protocol 1**, we obtained dialysate samples for baseline, VS1, VS2, A10-VS, and A100-VS. A previous study indicated that ACh measured by cardiac microdialysis in the left ventricle mainly reflected ACh released from the postganglionic nerve terminals and not from the parasympathetic ganglia (1 and see DISCUSSION for details).

**Protocol 4 (n = 5).** To examine the effects of ANG II on the baseline ACh level, we performed an additional protocol where the baseline ACh level was measured during intravenous infusion of ANG II at 10 μg·kg⁻¹·h⁻¹ (A10-base). In this protocol, we also obtained a dialysate sample using the perfusate without the cholinesterase inhibitor physostigmine before the usual dialysate sampling using the perfusate containing physostigmine.

**Protocol 5 (n = 5).** To avoid the pressor effect of ANG II, we administered an L-type Ca²⁺ channel blocker nifedipine (0.5–2.0 mg·kg⁻¹·h⁻¹) simultaneously with ANG II and obtained dialysate samples for VS, A10-VS, and A100-VS. In a previous study, intravenous administration of an L-type Ca²⁺ channel blocker alone did not affect the vagal stimulation-induced myocardial ACh release significantly (15).

**Statistical Analysis**

All data are presented as mean ± SE values. In **protocols 1 through 5**, myocardial interstitial ACh levels were compared among baseline, VS1, VS2, A10-VS, and A100-VS samples using a repeated-measures ANOVA (8). When there was a significant difference, Tukey’s test for all-pairwise comparisons was applied to identify the differences between any two of the samples. Differences were considered significant at P < 0.05. The mean AP value in the last 1 min of the 10-min vagal stimulation period was treated as the AP value during vagal stimulation. The AP data were compared using a repeated-measures ANOVA among baseline, during the two control stimulations (VS1 and VS2), and before and during vagal stimulation under the two different doses of intravenous ANG II administrations. When there was a significant difference, Dunnnett’s test for comparison against a single control was applied to identify differences from the baseline value. Differences were considered significant at P < 0.05. In

**Fig. 2.** Changes in dialysate ACh concentrations obtained from **protocol 1**. Vagal stimulation significantly increased the ACh levels. There was no significant difference in the ACh level between the 2 control trials (VS1 and VS2). The ACh level was significantly lower in A10-VS and A100-VS compared with that measured in VS1 and VS2. There was no significant difference in the ACh level between A10-VS and A100-VS. Values are presented as mean and SE. *P < 0.01 by Tukey’s test.

**Fig. 3.** Changes in dialysate ACh concentrations obtained from **protocol 2**. Vagal stimulation significantly increased the ACh levels. There was no significant difference in the ACh level among the 4 dialysate samples during vagal stimulation (VS1, VS2, A10-VS, and A100-VS). Values are presented as means and SE. *P < 0.01 by Tukey’s test.

**Fig. 4.** Changes in dialysate ACh concentrations obtained from **protocol 3**. Vagal stimulation significantly increased the ACh levels. There was no significant difference in the ACh level between the 2 control trials (VS1 and VS2). The ACh level was significantly lower in A10-VS and A100-VS compared with that measured in VS1 and VS2. There was no significant difference in the ACh level between A10-VS and A100-VS. Values are presented as means and SE. †P < 0.05 and *P < 0.01 by Tukey’s test.
ANGIOTENSIN II AND ACETYLCHOLINE

Table 1. Mean arterial pressure values before vagal stimulation and during the last 1 min of stimulation

<table>
<thead>
<tr>
<th>Protocol 1</th>
<th>Baseline</th>
<th>VS1</th>
<th>VS2</th>
<th>A10</th>
<th>A10-VS</th>
<th>A100</th>
<th>A100-VS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>102±11</td>
<td>93±17</td>
<td>91±17</td>
<td>132±9†</td>
<td>105±19</td>
<td>129±13†</td>
<td>105±21</td>
</tr>
<tr>
<td>Protocol 2</td>
<td>102±17</td>
<td>71±16*</td>
<td>69±16*</td>
<td>80±15</td>
<td>68±17*</td>
<td>86±19</td>
<td>72±18*</td>
</tr>
<tr>
<td>Protocol 3</td>
<td>102±13</td>
<td>100±17</td>
<td>92±17</td>
<td>139±11*</td>
<td>120±19</td>
<td>147±11*</td>
<td>122±21</td>
</tr>
</tbody>
</table>

Data are means ± SE obtained from baseline, two control trials (VS1 and VS2), before (A10) and during (A10-VS) vagal stimulation under iv administration of ANG II at 10 μg·kg⁻¹·h⁻¹, and before (A100) and during (A100-VS) vagal stimulation under iv administration of ANG II at 100 μg·kg⁻¹·h⁻¹. The heart was paced at 200 beats/min whenever vagal stimulation was applied. †P < 0.05 and *P < 0.01 from the respective baseline values by Dunnett’s test.

RESULTS

In protocol 1, vagal stimulation significantly increased myocardial interstitial ACh levels (Fig. 2). There was no significant difference between two control trials with an intervening interval of 15 min [VS1: 10.7±1.0 (SE) nM and VS2: 9.9±0.9 (SE) nM]. Intravenous administration of ANG II at 10 μg·kg⁻¹·h⁻¹ significantly attenuated the vagal stimulation-induced ACh release (A10-VS: 7.5±0.6 nM) to ~71% of VS1. Although the intravenous administration of ANG II at 100 μg·kg⁻¹·h⁻¹ also significantly attenuated the vagal stimulation-induced ACh release (A100-VS: 7.3±0.9 nM) to ~68% of VS1, the ACh levels were not different from those of A10-VS.

In protocol 2, vagal stimulation significantly increased myocardial interstitial ACh levels under control stimulations (VS1: 8.6±1.5 nM and VS2: 8.7±2.0 nM; Fig. 3). With a pretreatment of intravenous losartan, intravenous ANG II was unable to suppress the vagal stimulation-induced ACh release (A10-VS: 8.4±1.7 nM and A100-VS: 7.1±1.4 nM). Although the mean level of ACh tended to be lower in A100-VS compared with VS1 or VS2, the differences were not statistically significant.

In protocol 3, vagal stimulation significantly increased myocardial interstitial ACh levels under control stimulations (VS1: 8.0±0.8 nM and VS2: 7.9±0.8 nM; Fig. 4). Intravenous ANG II at either 10 μg·kg⁻¹·h⁻¹ or 100 μg·kg⁻¹·h⁻¹ significantly suppressed the vagal stimulation-induced ACh release to ~72% (A10-VS: 5.8±1.0 nM) and 62% (A100-VS: 5.0±0.7 nM) of that seen in VS1, respectively.

In protocol 1, the AP values before the vagal stimulation during the intravenous ANG II administrations (A10 and A100) were significantly higher than the baseline AP value (Table 1). The AP values during vagal stimulation (VS1, VS2, A10-VS, and A100-VS) were not different from the baseline AP value. In protocol 2, the AP value before the first administration of losartan was 126±14 mmHg. The AP values before the vagal stimulation during the intravenous ANG II administrations (A10 and A100) were not significantly different from the baseline AP value. The AP values during vagal stimulation (VS1, VS2, A10-VS, and A100-VS) were significantly lower than the baseline AP value. In protocol 3, the AP values before vagal stimulation during the intravenous ANG II administrations (A10 and A100) were significantly higher than the baseline AP value. The AP values during vagal stimulation (VS1, VS2, A10-VS, and A100-VS) did not differ statistically from the baseline AP value.

Figure 5 illustrates typical chromatograms obtained from one animal in protocol 4. The baseline ACh level was below the limit of determination (0.5 nM) when the perfusate did not contain physostigmine. Approximately 1 h after replacing the perfusate with Ringer solution containing physostigmine, the baseline ACh level was above the limit of determination. As shown in Table 2, vagal stimulation significantly increased the ACh level (VS). The intravenous administration of ANG II did not affect the baseline ACh level (A10-baseline) but significantly attenuated the ACh level during vagal stimulation (A10-VS).

![Typical chromatograms for the ACh measurements obtained from protocol 4](http://ajpheart.physiology.org/DownloadedFrom/10.25363_H2519.png)
In protocol 5, the pressor effect of ANG II was counteracted by the simultaneous intravenous infusion of nifedipine (Table 3). Under this condition, the intravenous administration of ANG II significantly attenuated the stimulation-induced ACh release to ~83% (A10-VS) and 72% (A100-VS) of that seen in VS.

**DISCUSSION**

The present study demonstrated that intravenous ANG II significantly inhibited the vagal stimulation-induced myocardial interstitial ACh release in the left ventricle in anesthetized cats. Intravenous administration of losartan abolished the inhibitory effect of ANG II on the stimulation-induced ACh release, suggesting that the inhibitory action of ANG II was mediated by AT1 receptors.

**Inhibitory Effect of ANG II on Myocardial Interstitial ACh Release**

Only a few reports have focused on the modulatory effects of ANG II on the parasympathetic nervous system (3, 5, 25, 26), all of which have used the heart rate reduction in response to vagal stimulation as a functional measurement to assess the peripheral vagal function. Although ANG II has been shown to inhibit the ACh release in the rat entorhinal cortex in vitro (4), the direct evidence for the inhibitory effect of ANG II on the ACh release in the peripheral vagal neurotransmission in vivo has been lacking. The present study demonstrated that intravenous ANG II inhibited the vagal nerve stimulation-induced ACh release in the left ventricle in vivo (Fig. 2). As for the sympathetic system in the heart, Lameris et al. (18) have previously demonstrated that ANG II does not affect the sympathetic nerve stimulation-induced norepinephrine release. The significant effect of ANG II on the sympathetic neurotransmission and the inhibitory effect of ANG II on the parasympathetic neurotransmission may provide the basis for a study by Takata et al. (26) in which ACE inhibitor enhanced cardiac vagal but not sympathetic neurotransmission.

An increased activity of the renin-angiotensin system is common in chronic heart failure and has been considered to be a stimulus for aggravation of the disease. Inhibition of the renin-angiotensin system by ACE inhibitors or by AT1 receptor blockers can prevent the ventricular remodeling and improve the survival rate (16, 17), suggesting that ANG II is indeed involved in the aggravation of heart failure. ACh, on the other hand, can exert a cardioprotective effect against myocardial ischemia in several experimental settings (12, 24, 29). If ANG II inhibits the peripheral vagal neurotransmission, blockade of ANG II would increase the vagal effect on the heart. Actually, Du et al. (5) demonstrated that losartan enhanced bradycardia induced by vagal stimulation in rats with chronic myocardial infarction. In that study, however, the ventricular effect of vagal stimulation was not assessed. The results of the present study indicate that ANG II inhibited the vagal neurotransmission in the ventricle. Blockade of ANG II is therefore expected to increase the vagal effect on the ventricular myocardium. The results of the present study also indicate that ANG II inhibited the vagal neurotransmission in the ventricle. Blockade of ANG II is therefore expected to increase the vagal effect on the ventricular myocardium when the vagal outflow from the central nervous system is unchanged. Although no literatures appear to be available as to the chronic effect of ACh on the prognosis of heart failure, electrical vagal stimulation was able to improve the survival rate of chronic heart failure in rats (19). In that study, the magnitude of the vagal stimulation was such that the heart rate decreased by only 20–30 beats/min in rats, suggesting that a modest increase in vagal tone would be sufficient to produce a cardioprotective effect. It is plausible that blockade of ANG II yields beneficial effects on chronic heart failure not only by antagonizing the sympathetic effects but also by enhancing the vagal effects on the ventricle.

Vagal stimulation was able to reduce the left ventricular contractility as assessed by end-systolic elastance only when sympathetic stimulation coexisted (20), suggesting that the effect of vagal stimulation on ventricular contractility would be secondary to sympathoinhibition. Accordingly, contribution of the inhibitory effect of ANG II on the stimulation-induced ACh release to the physiological regulation of ventricular contractility might be marginal. We think that the finding is important as a peripheral mechanism of vagal withdrawal in heart diseases accompanying the activation of the renin-angiotensin system.

### Table 2. Mean arterial pressure values and ACh concentrations obtained in protocol 4

<table>
<thead>
<tr>
<th></th>
<th>Physostigmine-free Baseline</th>
<th>Baseline</th>
<th>VS</th>
<th>A10-Baseline</th>
<th>A10-VS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh, nM</td>
<td>Not detected</td>
<td>12.7±1.1</td>
<td>10.6±1.1</td>
<td>9.2±1.5*</td>
<td>7.8±2.1*</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>111±11</td>
<td>109±12</td>
<td>103±6</td>
<td>148±3*</td>
<td>118±6</td>
</tr>
</tbody>
</table>

Data are means ± SE obtained from physostigmine-free baseline, baseline, control vagal stimulation (VS), and baseline (A10-baseline) and vagal stimulation (A10-VS) under iv administration of ANG II at 10 μg·kg⁻¹·h⁻¹. There was no significant difference in the ACh level between baseline and A10-baseline by a paired-t-test. The ACh level was significantly lower in A10-VS than in VS by a paired-t-test. Mean arterial pressure was significantly higher in A10-baseline compared with the physostigmine-free baseline value by Dunnett’s test. *P < 0.01.

### Table 3. Mean arterial pressure values and ACh concentrations obtained in protocol 5

<table>
<thead>
<tr>
<th></th>
<th>VS</th>
<th>A10-VS</th>
<th>A100-VS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh, nM</td>
<td>12.7±1.1</td>
<td>10.6±1.1</td>
<td>9.2±1.5*</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>83.4±12.2</td>
<td>68.4±6.3</td>
<td>70.4±9.5</td>
</tr>
</tbody>
</table>

Data are means ± SE from a control vagal stimulation trial (VS), during vagal stimulation under iv administration of ANG II at 10 μg·kg⁻¹·h⁻¹ (A10-VS) and during vagal stimulation under iv administration of ANG II at 100 μg·kg⁻¹·h⁻¹ (A100-VS). The heart was paced at 200 beats/min during vagal stimulation. In this protocol, the pressor effect of ANG II was counteracted by simultaneous iv administration of the L-type Ca²⁺ channel blocker nifedipine (0.5–2 mg·kg⁻¹·h⁻¹). *P < 0.05 and **P < 0.01 from the VS group by Tukey’s test. There was no significant difference between A10-VS and A100-VS in the ACh level. There were no significant differences in mean arterial pressure among the three trials.
Possible Site of the Inhibitory Action of ANG II on ACh Release

In protocol 3, we examined whether local administration of losartan was able to nullify the inhibitory effect of ANG II on the vagal stimulation-induced ACh release. The utility of local administration of pharmacological agents through the dialysis fiber has been confirmed previously. As an example, local administration of the Na+ channel inhibitor tetrodotoxin through the dialysis fiber completely blocked the nerve-stimulation-induced ACh release (14). With respect to the source for ACh, intravenous administration of the nicotinic antagonist hexamethonium bromide completely blocked the stimulation-induced ACh release, whereas local administration of hexamethonium bromide did not, suggesting the lack of parasympathetic ganglia in the vicinity of dialysis fiber (1). In support of our interpretation, a neuroanatomic findings indicates that three ganglia, away from the left anterior free wall targeted by the dialysis probe, provide the major source of left ventricular postganglionic innervation in cats (11). Therefore, the myocardial interstitial ACh measured by cardiac microdialysis in the left ventricle mainly reflects the ACh release from the postganglionic vagal nerve terminals. The results of protocol 3 indicate that losartan spread around the postganglionic vagal nerve terminals failed to abolish the inhibitory effect of ANG II on the stimulation-induced ACh release. Because intravenous administration of losartan was able to abolish the inhibitory effect of ANG II on the stimulation-induced ACh release (protocol 2), the site of this inhibitory action is likely the parasympathetic ganglia rather than at postganglionic vagal nerve terminals. The fact that AT1 receptors are rich in parasympathetic ganglia (2) would support our interpretation.

ANG II has a direct vasoconstrictive effect on the coronary artery (30). At the same time, however, the intravenous administration of ANG II tended to increase mean AP during vagal stimulation by -15 mmHg in protocol 1 (Table 1). Although it was statistically insignificant, if this increase in mean AP increased cardiac oxygen demand, the coronary blood flow might have been increased (27), resulting in an increased rate of washout in the myocardial tissue. The possibility cannot be ruled out that such a washout mechanism contributed to the reduction of stimulation-induced ACh release during ANG II administration. However, the baseline ACh level was not decreased by ANG II in protocol 4, suggesting that the washout rate did not increase significantly. In addition, even when the pressor effect of ANG II was counteracted by nifedipine, ANG II was still able to inhibit the vagal stimulation-induced ACh release in protocol 5. Therefore, we think that the change in washout rate was not a principal mechanism for the reduction of stimulation-induced ACh release by ANG II.

The mechanisms for the baseline ACh release under the vagotomized condition were not identified in the present study. In the motor nerve terminals, a so-called nonquantal release of ACh is documented, which is independent of nerve activity (6). Incorporation of the vesicular transport system in the membrane of the nerve terminals during an exocytosis process is considered to be responsible for the mechanism of nonquantal ACh release. A similar mechanism might contribute to the baseline ACh release in the vagal nerve terminals.

Several limitations need to be addressed. First, the dose of ANG II might have increased the plasma ANG II concentration beyond the physiological range. In this regard, the observed effect might be rather pharmacological or pathological than physiological. Nevertheless, because there are local synthesis and degradation of ANG II in the heart (21, 28), the inhibition of ACh release by ANG II could operate locally in the heart. Second, whether ANG II inhibited the ACh release from the preganglionic nerve terminals or it suppressed the excitability of the postganglionic nerve fibers to ACh was not identified in the present study. Third, the involvement of ANG II receptor subtype 2 (AT2 receptor) in the modulation of peripheral parasympathetic neurotransmission was not examined in the present study because intravenous losartan was able to abolish the inhibitory effect of ANG II on the stimulation-induced ACh release. However, if coactivation of AT1 and AT2 receptors is required for the inhibitory effect of ANG II, blockade of AT2 receptors would also abolish the inhibitory effect. Forth, we tested just one level of vagal stimulation. Whether the effect of ANG II on the stimulation-induced ACh release depends on the vagal stimulation intensity remains to be resolved.

In conclusion, intravenous ANG II reduced the vagal nerve stimulation-induced ACh release in the left ventricle. Intravenous losartan abolished the inhibitory effect of ANG II on the stimulation-induced ACh release, suggesting that this inhibition was mediated by AT1 receptors. Because local administration of losartan via dialysis fiber was unable to nullify the inhibitory effect of ANG II on the stimulation-induced ACh release, the site of this inhibitory action is likely parasympathetic ganglia. The present results imply that the beneficial effects of ACE inhibitors and AT1 receptor antagonists in the treatment of heart diseases may include not only the suppression of sympathetic activity but also the enhancement of vagal activity to the ventricle.

GRANTS

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