Angiotensin II Exerts Differential Actions on Renal Nerve Activity and Heart Rate

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Abstract Angiotensin II (Ang II) exerts complex actions on sympathetic nerve activity and heart rate, but these actions are incompletely understood. We performed three series of experiments in conscious rabbits to analyze the actions of exogenous and endogenous Ang II on renal sympathetic nerve activity and heart rate. (1) Graded intravenous doses of phenylephrine and Ang II suppressed renal sympathetic nerve activity to the same degree, whereas Ang II decreased heart rate much less than phenylephrine. (2) Ang II infusion at 10 ng/kg per minute increased mean arterial pressure by 13±2 mm Hg (P<.01) and decreased renal sympathetic nerve activity by 67±13% (P<.01) but did not change heart rate. In the same rabbits, nitroprusside and phenylephrine infusions were used to generate baroreceptor reflex curves. Ang II shifted the heart rate–mean arterial pressure curve to the right but did not alter the renal nerve activity–mean arterial pressure curve. (3) The Ang II type 1 receptor antagonist losartan decreased mean arterial pressure by 8±3 mm Hg (P<.01) and increased renal sympathetic nerve activity by 63±15% (P<.05) but did not change heart rate. Losartan shifted the heart rate–mean arterial pressure curve to the left but did not alter the renal nerve activity–mean arterial pressure curve. These results demonstrate that whereas exogenous Ang II resets the baroreceptor reflex control of heart rate to a higher pressure, it does not increase resting renal sympathetic nerve activity or alter the baroreceptor reflex control of renal nerve activity. The results also confirm that endogenous Ang II resets the reflex control of heart rate by stimulating Ang II type 1 receptors but fail to provide evidence for an action of endogenous Ang II on resting renal sympathetic nerve activity or on the baroreceptor reflex control of renal nerve activity. (Hypertension. 1994;24: 451-456.)

Key Words • angiotensin II • losartan • receptors, angiotensin • pressoreceptors • sympathetic nervous system • heart rate

It is generally accepted that angiotensin II (Ang II) modulates the baroreceptor reflex control of heart rate (HR). This modulation results from a resetting of the baroreceptor reflex control of HR to a higher pressure with or without a decrease in baroreceptor reflex gain. The resetting is apparently due to an action of Ang II at the area postrema that is mediated by Ang II type 1 (AT1) receptors. It can be elicited by endogenous and exogenous Ang II.

Ang II may also modulate the baroreceptor reflex control of sympathetic nerve activity, although less information is available and some of it is conflicting. The receptors that mediate effects of Ang II on sympathetic nerve activity have not been characterized, and the role of endogenous Ang II in the baroreceptor reflex control of sympathetic nerve activity has not been investigated.

The aims of the present study were to further investigate the effect of a mildly pressor dose of Ang II on resting renal sympathetic nerve activity (RSNA) and on the baroreceptor reflex control of RSNA and to determine the role of endogenous Ang II in the baroreceptor reflex control of RSNA by investigating the effect of blockade of the action of endogenous Ang II with the specific AT1 receptor antagonist losartan.

Methods

Experiments were conducted in male New Zealand White rabbits weighing 2.5 to 3.2 kg using procedures approved by the University of California–San Francisco Committee on Animal Research. The rabbits were housed singly in cages in a room with a constant temperature and a 12-hour light/dark cycle and fed a commercial diet containing 0.32% sodium (Purina rabbit chow, Ralston-Purina) with water ad libitum.

Surgical Procedures

For surgical preparation the rabbits were anesthetized with a mixture of xylazine (5 mg/kg IM, Lloyd Laboratories) and ketamine (50 mg/kg IM, Parke-Davis). Sterile technique was used.

Arterial and Venous Catheters

Two Tygon catheters were inserted into the right external jugular vein and their tips positioned in the vena cava. A catheter consisting of 10 cm medical grade silicone elastomer (Dow-Corning Corp) connected to PE-60 tubing was inserted into the right femoral artery and advanced into the lower abdominal aorta. The catheters were led subcutaneously to the back of the neck and protected in the pockets of a nylon mesh jacket. The rabbits were allowed at least 3 days to recover from surgery. Ampicillin (10 mg/d IV) diluted in sterile 0.9% saline was given during the recovery period, and the catheters were flushed with sterile heparinized isotonic saline (1000 U/mL) at least every other day. During the recovery period the rabbits were brought to the laboratory and accustomed to the experimental environment.

Renal Nerve Electrodes

Electrodes were placed on the left renal nerve for multifiber postganglionic recording of RSNA as described previously. In a shielded cage, the left kidney was exposed via a retroperitoneal approach through a left flank incision. With the use of
a dissecting microscope, a renal nerve was identified, isolated, and carefully dissected. Polytetrafluoroethylene-coated multistrand stainless wire electrodes (A-M System, Inc) were placed on the nerve. To insulate the electrodes and nerve from surrounding tissue, a small piece of Paralilm (American Can Co) was placed under the nerve. The nerve and recording electrode assembly was covered with a two-component silicon gel (Sil Gel 604, Wacker-Chemie Gimble) to prevent the nerve from drying. A ground lead was fixed to the tissue close to the electrodes. When the gel had hardened, the flank incision was closed, the electrodes were exteriorized at the back of the neck, and the rabbits were allowed to recover for 2 days. Ampicillin (10 mg/d IV) diluted in 0.9% sterile saline was given postoperatively.

Data Acquisition
On the day of an experiment, a rabbit was brought to the laboratory and placed in a stainless steel cage inside the shielded cage, and continuous recordings of arterial pressure, HR, and RSNA were begun. Arterial pressure and HR were monitored continuously with a pressure transducer (Cobe Laboratories, Inc) and a custom-built cardiovascular analyzer. RSNA was amplified (×10 000 to ×20 000) using a differential amplifier (Tektronix Inc) with a band-pass filter (low, 100 Hz; high, 3 kHz), and the action potentials were filtered, rectified, and integrated using a custom-built envelope detector. Renal neurograms were photographed from the screen of an oscilloscope (Tektronix). Background noise was determined when nerve activity was eliminated by increasing arterial pressure with an infusion of phenylephrine (40 μg/kg per minute). This value was subtracted from all experimental values of RSNA. Arterial pressure, HR, and integrated RSNA were digitized at 100 Hz, stored, analyzed with a PDP 11/23+ computer (Digital Equipment Corp), and displayed on a polygraph (Grass Instrument Co).

Experimental Protocols
Effects of Phenylephrine and Ang II on Resting RSNA and HR (n=6)
The purpose of this experiment was to compare the RSNA and HR responses to increases in arterial pressure produced by intravenous infusions of phenylephrine and Ang II (Peninsula Laboratories, Inc). Phenylephrine was infused in doses of 2.5 to 10 μg/kg per minute and Ang II in doses of 2.5 to 80 μg/kg per minute.

Effects of Ang II on Baroreceptor Reflex Control of RSNA and HR (n=9)
The purpose of this experiment was to investigate the effects of exogenous Ang II on the baroreceptor reflex control of RSNA and HR. After a control period of at least 20 minutes, a background infusion of Ang II at 10 ng/kg per minute IV was begun. Baroreceptor reflex curves were generated in the same rabbits with and without background infusion of Ang II.

Effects of Losartan on Baroreceptor Reflex Control of RSNA and HR (n=7)
The purpose of this experiment was to investigate the role of endogenous Ang II in the baroreceptor reflex control of RSNA and HR. This was accomplished using the AT1 receptor antagonist losartan (Du Pont). After a control period of at least 20 minutes, losartan was administered as a bolus of 5 mg/kg IV followed by infusion at 1 mg/kg per hour IV. Baroreceptor reflex curves were generated in the same rabbits with and without losartan administration.

Baroreceptor Reflex Curves
Baroreceptor reflex curves relating HR and RSNA to mean arterial pressure (MAP) were generated by measuring the HR and RSNA responses to changes in arterial pressure produced by intravenous infusions of both nitroprusside (Elkins-Sinn, Inc) and phenylephrine (Elkins-Sinn) at 2.5, 5, 10, and 20 μg/kg per minute. Each infusion lasted 3 to 5 minutes, and 15 minutes elapsed between each infusion. Arterial pressure, HR, and RSNA data collected during the last 1 to 2 minutes of each infusion were averaged and used to generate baroreceptor reflex curves.

Steady-state values of MAP, HR, and RSNA were analyzed using the four-parameter sigmoidal logistic function:

$$RSNA \text{ or } HR = \frac{A-D}{1 + \left(\frac{MAP}{C}\right)^B} + D$$

where A and D are the upper and lower plateaus of the baroreceptor reflex curve, B is the slope coefficient, and C is the MAP at the midpoint of the RSNA or HR range (BPm). The curves were fitted to the data using a nonlinear curve-fitting program (NONL, Island Products) based on the Marquardt-Levenberg algorithm. The baroreceptor reflex gain at BPm was calculated as:

$$Gain_m = -B(A-D)/4C$$

RSNA, HR, and MAP data for each group of experiments were averaged and the means fitted to construct a baroreceptor reflex curve for each treatment group.

Statistical Analysis
Results are expressed as mean±SEM. Data were analyzed using one-way ANOVA for repeated measures and the Newman-Keuls or Dunnett test. Comparisons between individual pairs of data were made with the paired t test. Changes were considered to be statistically significant at a value of $P<.05$.

Results
Fig 1 shows representative renal sympathetic neurograms. Figs 2 through 4 show representative records of the effects of phenylephrine, Ang II, and losartan on arterial pressure, HR, and RSNA.
Effects of Phenylephrine and Ang II on Resting RSNA and HR

Graded doses of phenylephrine produced dose-related increases in MAP from 83±6 to 106±3 mm Hg \((P<.01)\) that were accompanied by decreases in HR from 263±16 to 199±12 beats per minute \((P<.01)\) and RSNA by 77±4% \((P<.001)\) (Fig 5). Graded doses of Ang II produced dose-related increases in MAP from 85±5 to 105±4 mm Hg \((P<.05)\). In marked contrast to phenylephrine, Ang II did not decrease HR significantly (252±12 to 242±25 bpm) but decreased RSNA by 74±14% \((P<.05)\) (Fig 5).

Effects of Ang II on Baroreceptor Reflex Control of RSNA and HR

Ang II infusion at 10 ng/kg per minute IV increased MAP from 81±2 to 94±2 mm Hg \((P<.05)\) without changing HR (240±13 to 246±11 bpm) but increased RSNA by 67±13% \((P<.01)\). Ang II infusion shifted the HR-MAP curve to the right (Fig 6), as indicated by an increase in the BP\(_{50}\) of the curve from 75±2 to 90±4 mm Hg \((P<.05)\). The gain of the HR-MAP curve tended to decrease, but the change from −7.2±2.1 to −5.5±2.4 bpm/mm Hg was not statistically significant. In contrast to its effect on the HR-MAP curve, losartan did not reset the RSNA-MAP curve, nor did it alter the gain of the curve (Fig 6). Losartan did not alter the upper and lower plateaus of the HR-MAP and RSNA-MAP curves.

Effects of Losartan on Baroreceptor Reflex Control of RSNA and HR

Losartan decreased MAP from 81±2 to 73±3 mm Hg \((P<.01)\) but did not change HR (240±13 to 246±11 bpm) and increased RSNA by 63±15% \((P<.05)\). Losartan infusion shifted the HR-MAP curve to the left (Fig 6), as indicated by a decrease in the BP\(_{50}\) of the curve from 75±2 to 68±3 mm Hg \((P<.05)\). The gain of the HR-MAP curve tended to decrease, but the change from −7.2±2.1 to −5.5±2.4 bpm/mm Hg was not statistically significant. In contrast to its effect on the HR-MAP curve, losartan did not reset the RSNA-MAP curve, nor did it alter the gain of the curve (Fig 6). Losartan did not alter the upper and lower plateaus of the HR-MAP and RSNA-MAP curves.

Discussion

Effects of Ang II on Resting RSNA and HR

In the present study elevations in arterial pressure produced by intravenous infusions of graded doses of Ang II were accompanied by reciprocal decreases in RSNA. The curve relating RSNA to MAP during infusions of graded doses of Ang II was identical to that during infusions of graded doses of phenylephrine, suggesting that the Ang II–induced reduction in RSNA was simply a reflex response to the increase in arterial pressure. Although it has been reported that the activity of the sympathetic nervous system did not increase during Ang II infusion in humans as assessed by measurement of norepinephrine spillover\(^1\) or renal norepinephrine release,\(^2\) the present results provide direct evidence that Ang II does not increase RSNA. Indeed, the results indicate that the pressor response to Ang II is buffered by a decrease in RSNA. This result differs from the finding by Matsukawa et al\(^1\) that infusion of graded doses of Ang II (5 to 20 ng/kg per minute) in normotensive or hypertensive human subjects caused less suppression of muscle sympathetic nerve activity than phenylephrine. The discrepancy between their
results and ours may reflect the difference in the sympathetic nerve being recorded from, since nonuniformity of the sympathetic outflow has been demonstrated. In contrast to its effect on RSNA, infusions of graded doses of Ang II caused little or no reduction in HR, whereas infusions of graded doses of phenylephrine elicited marked bradycardia. These results are in good agreement with those of an earlier study showing that infusions of graded doses of Ang II cause little reduction in resting HR and induce no less reduction in RSNA than phenylephrine.

Effect of Ang II on Baroreceptor Reflex Control of RSNA and HR

We performed a second series of experiments to investigate the effect of exogenous Ang II on the baroreceptor reflex control of RSNA and HR. As anticipated from previous studies, Ang II reset the baroreceptor reflex control of HR to a higher pressure. There was a tendency for the gain of the reflex control of HR, Ang II had no significant effect on the baroreceptor reflex control of RSNA. Thus, the curve relating RSNA to MAP during background infusion of Ang II could be superimposed on the control curve. These results demonstrate that Ang II exerts a differential action on the baroreceptor reflex control of RSNA and HR and fail to provide evidence that Ang II enhances RSNA.

These observations differ from the results of Guo and Abboud, who found that background Ang II infusion at 400 ng/kg per minute IV in chloralose-anesthetized rabbits attenuated the reflex inhibition of lumbar sympathetic nerve activity in response to phenylephrine infusion but not in response to nitroprusside infusion. On the other hand, Matsumura et al observed that neither background intravenous nor intravertebral arterial infusion of Ang II in conscious rabbits altered the changes in RSNA elicited by increases or decreases in arterial pressure. The doses used by Matsumura et al were lower than those used by Guo and Abboud but were still high in physiological terms (intravenous, 10 to 40 ng/kg per minute; vertebral artery, 20 to 80 ng/kg per minute). Dorward and Rudd showed that intracerebroventricular injection of Ang II (10 to 100 ng) in conscious rabbits did not alter the RSNA response to increases in arterial pressure induced by inflating an aortic cuff but augmented the RSNA response to decreases in arterial pressure induced by inflating a vena caval cuff. The differences in the Ang II dose and route of administration, the sympathetic nerve being recorded from, and the use of anesthetics may account for the differences in the results obtained in these studies.
Role of Endogenous Ang II

It is known that the resetting of the baroreceptor reflex control of HR by Ang II is mediated by AT1 receptors and can be elicited by endogenous and exogenous Ang II. On the other hand, the receptors that mediate the action of Ang II on RSNA have not been characterized, and the role of endogenous Ang II in the baroreceptor reflex control of RSNA has not been investigated. For this reason, we performed additional experiments in which we assessed the effect of blockade of endogenous Ang II on the baroreceptor reflex control of RSNA using the AT1 receptor antagonist losartan.

Losartan decreased resting arterial pressure without increasing HR and reset the baroreceptor reflex control of HR to a lower pressure without changing its gain. There was a tendency for the gain of the reflex control of HR to decrease, but this was not statistically significant. The resetting of the baroreceptor reflex control of HR apparently results from an action of Ang II on AT1 receptors in the area postrema. This resetting can account for the ability of Ang II to increase arterial pressure without eliciting bradycardia as observed in the first series of experiments and in previous studies. This finding indicates that endogenous Ang II exerts a tonic action on the baroreceptor reflex control of HR, effectively increasing the set point around which the baroreceptor reflex regulates arterial pressure.

In marked contrast to its lack of effect on resting HR, losartan increased resting RSNA. Other investigators have found that inhibition of the renin-angiotensin system with the converting enzyme inhibitor captopril in conscious dogs decreases resting RSNA. However, this suppression was attributed to an increase in prostaglandin synthesis rather than to a reduction in circulating Ang II. Losartan did not alter the set point or gain of the baroreceptor reflex control of RSNA. Thus, the curve relating RSNA to MAP in the presence of losartan could be superimposed on the control curve and on the curve in the presence of exogenous Ang II. These results demonstrate that endogenous as well as exogenous Ang II exerts a differential action on the baroreceptor reflex control of RSNA and HR and that neither endogenous nor exogenous Ang II enhances RSNA. The differential action of Ang II on the baroreceptor reflex control of RSNA and HR could reflect the fact that Ang II suppresses the increase in cardiac vagal efferent activity that is normally elicited by an increase in arterial pressure but does not suppress the decrease in efferent RSNA that is normally elicited by an increase in arterial pressure.

It is important to note that the present investigation was concerned only with short-term effects of Ang II on RSNA, and it is possible that long-term Ang II administration would have different effects. However, Cox and Bishop have reported that Ang II infusion (50 ng/kg per minute) in rabbits caused marked suppression of RSNA even after 2 to 9 days. In addition, it should be emphasized that the action of Ang II to reset the baroreceptor reflex control of HR was readily demonstrable within the time course of the present study. In the present study RSNA was recorded as an index of the action of Ang II on the sympathetic nervous system. Information about the effect of Ang II on RSNA is particularly important in terms of blood pressure control because the renal sympathetic nerves contribute significantly to the regulation of renin secretion, sodium excretion, and renal hemodynamics. However, it is possible that Ang II might have different effects on activity in other sympathetic nerves, eg, the cardiac, lumbar, and muscle sympathetic nerves, and so the present conclusions should be limited to the renal nerves.

In summary, the present results demonstrate that endogenous as well as exogenous Ang II exerts a differential action on the baroreceptor reflex control of RSNA and HR. The alterations in arterial pressure produced by exogenous Ang II or blockade of endogenous Ang II were accompanied by little or no changes in HR, reflecting the action of Ang II to reset the baroreceptor reflex control of HR. This action of Ang II effectively enhances the pressor action of the peptide. On the other hand, the changes in arterial pressure produced by exogenous Ang II or blockade of endogenous Ang II were accompanied by reciprocal changes in RSNA, and no evidence was found for an action of Ang II on the baroreceptor reflex control of RSNA. Thus, no evidence was found that renal sympathetic stimulation contributes to the pressor response to Ang II.

In previous studies we observed that blockade of the renin-angiotensin system with an angiotensin-converting enzyme inhibitor (captopril) or an angiotensin receptor antagonist (losartan) markedly reduced the cardiovascular and renal sympathetic nerve responses to bilateral carotid occlusion in conscious rabbits. We interpreted these findings as evidence that endogenous Ang II facilitates the cardiovascular and sympathetic responses to the sympathetic stimulation evoked by carotid occlusion. In contrast, the present results indicate that neither endogenous nor exogenous Ang II increases sympathetic activity. Our previous observations and the present results are thus consistent with the earlier conclusion that although Ang II can enhance or facilitate sympathetic responses, it does not itself cause sympathetic stimulation. Nevertheless, additional research is needed to further delineate the complex interactions between Ang II and the sympathetic nervous system.

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References

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