Importance of Endogenous Angiotensin II in the Cardiovascular Responses to Sympathetic Stimulation in Conscious Rabbits

Jeffry S. Isaacson and Ian A. Reid

Pharmacological evidence indicates that angiotensin (Ang II) converting enzyme inhibitors attenuate cardiovascular responses to sympathetic stimulation. To investigate the physiological significance of this attenuation, the pressor and heart rate responses to bilateral carotid occlusion (BCO) were studied before and after administration of captopril and again during Ang II replacement in conscious, aortic nerve–sectioned rabbits with chronically implanted carotid occluders. In the control period, BCO produced increases ($p<0.05$) in mean arterial pressure (MAP) and heart rate (HR) of 37.3±3.0 mm Hg and 21.7±5.4 beats/min from baseline values of 79.1±2.5 mm Hg and 255.4±16.7 beats/min. Captopril (5 mg/kg i.v.) markedly reduced ($p<0.05$) both the pressor (10.2±2.6 mm Hg) and HR (5.0±4.0 beats/min) responses to BCO, in parallel with a decrease in plasma Ang II of 75%. Infusion of a subpressor dose of Ang II (5–25 ng/kg/min i.v.) increased plasma Ang II to precaptopril levels and fully restored ($p<0.05$) the pressor (33.0±5.7 mm Hg) and HR (19.8±7.7 beats/min) responses to BCO. In two additional series of experiments, the mechanism of the effects of captopril and Ang II were investigated. In the first series, cardiac baroreflex curves (pulse interval versus MAP) were generated by increasing or decreasing blood pressure with phenylephrine or nitroprusside (5–20 μg/kg/min i.v.). The slope of the linear region of the curve (2.9 msec/mm Hg) was not changed significantly by captopril treatment (3.1 msec/mm Hg) or Ang II replacement (3.2 msec/mm Hg), indicating that cardiac baroreflex sensitivity was not altered by blockade of the renin-angiotensin system. In the second series, the effect of captopril on the pressor response to exogenous norepinephrine (0.1–2.5 μg/kg/min i.v.) was tested. The response was reduced by less than 40%, indicating only a modest postsynaptic component to the action of captopril. These results provide physiological evidence for an important action of endogenous Ang II in facilitating the cardiovascular responses to sympathetic stimulation in conscious rabbits. This facilitation is not due to an action upon the baroreflex per se but results, at least in part, from a presynaptic action of Ang II. (Circulation Research 1990;66:662–671)

Angiotensin II (Ang II) exerts potent effects on blood pressure (BP) via its direct vasoconstrictor action and via its effects on aldosterone secretion and sodium excretion.1 The peptide can also affect BP regulation through a variety of effects on the autonomic nervous system. It acts centrally to increase sympathetic nerve activity2 and decrease vagal tone to the heart.3-4 It also exerts a variety of actions on the peripheral sympathetic nervous system.5 Specifically, it facilitates adrenergic function by potentiating the release6-7 and inhibiting the reuptake8-9 of norepinephrine from sympathetic nerve terminals, by stimulating the release of catecholamines from the adrenal medulla,10,11 and by increasing vascular responsiveness to norepinephrine.12-14 Finally, Ang II acts to reset15 or attenuate16,17 baroreceptor reflexes, and this is apparently responsible for the absence of bradycardia when BP is increased by Ang II.

The effects of endogenous Ang II on peripheral sympathetic function and the baroreflex have commonly been investigated by blocking the renin-angiotensin system with angiotensin converting enzyme inhibitors or Ang II receptor antagonists. There have been several reports that the administration of these agents attenuates sympathetically mediated vascular responses by actions at both the presynaptic and postsynaptic levels,18-21 effects opposite to those elicited by exogenous Ang II. Similarly, there
is evidence that converting enzyme inhibitors reset the cardiac baroreflex in a direction opposite to that produced by Ang II.22 Thus, it appears that endogenous Ang II exerts the same effects upon the sympathetic nervous system and baroreflex as does endogenous Ang II.

Despite this progress, the physiological importance of the effects of Ang II on the sympathetic nervous system remains unclear. Many studies of the influence of endogenous Ang II on the sympathetic nervous system have been performed in anesthetized or pithed animals in which cardiovascular, endocrine, and neural function is markedly altered. Anesthesia or acute surgical stress frequently increases plasma Ang II concentration well above the normal physiological range, but in most investigations, circulating levels of Ang II were not measured. In some cases, the actions of endogenous Ang II have been explored in isolated perfused vascular beds, including the dog paw23 and rat mesenteric bed,24 but it is not clear if these experiments reflect actions of Ang II throughout the systemic circulation.

The aim of the present study was to investigate the effects of endogenous Ang II on the sympathetic nervous system in a more physiological setting. Conscious rabbits, with vascular balloon occluders chronically implanted around both carotid arteries, were used to investigate cardiovascular responses to endogenous sympathetic stimulation produced by bilateral carotid occlusion. The pressor and HR responses to carotid occlusion were examined before and after blockade of the renin-angiotensin system with the converting enzyme inhibitor captopril. The responses to carotid occlusion were subsequently studied after restoration of circulating Ang II by the infusion of a subpressor dose of Ang II. To assess a possible postsynaptic influence of Ang II, the effects of captopril treatment and subsequent Ang II replacement on the pressor and HR responses to exogenous norepinephrine were studied. Finally, the effects of converting enzyme inhibition and Ang II replacement on the cardiac baroreflex were investigated.

Materials and Methods

Sixteen male New Zealand white rabbits, 2.3–3.5 kg in body weight, were used in this study. The animals were fed a standard commercial diet (Purina Rabbit Chow, St. Louis, Missouri) supplemented with alfalfa and were allowed water ad libitum. All procedures used in this study were approved by the University of California, San Francisco Committee on Animal Research.

Surgical Procedures

The rabbits were tranquilized with acepromazine maleate (2 mg/kg i.m., Promace, Aveco, Fort Dodge, Iowa) and anesthetized with sodium pentobarbital (20 mg/kg i.v.) administered via a marginal ear vein. Under sterile conditions, the right femoral artery was exposed and a Tygon catheter (0.04 mm i.d.) was inserted into the abdominal aorta for blood sampling and recording arterial BP and HR. A branch of the right or left jugular vein was exposed through a midline neck incision, and two Tygon catheters (0.03 mm i.d.) were advanced to the inferior vena cava for administration of drugs and hormones. Both common carotid arteries were isolated at the midcervical level and fitted with inflatable balloon vascular occluders (2 mm i.d., In Vivo Metric, Healdsburg, California). The aortic nerves were identified near their junction with the vagal and superior laryngeal nerves and sectioned bilaterally. All cannulas and tubing were led subcutaneously to an area between the scapulae where they were exteriorized and protected by a nylon jacket (Medical Arts, Los Angeles, California). During the 2 days after surgery, the rabbits were treated with penicillin (Penicillin G Procaine, Hanford, Syracuse, New York, 300,000 U/day i.m.). Cannulas were flushed daily with sterile isotonic saline and filled with heparinized saline (100 U/ml). The animals were allowed at least 2 days for recovery before experiments were begun.

Experimental Protocols

On the day of each experiment, rabbits were brought to the laboratory, where they sat in covered stainless steel cages in a quiet area. Arterial BP and HR were recorded continuously via the femoral artery catheter using Statham or Cobe pressure transducers and a Grass polygraph (Grass Instruments, Quincy, Massachusetts). In some experiments, cardiovascular data were digitized at 100 Hz and collected and analyzed using a PDP 11/23 Plus computer system (DEC, Maynard, Massachusetts). Arterial blood samples for analysis were drawn from the femoral catheter and replaced with an equal volume of sterile isotonic saline. Blood samples (3.6 ml) were placed in chilled tubes containing 0.4 ml 0.3 M ethylenediaminetetraacetic acid (EDTA) and immediately centrifuged at 4°C. Plasma was frozen until it was analyzed for plasma renin activity (PRA) and Ang II. PRA was measured using a radioimmunoassay for angiotensin I (Ang I) and was expressed as nanograms of Ang I generated per milliliter of plasma during a 2-hour incubation at 37°C and pH 6.5.25 Plasma immunoreactive Ang II concentration was measured by radioimmunoassay as described previously26 using the Ang II antibody CD3.27 All drugs and hormones were dissolved in saline and injected intravenously either as an infusion or a bolus. The rabbits were allowed at least 30 minutes for BP and HR to stabilize before experiments were started.

The experiments were performed according to the following protocols:

Effects of captopril and Ang II on the blood pressure and heart rate responses to bilateral carotid occlusion. The BP and HR responses to carotid occlusion were measured during three treatment periods in each of six rabbits: 1) control, 2) after blockade of the renin-angiotensin system with the angiotensin converting enzyme inhibitor captopril (Squibb, Prince-
ton, New Jersey), and 3) during intravenous infusion of Ang II (Peninsula Labs, Belmont, California) to replace endogenous Ang II. Three carotid occlusions were performed in each treatment period.

When BP and HR had stabilized, the carotid arteries were occluded for 4 minutes by inflating the occluders with a volume of saline (approximately 0.35 ml) sufficient to provide 275–300 mm Hg pressure. The responses to carotid occlusion were recorded in the steady state as the average mean arterial pressure (MAP) and HR during the last 3 minutes of the occlusion period. Following a 10-minute recovery period, when BP and HR returned to their preocclusion values, a second occlusion was performed. After another 10-minute recovery period, a third occlusion was performed.

Captopril (5 mg/kg i.v.) was injected as a 1.5-ml bolus after recovery from the third carotid occlusion performed during the control period. Twenty to 30 minutes after captopril administration, the sequence of three carotid occlusions was repeated. To test the effectiveness of the converting enzyme inhibitor, the BP response to the injection of Ang I (Peninsula Labs) as a 200-ng/g bolus in 0.5 ml was tested before and 10–90 minutes after captopril treatment.

An intravenous infusion of Ang II at 5 ng/kg/min in 0.0136 ml/min was started after the third postcaptopril carotid occlusion. Fifteen minutes after the start of the Ang II infusion, another series of three carotid occlusions was performed. In two rabbits, the dose of Ang II was subsequently increased to approximately 10 ng/kg/min, and in a third rabbit it was increased to 25 ng/kg/min. The sequence of three occlusions was then repeated. The effect of Ang II administration was also tested in four rabbits that had not received captopril.

An arterial blood sample was collected immediately after the first occlusion in each experiment, after the third occlusion following captopril, and after the last carotid occlusion of the Ang II treatment period.

In a control experiment, a rabbit received a saline bolus (1.5 ml) in place of captopril and a saline infusion (0.0136 ml/min) substituted for Ang II. Three series of three carotid occlusions were performed as just described. In another experiment, the responses to carotid occlusion were studied before and after captopril, and again during Ang II infusion as described above. The infusion of Ang II was then stopped, and the effects of carotid occlusion were studied 20, 30, and 40 minutes later. In an additional experiment in one rabbit, the responses to carotid occlusion were examined before and after captopril. After the third occlusion after captopril, phenylephrine (Neo-Synephrine, Sterling Drug, New York, New York) was infused (25 µg/kg/min, 0.051 ml/min i.v.) instead of Ang II. Ten minutes after the start of the phenylephrine infusion, two carotid occlusions were performed. The phenylephrine infusion was then discontinued before an infusion of Ang II (10 ng/kg/min) was started and the responses to three occlusions assessed as described above.

Effects of captopril and Ang II on the cardiac baroreflex. The HR-BP baroreflex was assessed by examining the relation between pulse interval and MAP in four rabbits. In these experiments, BP was lowered by intravenous infusions of sodium nitroprusside (Elkins-Sinn, Cherry Hill, New Jersey) in doses of 5, 10, and 20 µg/kg/min i.v. at 0.051 ml/min and was increased by infusions of phenylephrine in doses of 5, 10, and 20 µg/kg/min i.v. at 0.051 ml/min. Each infusion lasted 2 minutes. BP and HR were recorded continuously during the infusion of each dose, and 10 minutes was allowed between each infusion for BP and HR to return to baseline levels. After the six infusions during the control period, captopril was administered as described above. The series of six infusions was repeated 15 minutes later. Ang II was then infused (10 ng/kg/min i.v., 0.0136 ml/min), and the series of infusions was repeated for a third time.

Effects of captopril and Ang II on the pressor and heart rate responses to norepinephrine. In five rabbits, the pressor and HR responses to norepinephrine (Levophed, Sterling Drug) were studied before and after converting enzyme blockade with captopril and again during the replacement of Ang II.

After HR and BP had stabilized, 5-minute intravenous infusions of norepinephrine in doses of 0.1, 0.5, and 2.5 µg/kg/min (0.051 ml/min) were delivered in random order. Ten minutes was allowed for BP and HR to return to baseline between each infusion. Captopril (5 mg/kg i.v. as a 1.5 ml bolus) was injected after the last control norepinephrine infusion, and the series of three infusions was repeated 15 minutes later. An infusion of Ang II (10 ng/kg/min i.v., 0.0136 ml/min) was started after the last norepinephrine infusion of the captopril period. Fifteen minutes later, the responses to the three doses of norepinephrine were recorded.

Statistical Analysis

Data were analyzed using one-way and two-way analyses of variance for repeated measures and Newman-Keuls multiple range test.26 Baroreflex curves were fitted by regression analysis using the least-squares method, and their slopes were compared statistically. For all tests, a value of p<0.05 was used as the level of significance. Except where indicated, all results are expressed as mean±SEM.

Results

Effects of Captopril and Ang II on the Blood Pressure and Heart Rate Responses to Bilateral Carotid Occlusion

The effects of carotid occlusion, captopril, and Ang II on BP and HR are shown in Figures 1–3. Responses in a representative rabbit are shown in Figure 1, and data for six rabbits are summarized in Figures 2 and 3. Because the BP and HR responses
to carotid occlusion were very reproducible, the data for the three occlusions in each experimental period (Control, Captopril, and Ang II) were averaged.

Figure 1. Blood pressure responses to bilateral carotid occlusion (duration = 4 minutes) in a representative rabbit before and after captopril treatment (5 mg/kg i.v.) and during Ang II replacement (10 ng/kg/min i.v.).

Figure 2. Effect of bilateral carotid occlusion on mean arterial blood pressure (MABP) and heart rate before and after captopril treatment and during Ang II replacement. Bars indicate the mean ± SEM of observations made in six rabbits. +p < 0.05 vs. baseline; *p < 0.05 vs. control baseline.

Figure 3. Pressor (MABP) and heart rate (HR) responses to bilateral carotid occlusion before and after captopril treatment and during Ang II replacement. Bars indicate the mean ± SEM of observations made in six rabbits. *p < 0.05 vs. control; +p < 0.05 vs. captopril.

Effects of Carotid Occlusion

In the control period, baseline MAP averaged 79.1 ± 2.5 mm Hg. During carotid occlusion, MAP rapidly increased to 116.4 ± 3.7 mm Hg (p < 0.05, Figure 2), an increase of 37.3 ± 3.0 mm Hg (Figure 3). The pressor response to carotid occlusion was accompanied by an increase in HR from 255.4 ± 16.7 to 277.1 ± 12.4 beats/min (p < 0.05, Figure 2), an increase of 21.7 ± 5.4 beats/min (Figure 3). BP and HR rapidly returned to baseline values after release of the occlusion.

Effects of Captopril

Captopril decreased baseline BP in three rabbits but had essentially no effect (<5 mm Hg) on BP in the other three (see Figure 1). Overall, baseline MAP decreased from 79.1 ± 2.5 to 66.4 ± 6.4 mm Hg (p < 0.05, Figure 2). Baseline HR increased slightly in some rabbits, but the overall increase from 255.4 ± 16.7 to 266.4 ± 12.9 beats/min was not statistically significant. Captopril treatment greatly reduced the BP and HR responses to carotid occlusion (Figures 1–3). MAP increased to 76.6 ± 8.1 mm Hg (p < 0.05, Figure 2), an increase of only 10.2 ± 2.6 mm Hg, which was significantly less than the increase during the control period (p < 0.05, Figure 3). HR also changed little in response to carotid occlusion after captopril, reaching only 271.0 ± 13.8 beats/min (Figure 2). This increase in HR of 5.0 ± 4 beats/min was significantly less than the rise during the control period (p < 0.05, Figure 3).
The effectiveness of converting enzyme inhibition by captopril was assessed by measuring the pressor response to intravenous injection of Ang I. In six rabbits, Ang I (200 ng/kg) administered during the control period increased MAP by 25.3±2.8 mm Hg. In all rabbits, this response was completely blocked 10 minutes after the administration of captopril and was still blocked 1 hour later. In other experiments, the pressor response to Ang I was at least 95% blocked 3 hours after captopril injection.

Effects of Ang II

Infusion of subpressor doses of Ang II completely restored both the BP and HR responses to carotid occlusion. During infusion of Ang II in the six rabbits, baseline BP averaged 67.4±6 mm Hg, a value not significantly different from the baseline value during the captopril period (Figure 2). Carotid occlusion during Ang II replacement increased MAP to 100.3±11.0 mm Hg (p<0.05, Figure 2), and this increase of 33.0±5.7 mm Hg was significantly greater than that observed during the captopril treatment period (p<0.05, Figure 3). Baseline HR was also not changed significantly by Ang II replacement. Carotid occlusion during Ang II replacement increased HR from 255.3±12.5 to 275.1±7.9 beats/min (p<0.05, Figure 2), an increase of 19.8±7.7 beats/min (p<0.05, Figure 3), which was significantly greater than the HR response to carotid occlusion during the captopril period (p<0.05, Figure 3). Administration of Ang II in four rabbits that had not received captopril did not modify the pressor or HR responses to carotid occlusion.

In an additional experiment in two rabbits, the dose dependence of the restoration of the responses to carotid occlusion by Ang II was explored. Figure 4 demonstrates the restoration of responses to carotid occlusion after captopril treatment with two doses of Ang II, 5 and 12.5 ng/kg/min. In the control period, carotid occlusion increased MAP by 37.5±1.7 mm Hg. Captopril reduced this response by approximately 50% to 18.0±3.0 mm Hg. Infusion of Ang II at 5 ng/kg/min increased the pressor response to 31.7±8.2 mm Hg, whereas infusion at 12.5 ng/kg/min increased the response to 45.6±4.8 mm Hg. Ang II also produced a dose-related restoration of the HR response to carotid occlusion (Figure 4). Similar results were obtained in another rabbit when Ang II was infused in doses of 2.5, 12.5, and 25 ng/kg/min.

In another experiment, the effects of carotid occlusion were reexamined after the Ang II infusion was stopped. In this experiment, carotid occlusion in the control period increased MAP by 42.7±2.5 mm Hg. After captopril administration, the response to carotid occlusion was reduced to only 4.3±1.4 mm Hg. Infusion of Ang II at 5 ng/kg/min restored the response to 33.0±2.0 mm Hg. The infusion of Ang II was stopped and the effects of carotid occlusion were studied 20, 30, and 40 minutes later. These occlusions produced an average increase in MAP of only 16.7±4.3 mm Hg.

**Time Control**

Figure 5 represents an experiment in which saline was administered instead of captopril and Ang II. Throughout the course of the experiment, the BP and HR responses to repeated carotid occlusions were very reproducible.

**Comparison of the Effects of Phenylephrine and Ang II**

The possibility that the attenuation of the responses to carotid occlusion by captopril was partly due to decreased BP was also investigated. In an experiment in one rabbit, baseline MAP during the control period was 88.2±1.8 mm Hg and increased by 28±2.1 to 116.2±2.9 mm Hg during carotid occlusion. Captopril treatment decreased baseline MAP to 65.9±1.6 mm Hg and reduced the pressor response to carotid occlusion to 13.5±3.0 mm Hg (p<0.05). Phenylephrine was then infused at 25 μg/kg/min. The phenylephrine infusion increased baseline MAP to 103.5±5 mm Hg but failed to restore the pressor response to carotid occlusion (10.2±2.7 mm Hg). The phenylephrine infusion was then discontinued and substituted with an infusion of Ang II at 10 ng/kg/min. During Ang II replacement, baseline MAP was 83.7±1.2 mm Hg, and the pressor response to carotid occlusion was increased to 20±1.5 mm Hg.

**Plasma Renin Activity and Ang II Concentration**

During the control period, plasma Ang II concentration ranged from 11 to 266 pg/ml and averaged 85.4±39
pg/ml (Figure 6). The corresponding range for PRA was 10–99 ng Ang I/ml/2 hr, and the mean value was 41.9±14.3 ng Ang I/ml/2 hr. There was a close correlation between plasma Ang II concentration and PRA (plasma Ang II=1.5×PRA+2.0 \(r=0.92, \ p<0.05\)) (Figure 7). A similar relation has been observed in humans \(^{29}\) and dogs. \(^{26}\) After captopril treatment, plasma Ang II concentration decreased to 23.4±4.7 pg/ml \(p<0.05\) (Figure 6) and PRA increased to 186.2±70.2 ng Ang I/ml/2 hr \(p<0.05\). After captopril, there was no longer a significant correlation between plasma Ang II concentration and PRA (Figure 7). The failure of plasma Ang II concentration to decrease to zero after captopril probably reflects the 2\% cross-reactivity of the Ang II antibody with Ang I. \(^{27}\) Ang II infusion (5–25 ng/kg/min) increased plasma Ang II concentration to within the range measured in the control period, averaging 104.6±42.2 pg/ml, and decreased PRA to 46.5±20.6 ng Ang I/ml/2 hr.

**Effects of Captopril and Ang II on the Cardiac Baroreflex**

In four rabbits, the effects on BP and pulse interval (PI) of the infusion of three doses of phenylephrine and three doses of nitroprusside were studied before and after captopril treatment and again during Ang II infusion. The results are summarized in Figure 8.

In the control period, MAP ranged from 60 to 119 mm Hg during the nitroprusside and phenylephrine infusion; PI ranged from 190 to 373 msec. The relation between PI and MAP could be described by the equation PI=2.94×MAP+13.1 \(r=0.98, \ p<0.05\). After captopril treatment, MAP ranged from 42 to 113 mm Hg during the infusions of nitroprusside and phenylephrine, and PI ranged from 192 to 399 msec. The relation between PI and MAP was shifted to the left of the control baroreflex curve, whereas the slope remained essentially unchanged (PI=3.19×MAP+42.8 \(r=0.99, \ p<0.05\)). During Ang II replacement, MAP ranged from 66 to 130 mm Hg and PI ranged from 200 to 432 msec. The regression line (PI=3.17×MAP−11.1 \(r=0.97, \ p<0.05\)) was shifted back to the right, virtually overlapping the line obtained during the control period. The slope of the baroreflex line during Ang II replacement was not significantly different from the slopes of the control or captopril periods.

**Effects of Captopril and Ang II on the Pressor and Heart Rate Responses to Norepinephrine**

In five rabbits, the BP and HR responses to three doses of norepinephrine were studied before and after treatment with captopril and again during infusion of Ang II. The results are summarized in Figure 9.

In the control period, the three doses of norepinephrine increased MAP by 3.0±1.9, 13.3±1.9, and 36.4±3.4 mm Hg, respectively \(p<0.05\). These increases were accompanied by decreases in HR of
9.6±4.2, 18.0±8.2, and 79.8±22.9 beats/min, respectively (p<0.05). Captopril treatment reduced the pressor responses to 1.0±1.2, 8.1±0.7, and 28.6±2.3 mm Hg, respectively, but this reduction was significant only at the highest dose of norepinephrine (Figure 9). In contrast, the HR responses to norepinephrine were slightly but not significantly potentiated during captopril treatment.

The pressor and HR responses to norepinephrine were restored by infusion of Ang II at 10 ng/kg/min (Figure 9). During the Ang II infusion, the BP and HR responses to norepinephrine were not significantly different from the responses of the control period except for the HR response to the highest dose of norepinephrine, which was less than the responses in the control and captopril periods.

Discussion

Bilateral carotid occlusion is an effective means of increasing sympathetic drive to the cardiovascular system and, in turn, increasing arterial BP. In rabbits this response can conveniently be increased by sectioning the aortic nerve, which eliminates the buffering input from aortic arch baroreceptors.30

In the present study in conscious, aortic nerve-sectioned rabbits, bilateral carotid occlusion elicited rapid and highly reproducible increases in BP and HR. These increases were maintained for the duration of the 4-minute occlusion and fell rapidly to preocclusion levels after release of the occlusion. In some cases, the off-response was accompanied by an overshoot in both BP and HR to below preocclusion levels, followed by a return to baseline within 5 minutes. The increases in BP in the present study were similar to the changes observed by Yamazaki and Sagawa30 in conscious aortic-denervated rabbits.

In the carotid occlusion experiments, PRA and Ang II concentration were within the normal physiological range in some rabbits and elevated in others. This variability probably reflects the variable and often short (approximately 2 days) time between surgical preparation and the initial experiments. Sectioning the aortic nerves may have also contributed to the elevated PRA in some rabbits. The effects of carotid occlusion on PRA and Ang II concentration were not investigated, but we have observed previously that carotid occlusion does not increase renin secretion in dogs unless renal perfusion pressure is held constant.31 Furthermore, in preliminary experiments (F. Hreash and I.A. Reid, unpublished observations), we have observed that PRA actually decreases during carotid occlusion in conscious rabbits.

The major finding in the present study was that both the pressor and HR responses to carotid occlusion were dramatically reduced after captopril treatment. After captopril treatment, the increases in BP and HR produced by carotid occlusion averaged only 25% of the control responses. Thus, blockade of converting enzyme in conscious rabbits results in a marked inhibition of the cardiovascular responses to a reflex increase in sympathetic activity.
The inhibitory effect of captopril in the present study could have been due to inhibition of Ang II formation or to some other effect, such as decreased metabolism of bradykinin. It was clear that captopril did block Ang II formation because there was a marked reduction in plasma Ang II concentration and because the pressor response to Ang I was completely blocked. Nevertheless, to determine whether the inhibition of the responses to carotid occlusion by captopril was due to inhibition of Ang II formation, the effect of Ang II replacement was investigated. Replacement of Ang II in low, subpressor doses returned plasma Ang II concentration to its precaptopril level and fully restored the BP and HR responses to carotid occlusion. The restoration of the responses to carotid occlusion by Ang II was dependent on the dose of Ang II. In addition, the restoration of the pressor response by Ang II was shown to be reversible.

Some experiments were performed as soon as 2 days after surgery. The effect of captopril to reduce the responses to carotid occlusion could usually be demonstrated in these rabbits (and in others not included in this study) for a number of days following the initial experiment. In one rabbit in this group, however, the ability of captopril to attenuate the responses to carotid occlusion was lost several days after the first experiment. In addition, one rabbit, not included in this study, failed to respond to captopril treatment on the initial trial 2 days after surgery. However, after sodium depletion, with a regimen known to increase renin release and plasma Ang II concentration, both rabbits demonstrated characteristically attenuated responses to carotid occlusion after captopril administration.

One possibility that should be considered is that the blockade of the responses to carotid occlusion by captopril was simply the result of a decrease in baseline BP. If this resulted in maximal "unloading" of the carotid sinus baroreceptors, the baroreceptors would be unable to sense the further drop in carotid sinus pressure caused by carotid occlusion. However, the results indicate that another mechanism must be involved because in some rabbits the responses to carotid occlusion were markedly reduced even when baseline BP was not changed by captopril. Moreover, the restoration of the responses to carotid occlusion by Ang II occurred without an increase in baseline arterial pressure. However, to further explore the possible dependence of the responses to carotid occlusion on baseline BP, we investigated the effect of elevating baseline BP with phenylephrine after captopril treatment. Phenylephrine produced a large increase in BP but failed to reverse the attenuated responses to carotid occlusion. Ang II, on the other hand, completely restored the responses.

Taken together, these results suggest that the attenuation of the responses to carotid occlusion by captopril resulted from a reduction in endogenous Ang II levels. Whether the attenuation was due to the decrease in circulating Ang II levels or to decreased Ang II formation in vascular or other tissues remains to be determined. The results are consistent with previous pharmacological studies in which responses to increased sympathetic activity were reduced after blockade of the renin-angiotensin system and indicate an important physiological role for endogenous Ang II in facilitating the cardiovascular responses to sympathetic stimulation.

The present findings differ from results obtained in conscious dogs by Rocchini et al.35 These investigators reported that sodium depletion decreased the pressor response to carotid occlusion and that teprotide, another converting enzyme inhibitor, had no effect on the responses to carotid occlusion. They concluded that sodium depletion attenuated the pressor response to carotid occlusion via a mechanism that is independent of the actions of Ang II. It is possible that teprotide may not have the same effects as captopril.38,39 It is surprising, however, that chronic sodium depletion, which increased plasma renin activity, blunted the pressor response to carotid occlusion. This finding may reflect an additional effect of sodium depletion that could influence the cardiovascular responses to sympathetic stimulation.

Additional experiments were performed to investigate the site where and mechanism by which captopril attenuates the cardiovascular responses to carotid occlusion. One possibility was that captopril attenuated the responses by inhibiting the baroreflex. To test this possibility, cardiac baroreflex curves were generated by measuring the HR responses to increases or decreases in BP and plotting the relation between PI and MAP. A displacement of the curve to the left or right reflects a change in the "setpoint" of the baroreflex, whereas a change in slope indicates a change in baroreflex sensitivity. Captopril treatment shifted the linear region of the baroreflex curve to the left but did not change the slope of the curve (Figure 8). Thus the sensitivity of the baroreflex was not changed by captopril treatment. The displacement of the curve to the left indicates a resetting of the baroreflex setpoint to a new, lower resting pressure. Replacement of Ang II shifted the baroreflex curve to the right, nearly superimposing it upon the control baroreflex curve, without changing the slope of the line. Therefore, Ang II replacement shifted the setpoint back to its original value without a change in baroreflex sensitivity.

The present observations of the effects of captopril and Ang II replacement on the baroreflex compare favorably with the results of Hatton et al.,22 who observed that inhibition of converting enzyme in sodium-depleted dogs changed the setpoint but not the sensitivity of the cardiac baroreflex. This effect of captopril explains the ability of converting enzyme inhibitors to decrease BP without causing tachycardia.22 However, the effect of captopril to alter the setpoint, but not the sensitivity, of the baroreflex cannot account for the marked inhibition of the pressor and HR responses to carotid occlusion observed in the present study.
A second possibility is that captopril acted postsynaptically to inhibit cardiovascular responses to endogenous norepinephrine. This possibility was tested by investigating the effects of captopril and Ang II replacement on the pressor response to exogenous norepinephrine. Captopril caused a slight reduction in the pressor response to norepinephrine, and this modest attenuation could be reversed by the subsequent infusion of Ang II. These results are in good agreement with other studies showing that endogenous Ang II only slightly facilitates the pressor response to sympathetic stimulation at a postsynaptic site in the vasculature. Thus, the effectiveness of captopril in the present study is unlikely to be solely the result of the removal of a postsynaptic action of Ang II, although such an action may be involved.

A third, and most likely, possibility is that the blockade of the cardiovascular responses to carotid occlusion resulted from the removal of the well-known presynaptic effect of Ang II to facilitate norepinephrine release at sympathetic nerve endings. This mechanism appears to be responsible for the inhibitory effect of blockade of the renin-angiotensin system on vasoconstrictor responses observed by others in intact animals and perfused vascular beds. This presynaptic action could be exerted at various sites, including sympathetic nerve endings, autonomic ganglia, or the central nervous system, but the present results do not distinguish between these possibilities.

In conclusion, the present results demonstrate that blockade of the renin-angiotensin system by the converting enzyme inhibitor captopril markedly attenuates the cardiovascular responses to bilateral carotid occlusion in conscious rabbits. This inhibitory effect appears to be due to blockade of Ang II formation rather than to another action of the drug. It is not due to inhibition of the baroreflex itself, but is apparently the result of the removal of a presynaptic, and possibly postsynaptic, action of Ang II to facilitate sympathetic transmission. The finding that Ang II did not facilitate the cardiovascular responses to carotid occlusion in animals that had not received captopril suggests that the basal or moderately elevated endogenous Ang II levels in these rabbits are sufficient to produce maximal or near-maximal facilitation of sympathetic transmission. Taken together, the results provide evidence that this Ang II-sympathetic interaction is physiologically important in cardiovascular regulation.

Acknowledgments
The expert technical assistance of Ms. Lance Chou and Ms. Dina San Juan is gratefully acknowledged.

References


**KEY WORDS**
- angiotensin II
- carotid occlusion
- captopril
- blood pressure
Importance of endogenous angiotensin II in the cardiovascular responses to sympathetic stimulation in conscious rabbits.

J S Isaacson and I A Reid

doi: 10.1161/01.RES.66.3.662

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1990 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/66/3/662

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/