SUMMARY To determine the importance of the direct and the indirect pressor and vasoconstrictor actions of angiotensin II (ANG II), experiments were conducted in conscious dogs 2 to 8 weeks after instrumentation with aortic catheters and aortic electromagnetic flow probes to measure arterial pressure and cardiac output. Total peripheral resistance was calculated by an on-line digital computer. Pretreatment with propranolol eliminated complicating inotropic effects of norepinephrine, released by the indirect actions of ANG II. The pressor and vasoconstrictor responses after ganglionic blockade, in either the presence or absence of arterial baroreceptor nerves, were considered to be the direct effects of ANG II. In conscious dogs, systemically administered ANG II (32 ng/kg bolus) increased mean arterial pressure by 38 ± 3 mm Hg, total peripheral resistance by 37 ± 2 mm Hg/L/minute, and decreased heart rate by 15 ± 2 beats/minute. After arterial baroreceptor denervation, administration of ANG II increased mean arterial pressure by 88 ± 7 mm Hg, total peripheral resistance by 54 ± 4 mm Hg/L/minute, and heart rate by 12 ± 2 beats/minute. After arterial baroreceptor denervation and ganglionic blockade with hexamethonium, administration of ANG II increased mean arterial pressure by 53 ± 8 mm Hg, total peripheral resistance by 27 ± 3 mm Hg/L/minute, and left heart rate unchanged. These results indicate that in the conscious dog without baroreflex buffering nearly one-half of the pressor and vasoconstrictor actions of angiotensin are not direct, but are mediated by the autonomic nervous system. (Hypertension 7: 253-261, 1985)

KEY WORDS • angiotensin II • arterial baroreceptor denervation • autonomic and sympathetic nervous system • ganglionic blockade • cardiac output

ANGIOTENSIN II (ANG II) is a potent endogenous vasoconstrictor hormone that has been implicated in the physiological regulation of blood pressure1 and in certain pathological conditions, such as renal hypertension2-3 and heart failure.4-5 It is known to have multiple central and peripheral interactions with the autonomic nervous system that augment sympathetic vasoconstrictor tone and inhibit parasympathetic tone.6-12 Specifically, at least two central nervous system receptor sites have been shown to elicit an indirect, sympathetically mediated vasoconstriction in response to ANG II.13,14 A peripheral facilitation of norepinephrine release and augmented sensitivity to norepinephrine by ANG II also have been demonstrated.15 16 Thus modulation of the pressor and vasoconstrictor activity of ANG II in intact animals is complex; its direct vasoconstrictor activity should be augmented by these indirect actions, while arterial baroreflexes should elicit an opposing effect (i.e., buffering of the pressor and vasoconstrictor actions).

The relative importance of the direct and indirect actions of ANG II in intact animals, the modulation of these actions by arterial baroreflexes, and their respective contributions to the observed pressor and vasoconstrictor responses induced by ANG II are not known. The goal of this study was to quantitate the contributions of the direct and indirect vasoconstriction induced by systemically administered ANG II in healthy, conscious dogs. To assess the direct pressor and vasoconstrictor effects, ANG II was administered after α-adrenergic or ganglionic blockade, with the
arterial baroreceptors either intact or chronically denervated.

Methods

Healthy, conscious, adult mongrel dogs (20–30 kg) were studied 2 to 8 weeks after operation. All operations were performed with sterile surgical technique. The dogs were anesthetized with sodium pentobarbital (30 mg/kg) and instrumented through a thoracotomy in the fourth left intercostal space. A Tygon catheter was implanted in the descending thoracic aorta and an electromagnetic flow transducer (Zepeda Instruments, Seattle, WA) was placed around the ascending aorta. Six dogs underwent additional arterial baroreceptor denervation (ABD) either by the technique of Edis and Shepherd or by surgical stripping of the aortic arch and carotid sinuses, followed by painting of these regions with 1% phenol. Care of the animals was in accordance with the recommendations of the American Association for Accreditation of Laboratory Animal Care and met all standards prescribed by the National Institutes for Health.

After the dogs had recovered from the operation, they were studied unsedated while lying on their right side in a quiet, darkened room. Arterial pressure was measured with a Statham P23ID strain gauge manometer, and aortic blood flow was measured with a Boston toner square wave electromagnetic flowmeter (Benton Instruments, Cupertino, CA). The signals were recorded on a multichannel tape recorder and played back on a direct-writing oscillograph (Gould-Brush, Mark 200, Cleveland, OH). Mean arterial pressure and cardiac output (minus coronary blood flow) were derived by resistance-capacitance filters with an 8-second time constant. Total peripheral resistance was calculated on line by a PDP 11/34 digital computer (Digital Equipment, Maynard, MA) as the quotient of the mean arterial pressure and mean aortic blood flow. A cardiometer (Beckman Instruments, type 9857B, Palo Alto, CA), triggered by the signal from the arterial pressure pulse, provided a continuous record of heart rate.

Protocols

The dogs received graded, intravenous bolus injections of ANG II (Hypertensin; Ciba Pharmaceutical Co., Summit, NJ), 8, 16 and 32 ng/kg. All experiments were performed in the presence of $\beta$-adrenergic blockade induced with propranolol (Inderal); 1.0 to 2.0 mg/kg, to eliminate complicating inotropic and chronotropic effects of catecholamines released from the sympathetic nervous system by ANG II, while leaving the parasympathetically mediated effects on heart rate and contractility intact. Adequacy of the $\beta$-adrenergic blockade was tested with an injection of isoproterenol (Isuprel), 0.1 $\mu$g/kg, which increased heart rate by $64 \pm 9$ beats/minute and decreased mean arterial pressure by $15 \pm 2$ mm Hg before $\beta$-adrenergic blockade, and did not change either heart rate or mean arterial pressure after $\beta$-adrenergic blockade.

To determine the extent to which the autonomic nervous system modulates the pressor and vasoconstrictor responses to graded doses of ANG II, and to determine the magnitude of the direct vasoconstrictor effect of ANG II, conscious dogs were studied before and after ganglionic blockade with hexamethonium bromide (Sigma Chemical Co., St. Louis, MO), 40 mg/kg. Adequacy of the ganglionic blockade was tested by administering nitroglycerin (nitroglycerin U.S.P.; Eli Lilly and Co., Indianapolis, IN), 5 $\mu$g/kg. Before ganglionic blockade nitroglycerin administration decreased mean arterial pressure by $16 \pm 2$ mm Hg and increased heart rate by $66 \pm 9$ beats/minute. After ganglionic blockade the nitroglycerin decreased arterial pressure by $27 \pm 3$ mm Hg, but left heart rate unchanged.

To determine whether ANG II elicited an indirect, peripheral, $\alpha$-adrenergically mediated vasoconstriction, independent of central or reflex pathways — as has been suggested by Cline — responses to administration of ANG II, 32 ng/kg, were examined in dogs after ganglionic blockade and after sequential $\alpha_1$-adrenergic blockade and combined $\alpha_1$- and $\alpha_2$-adrenergic blockade. Selective $\alpha_1$-adrenergic blockade was induced with prazosin (Minipress), 1.0 to 2.0 mg/kg. Combined $\alpha_1$- and $\alpha_2$-adrenergic blockade was induced with phenolamine (Regitine), 1.0 mg/kg, and maintained with an infusion of phenolamine at 1.0 mg/minute. Adequacy of the $\alpha_1$-adrenergic blockade was demonstrated by absence of the pressor or vasoconstrictor response to phenylephrine (Neo-Synephrine), 5 $\mu$g/kg. Adequacy of the $\alpha_1$- and $\alpha_2$-adrenergic blockade was demonstrated by absence of the pressor and vasoconstrictor responses to norepinephrine (Levophed), 0.2 $\mu$g/kg.

To evaluate the effects of arterial baroreflex buffering, graded doses of ANG II were administered to dogs with ABD. Adequacy of the ABD was tested with an injection of phenylephrine, 5 $\mu$g/kg, which increased mean arterial pressure by $66 \pm 7$ mm Hg and had no effect on heart rate. To determine the extent to which the pressor and vasoconstrictor responses to ANG II, observed in the dogs with ABD, were due to the direct and indirect sympathetically mediated effects of ANG II, graded doses of ANG II were administered after additional $\alpha_1$-adrenergic blockade with prazosin, 1.0 to 2.0 mg/kg. Adequacy of the $\alpha_1$-adrenergic blockade was again confirmed by the absence of pressor and vasoconstrictor responses to an intravenous bolus injection of phenylephrine, 5.0 $\mu$g/kg. To determine whether other autonomically mediated actions participated in the modulation of the pressor and vasoconstrictor responses to graded doses of ANG II, and to determine the magnitude of the direct vasoconstrictor responses to ANG II, we studied a series of dogs with ABD and additional ganglionic blockade with hexamethonium bromide, 40 mg/kg. To obtain stable baseline values, dogs with ABD were studied at night, when there were a minimum of extraneous disturbances, which could induce large fluctuations in the mean arterial pressure. The animals with an intact baroreflex were studied either at night or during the
day, as minor disturbances did not appear to affect the hemodynamic status of these dogs as profoundly as in the dogs with ABD. Further, the responses in the dogs studied at night with an intact baroreflex were similar to those studied during the day.

To evaluate the possibility that the lower baseline arterial pressure observed in the dogs after ganglionic blockade, had stimulated endogenous production of renin and ANG II, and by this mechanism reduced the pressor and vasoconstrictor responses to the systemically administered ANG II, peripheral venous plasma renin activity was measured by radioimmunoassay, originally described by Haber and colleagues.20 Plasma renin levels were measured in dogs after β-adrenergic blockade, before and after additional ganglionic blockade with hexamethonium.

To determine whether the small amount of augmented renin production observed in dogs after ganglionic blockade had a significant effect on the responses to ANG II, dogs were studied after converting-enzyme inhibition with captopril, 1.0 mg/kg, to block endogenous ANG II production. We studied the responses to ANG II (32 ng/kg) in the presence of β-adrenergic blockade in dogs with and without ABD, before and after additional ganglionic blockade to block autonomically mediated actions of the exogenously administered ANG II. Adequacy of the converting-enzyme inhibition was tested with angiotensin I, 32 ng/kg, which increased arterial pressure by 25 ± 1 mm Hg before, and did not change arterial pressure after, treatment with captopril.

As it was observed that larger doses of ANG II administered within 5 minutes of the previous dose elicited substantial reductions in the pressor and vasoconstrictor responses, at least 15 minutes was allowed to pass between injections of ANG II. When the same dose of ANG II was administered repeatedly, at 15-minute intervals, reproducibility of the pressor and vasoconstrictor responses was observed. Furthermore, an interval of at least 48 hours was allowed between protocols so that the dogs could recover from the various pharmacological blockades.

**Analysis of Data**

Baseline values and the changes from baseline were recorded for each equivalent dose of ANG II. The baseline values of the multiple groups were initially compared with one-way analysis of variance (p < 0.01). When significant differences were found between groups, the Bonferroni correction for five groups was used to make reciprocal comparisons between the individual groups.21 Linear regression analysis of the log of the ANG II dose versus the pressor or vasoconstrictor responses was performed with the least-squares method, and the dose-response curves for each of the experimental conditions were compared. Analysis of variance was used to compare the slopes of the lines, and analysis of covariance for multiple groups was used to compare the vertical position of the lines assuming a common slope.22 Analysis of covariance for two groups was used to make comparisons between specific groups if the multiple-group analysis suggested significant differences. All results are expressed as the average of the means ± SE. Differences were considered significant at p < 0.05.

**Results**

Responses to graded doses of ANG II are shown in the figures, and baseline values are presented in Table 1. For clarity, only the responses to ANG II, 32 ng/kg, will be discussed quantitatively. The control values listed in the table represent the average of the means ± SE for the control values of each of the individual doses. A representative experiment showing the pressor and vasoconstrictor responses to ANG II in an intact dog, before and after addition of ganglionic blockade, is shown in Figure 1.

**Effects of Ganglionic Blockade in Intact Dogs**

After β-adrenergic blockade in six dogs, administration of ANG II (32 ng/kg) increased mean arterial pressure by 38 ± 3 from 85 ± 4 mm Hg, increased total peripheral resistance by 37 ± 2 from 35 ± 3 mm Hg/L/minute, decreased cardiac output by 0.766 ±

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**Table 1. Baseline Values**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Beta-adrenergic blockade</th>
<th>Beta-adrenergic and ganglionic blockades</th>
<th>ABD and Beta-adrenergic blockade</th>
<th>ABD and Beta-adrenergic and Beta-adrenergic blockade</th>
<th>ABD and Beta-adrenergic and Beta-adrenergic blockade</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of dogs</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>AP (mm Hg)</td>
<td>85 ± 4</td>
<td>79 ± 4</td>
<td>72 ± 4</td>
<td>67 ± 4</td>
<td>60 ± 4*</td>
</tr>
<tr>
<td>CO (ml/min)</td>
<td>2501 ± 173</td>
<td>2651 ± 199</td>
<td>2332 ± 172</td>
<td>2483 ± 254</td>
<td>2417 ± 182</td>
</tr>
<tr>
<td>TPR (mm Hg/L/minute)</td>
<td>34.6 ± 2.5</td>
<td>30.3 ± 3.3</td>
<td>31.9 ± 3.1</td>
<td>27.9 ± 3.6</td>
<td>25.3 ± 1.5</td>
</tr>
<tr>
<td>HR (beats/minute)</td>
<td>77 ± 4</td>
<td>134 ± 5*</td>
<td>134 ± 5*</td>
<td>93 ± 6*</td>
<td>101 ± 5*</td>
</tr>
</tbody>
</table>

*Different from β-adrenergic blockade, p < 0.05.
†Different from β-adrenergic and ganglionic blockades, p < 0.05.
ABD = arterial baroreceptor denervation; AP = arterial pressure, CO = cardiac output, HR = heart rate; TPR = total peripheral resistance.
FIGURE 1. A representative experiment showing the responses to administration of ANG II, 32 ng/kg, in an intact dog, after β-adrenergic blockade, before (A) and after (B) additional ganglionic blockade.

0.110 from 2.516 ± 0.183 L/minute, and decreased heart rate by 15 ± 2 from 78 ± 3 beats/minute. After addition of ganglionic blockade, administration of ANG II (32 ng/kg) increased mean arterial pressure by 48 ± 4 from 79 ± 9 mm Hg, increased total peripheral resistance by 23 ± 1 from 30 ± 4 mm Hg/L/minute, decreased cardiac output by 0.273 ± 0.049 L/minute from 2.685 ± 0.194 L/minute, and left heart rate unchanged from 133 ± 6 beats/minute. The control values for mean arterial pressure, total peripheral resistance, and cardiac output were not significantly different in the dogs after ganglionic blockade, while values for heart rate were significantly greater (p < 0.01). Ganglionic blockade increased the pressor responses by 28 ± 10%, reduced the vasoconstrictor responses by 37 ± 3%, and eliminated the heart rate response.

Linear regression analysis for the responses to ANG II in β-adrenergically blocked animals shows the pressor response (change in arterial pressure, ΔAP) = 31.6 log dose ANG II – 10.4 (r = 0.75) and the vasoconstrictor response (change in total peripheral resistance, ΔTPR) = 37.9 log dose ANG II – 20.5 (r = 0.91). After addition of ganglionic blockade ΔAP = 38.8 log dose ANG II – 10.9 (r = 0.70) and ΔTPR = 20.5 log dose ANG II – 8.4 (r = 0.86). Analysis of variance shows a significant difference in the slopes of the vasoconstrictor dose-response curve (p < 0.01), but not in the pressor dose-response curve. Analysis of covariance, assuming a common slope, shows a significant increase in the pressor (p < 0.01) and reduction in the vasoconstrictor (p < 0.01) dose-response curves (Figures 2 and 3). Thus pharmacological blockade of the autonomic nervous system significantly reduced the vasoconstrictor responses to ANG II.

Effects of α₁- and α₂-Adrenergic Blockades After Ganglionic Blockade

After β-adrenergic and ganglionic blockades in three dogs, administration of ANG II (32 ng/kg) increased mean arterial pressure by 55 ± 10 from 92 ± 2 mm Hg, increased total peripheral resistance by 27 ± 2 from 34 ± 7 mm Hg/L/minute, decreased cardiac output by 0.397 ± 0.180 from 2.857 ± 0.493 L/minute, and left heart rate unchanged from 131 ± 6 beats/minute. After addition of α₁-adrenergic blockade in these 3 dogs, administration of ANG II (32 ng/kg) increased mean arterial pressure by 56 ± 9 from 92 ± 3 mm Hg, increased total peripheral resistance by 28 ± 3 from 33 ± 4 mm Hg/L/minute, decreased cardiac output by 0.418 ± 0.150 from 2.861 ± 0.372 L/minute, and left heart rate unchanged from 123 ± 6 beats/minute. After addition of α₁- and α₂-adrenergic blockades in these three dogs, administration of ANG II (32 ng/kg) increased mean arterial pressure by 49 ± 7 from 109 ± 1 mm Hg, increased total peripheral resistance by 31 ± 3 from 40 ± 6 mm Hg/L/minute, decreased cardiac output by 0.609 ± 0.226 from 2.839 ± 0.435 L/minute, and left heart rate unchanged from 112 ± 5 beats/minute. Thus, after ganglionic blockade, the responses to ANG II were not
Effects of Arterial Baroreceptor Denervation

In six dogs with ABD, administration of ANG II (32 ng/kg) in the presence of β-adrenergic blockade increased mean arterial pressure by 88 ± 7 from 71 ± 3 mm Hg, increased total peripheral resistance by 54 ± 4 from 32 ± 3 mm Hg/L/minute, decreased cardiac output 0.400 ± 0.066 from 2.275 ± 0.176 L/minute, and increased heart rate by 12 ± 2 from 87 ± 5 beats/minute. The control values for mean arterial pressure, total peripheral resistance, cardiac output, and heart rate in dogs with ABD were similar to the values in dogs with an intact arterial baroreflex. In dogs with ABD, the pressor response was 138 ± 25% greater and the vasoconstrictor response to ANG II administration was 49 ± 15% greater than the responses in the animals with intact arterial baroreflexes. Furthermore, heart rate actually increased in the dogs with ABD.

Linear regression analysis of the responses to ANG II administration in dogs with ABD and β-adrenergic blockade show AAP = 65.1 log dose ANG II - 8.8 (r = 0.67) and ATPR = 47.0 log dose ANG II - 16.9 (r = 0.80). The slopes of pressor and vasoconstrictor dose-response curves of dogs with intact or chronically denervated baroreflexes were similar. Analysis of covariance showed a significant increase (p < 0.01) in both the pressor and vasoconstrictor responses to ANG II after ABD (Figures 2 and 3). Thus the arterial baroreflex potently inhibited the pressor and vasoconstrictor responses to ANG II.

Effects of α₁-Adrenergic Blockade After Arterial Baroreceptor Denervation

In five dogs with ABD and additional α₁-adrenergic blockade, administration of ANG II (32 ng/kg) increased mean arterial pressure by 56 ± 3 from 69 ± 5 mm Hg, increased total peripheral resistance by 28 ± 2 from 29 ± 4 mm Hg/L/minute, decreased cardiac output by 0.170 ± 0.037 from 2.410 ± 0.214 L/minute, and increased heart rate by 5 ± 1 from 92 ± 6 beats/minute in the presence of β-adrenergic blockade. After α₁-adrenergic receptor blockade, the control values for mean arterial pressure, total peripheral resistance, cardiac output, and heart rate were not significantly different from those in the dogs after ABD alone. The α₁-adrenergic receptor blockade reduced the arterial pressor responses by 31 ± 4% and the vasoconstrictor responses by 46 ± 4% in dogs after ABD.

Linear regression analysis showed ΔAP = 57.8 log dose ANG II - 29.9 (r = 0.94) and ΔTPR = 31.4 log dose ANG II - 18.9 (r = 0.91). Analysis of variance showed no significant difference in the slopes of the pressor or vasoconstrictor dose-response curves. Analysis of covariance showed that with the addition of α₁-adrenergic blockade, the pressor and vasoconstrictor dose-response curves were significantly less (p <
0.01) than in dogs with ABD and β-adrenergic blockade alone (Figures 4 and 5). Thus, in dogs with ABD, the indirect sympathetically mediated action of ANG II potently augmented the direct pressor and vasoconstrictor responses.

**Effects of Ganglionic Blockade After Arterial Baroreceptor Denervation**

In six dogs with ABD, β-adrenergic blockade and additional ganglionic blockade, administration of ANG II (32 ng/kg) increased mean arterial pressure by 53 ± 8 from 61 ± 4 mm Hg, increased total peripheral resistance by 27 ± 3 from 25 ± 1 mm Hg/L/minute, decreased cardiac output by 0.273 ± 0.049 from 2.685 ± 0.194 L/minute, and left heart rate unchanged from 101 ± 5 beats/minute. A representative response to ANG II (32 ng/kg) in a dog with ABD before and after ganglionic blockade is shown in Figure 6. With addition of ganglionic blockade, the control values for mean arterial pressure, total peripheral resistance, and heart rate were similar to the control values in the dogs with ABD and β-adrenergic blockade alone. Ganglionic blockade reduced the arterial pressor response by 39 ± 9%, reduced the vasoconstrictor responses by 50 ± 6%, and eliminated the heart rate response when compared with the responses in dogs with ABD and β-adrenergic blockade alone.

Linear regression analysis showed ΔAP = 54.3 log dose ANG II - 29.9 (r = 0.71) and ΔTPR = 29.7 log dose ANG II - 18.5 (r = 0.76). Analysis of variance showed that the slopes of the pressor and vasoconstrictor dose-response curves to ANG II in dogs with ABD and β-adrenergic blockade were similar, before or after ganglionic blockade. Analysis of covariance in these dogs showed a significant reduction (p < 0.01) in the pressor and vasoconstrictor dose-response curves with addition of ganglionic blockade. The dose-response curves for the pressor and vasoconstrictor responses to ANG II in dogs with ABD and ganglionic blockade were similar to the responses in the dogs with ABD and α-adrenergic blockade (Figures 4 and 5). Thus both ganglionic and α-adrenergic blockade reduced the pressor and vasoconstrictor responses to ANG II to a similar degree in dogs with and without ABD. Ganglionic blockade reduced the arterial pressor response by 39 ± 9%, reduced the vasoconstrictor responses by 50 ± 6%, and eliminated the heart rate response when compared with the responses in dogs with ABD and β-adrenergic blockade alone.

Effect of Ganglionic Blockade on the Renin-Angiotensin System

In five dogs with an intact arterial baroreflex, in the presence of β-adrenergic blockade the addition of ganglionic blockade increased peripheral venous plasma renin activity from 0.70 ± 0.24 to 1.91 ± 0.54 ng/ml/minute (p < 0.05). Thus ganglionic blockade induced a small, but statistically significant, increase in plasma renin activity.

**Effect of Converting-Enzyme Inhibition**

After converting-enzyme inhibition with captopril in two dogs, administration of ANG II (32 ng/kg) in
Figure 6. A representative experiment showing the responses to administration of ANG II (32 ng/kg) in a dog with ABD after β-adrenergic blockade, before (A) and after (B) addition of ganglionic blockade with hexamethonium. There is a marked reduction in both pressor and the vasoconstrictor responses to ANG II in this dog with addition of ganglionic blockade.

The presence of β-adrenergic blockade increased mean arterial pressure by 40 from 92 mm Hg, increased total peripheral resistance by 47 from 36 mm Hg/L/minute, decreased cardiac output by 1.073 from 2.718 L/minute, and decreased heart rate by 19 from 90 beats/minute. After addition of ganglionic blockade, administration of ANG II (32 ng/kg) increased mean arterial pressure by 62 from 91 mm Hg, increased total peripheral resistance by 30 from 34 mm Hg/L/minute, decreased cardiac output by 0.157 from 2.785 L/minute, and left heart rate unchanged from 151 beats/minute. Thus ganglionic blockade reduced the vasoconstrictor response to ANG II by 36%, even after pretreatment with captopril. In one additional dog with ABD, after converting-enzyme blockade, administration of ANG II (32 ng/kg) in the presence of β-adrenergic blockade increased mean arterial pressure by 100 from 70 mm Hg, increased total peripheral resistance by 53 from 24 mm Hg/L/minute, decreased cardiac output by 0.740 from 2.963 L/minute, and increased heart rate by 13 from 109 beats/minute. After addition of ganglionic blockade, administration of ANG II (32 ng/kg) increased mean arterial pressure by 55 from 50 mm Hg, increased total peripheral resistance by 19 from 17 mm Hg/L/minute, and left cardiac output unchanged from 2.881 L/minute and heart rate unchanged from 118 beats/minute. In this dog ganglionic blockade reduced the pressor response by 45% and the vasoconstrictor response by 64%, even after pretreatment with captopril. Thus slight differences in endogenous ANG II production in the dogs before and after ganglionic blockade did not account for the reduction in the vasoconstrictor responses to ANG II observed after addition of ganglionic blockade.

Discussion

It is well known that ANG II exerts potent direct and indirect effects on the cardiovascular system. Gildenberg and colleagues and Reid and co-workers, using carotid or vertebral arterial infusions of ANG II, demonstrated that there are at least two central nervous system receptor sites for ANG II, which elicit an indirect, sympathetically mediated vasoconstriction. Harle and associates demonstrated in rats that this indirect vasoconstrictor action of ANG II (administered into the lateral ventricles of the brain) can be eliminated by disruption of the neural pathways extending from the anteroventral third ventricle (AV3V) region. Furthermore, ANG II has been shown to induce a peripheral facilitation of norepinephrine release when sympathetic nerves were stimulated and augment peripheral responsiveness to norepinephrine in isolated vascular strips. In addition to the indirect vasoconstrictor actions of ANG II, a positive chronotropic effect
has been observed. This effect appears to be mediated by receptors in the area postrema in dogs and is particularly prominent in greyhounds. Lumbers and colleagues, using nerve recording techniques, and Lee and co-workers demonstrated that centrally administered ANG II reduces efferent vagal nerve output. A positive chronotropic effect has been observed in response to systemically administered ANG II in conscious dogs after ABD and is probably vagally mediated. Thus, in addition to the direct vasoconstrictor actions, systemically administered ANG II has been shown to elicit an indirect, autonomically mediated vasoconstriction and a positive chronotropic effect, which produce the observed pressor responses. In the intact animal, however, the arterial baroreflex complicates these responses to systemically administered ANG II by buffering both the pressor and the vasoconstrictor responses.

To determine the relative importance of the direct and indirect actions of ANG II, the responses to systemically administered ANG II were compared before and after central or reflex effects were eliminated by ganglionic blockade (i.e., only the direct effects of ANG II remained). To specifically eliminate the complicating influence of arterial baroreflex buffering, experiments were also conducted in conscious dogs with chronic ABD. The major finding of the present investigation is that, in dogs with ABD, the magnitude of the indirect vasoconstriction induced by ANG II is as important as its direct vasoconstrictor action. This conclusion was based on finding approximately twice the rise in total peripheral resistance in dogs with ABD before, as compared with after, ganglionic blockade. Moreover, these experiments were not complicated by the opposing effects of baroreflex buffering. This conclusion was further supported by experiments in dogs with ABD after \(\alpha\)-adrenergic blockade, in which total peripheral resistance responses to ANG II after \(\alpha\)-adrenergic receptor blockade were approximately 50% of the values before blockade. These experiments also indicate that the indirect vasoconstrictor actions are \(\alpha\)-adrenergically mediated. Our data demonstrate, using pharmacological blockades of the sympathetically mediated effects of ANG II, the potential importance of the indirect actions of ANG II. The findings of Ferrari and colleagues, who found a reduction in the pressor responses to ANG II in dogs after ablation of the area postrema, and of Falcon and associates, who found a reduction in the pressor responses to intraventricular injections of ANG II in conscious rats after chemical sympathectomy with 6-hydroxydopamine, support our conclusions. This potent, indirect augmentation by the sympathetic nervous system of the direct vasoconstrictor actions of ANG II may be particularly important in conditions in which the arterial baroreflex sensitivity is diminished, such as hypertension and heart failure.

Cline has suggested that ANG II may have a direct effect on peripheral \(\alpha\)-adrenergic receptors or may induce release of norepinephrine at the peripheral nerve terminals in the presence of ganglionic blockade. To study this possibility, responses to ANG II were compared before and after additional \(\alpha\)-adrenergic receptor blockade in dogs with ganglionic blockade. As no further reduction in the vasoconstrictor responses were observed, these potential mechanisms appear to play little role in the responses to ANG II in the physiological dose range used in this study. Although there appears to be a postganglionic facilitation of peripherally released norepinephrine by ANG II, a significant role for this action on the vasoconstrictor responses, independent of sympathetic neural conduct, seems unlikely for the doses of ANG II used in these experiments.

It was considered that the depressed responses to ANG II, observed after ganglionic blockade, could have been due to elevated baseline levels of endogenous ANG II, which were stimulated by the lower arterial pressure. To examine this possibility, we measured plasma renin activity in conscious, \(\beta\)-adrenergically blockaded dogs before and after ganglionic blockade. Although it was unlikely that the slight rise in plasma renin activity, and consequent endogenous ANG II production, could have substantially affected responses to ANG II, we still tested this possibility by examining responses to ANG II before and after ganglionic blockade in the presence of converting-enzyme inhibition where endogenous renin would not produce ANG II. Even in the presence of converting-enzyme inhibition by captopril, the reduction in the response to ANG II induced by ganglionic blockade in the presence of \(\beta\)-adrenergic blockade still occurred in dogs with or without ABD. Hence it is unlikely that the slight increase in endogenous ANG II production, induced by ganglionic blockade, was responsible for this observation. Thus the pressor and vasoconstrictor responses to ANG II after ganglionic blockade approximate its direct actions.

The positive chronotropic effect we observed in the dogs with ABD probably was due to the indirect, vagolytic actions of ANG II suggested by Lumbers and colleagues, as this chronotropic effect occurred in the presence of \(\beta\)-adrenergic blockade and was eliminated by ganglionic blockade. It is likely that this factor contributed to the greater augmentation of the arterial pressor response, as compared with the enhanced vasoconstrictor response, in dogs after ABD.

**Conclusion**

The present study provides evidence that the indirect, autonomically mediated pressor and vasoconstrictor actions of ANG II in the conscious animal are as important as the direct actions of ANG II; however, the relative importance of the direct and indirect actions of ANG II can differ in pathological states.

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ANGIOTENSIN: DIRECT VERSUS INDIRECT ACTIONS/Fujii and Vainer

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