Effect of Angiotensin II on Baroreceptor Reflex Control of Heart Rate in Conscious Baboons

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SUMMARY Studies of the baroreceptor–heart rate reflex were performed in four conscious, unrestrained male baboons to determine whether changes in circulating angiotensin II within the physiological range are associated with alterations in baroreceptor reflex sensitivity. With the animals on a high sodium intake, studies were performed before and during graded angiotensin II infusion (10 and 20 ng/kg/min). To separate effects on baroreceptor reflex function mediated by angiotensin II–induced increases in arterial pressure, these studies were repeated on a different day with simultaneous glyceryl trinitrate infusion to prevent increases in pressure during angiotensin II infusion. With the animals on a low sodium intake, studies were performed before and after angiotensin converting enzyme inhibition with captopril (1 and 5 mg/kg). These studies were also repeated on a separate day during simultaneous phenylephrine infusion to prevent a decrease in pressure with captopril. Reduction in sodium intake had no significant effect on arterial pressure, heart rate, or plasma volume, although arterial plasma angiotensin II concentration and renin activity were significantly increased (p<0.01). Infusion of angiotensin II produced a significant reduction in baroreceptor reflex sensitivity (p<0.01), and converting enzyme inhibition produced a significant increase (p<0.05). These effects accompanied significant increases and decreases in arterial angiotensin II concentration, respectively (p<0.01), but were independent of angiotensin II–related changes in arterial pressure. The data indicate that physiological variations in circulating angiotensin II have a direct effect on sensitivity of the baroreceptor–heart rate reflex. (Hypertension 10: 628–634, 1987)

KEY WORDS • angiotensin • captopril • baboon • baroreceptor reflex • converting enzyme inhibitor • heart rate

The role of angiotensin II (Ang II) in cardiovascular homeostasis extends beyond its direct vasoconstrictor effect and its stimulation of aldosterone secretion. There is a complex interaction between the renin-angiotensin system and the autonomic nervous system in control of the circulation that involves both direct sympathetic effects on renal renin release1 and positive feedback by Ang II on the autonomic nervous system at peripheral and central sites.2,3 In particular, Ang II may modulate baroreceptor reflex control of heart rate (HR). Reflex bradycardia is less pronounced in response to Ang II–induced increases in arterial pressure than with other vasoconstrictors,4,5 while treatment of hypertension by converting enzyme inhibition is not accompanied by the reflex tachycardia seen with direct vasodilator drugs.6–8 This study was undertaken to test whether changes in circulating Ang II within the physiological range alter baroreceptor reflex sensitivity in the conscious primate, independent of changes in arterial pressure.

Materials and Methods

Studies were performed in four locally bred, conscious, unrestrained adult male baboons (Papio hamadryas) weighing from 19.3 to 28.8 kg. The studies were reviewed and approved by the Animal Ethics Review Committee of Royal Prince Alfred Hospital. Arterial (single-lumen) and venous (double-lumen) catheters had been placed in the animals' iliac vessels 3 weeks before the studies commenced, using a surgical technique previously described.9 During studies, the catheters were connected to drug infusion and blood

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sampling devices by extension tubes in a flexible stainless steel tether. The baboons were conditioned to this study system for at least 1 month before operation, and all studies were performed with animals sitting quietly in an upright position. Systemic arterial pressure was recorded continuously using a pressure transducer (Model 4-327-I, Bell and Howell, Pasadena, CA, USA) positioned at the midthoracic level and connected to a chart recorder (Neotrace 600, Neomedix Systems, Sydney, Australia). HR was calculated from the pulse interval of the phasic arterial pressure tracings, recorded at a paper speed of 25 mm/sec.

The baroreceptor-HR reflex was assessed using a modification of the ramp method. The suitability of this method for assessment of responses to baroreceptor stimulation has been reported previously. Acute changes in systolic arterial blood pressure (SBP) were elicited by rapid intravenous injections of phenylephrine, 0.01 mg/kg, or glyceryl trinitrate, 0.01 mg/kg (Figure 1). Both phenylephrine and glyceryl trinitrate have been shown to have no effect on HR independent of their vasopressor and vasodilator effects, respectively. Two injections of each drug were given. HR was plotted against the preceding SBP on a beat-to-beat basis during acute changes in pressure. Slope of the regression line of HR on SBP, derived by the least-squares method, was used as an index of baroreceptor sensitivity (Figures 2 and 3).

The treatment protocol used to vary the baboons' circulating Ang II concentration is summarized in Table 1. This protocol entailed manipulation of dietary sodium intake to alter endogenous Ang II production. Exogenous Ang II or converting enzyme inhibition was then employed to raise or lower circulating Ang II. High sodium intake (approximately 100 mmol/day) was achieved by addition of sodium chloride to the usual diet of bread, nuts, primate pellets, and fruit containing 30 to 50 mmol of sodium per day. For low sodium intake (5–10 mmol/day) sodium-free bread replaced the baboons' usual daily bread ration. Each diet was given for 2 weeks before commencement of the studies. At the beginning of the low sodium diet, sodium loss was accelerated by administering furosemide (furosemide injection, B.P., Astra), 20 mg i.v. for 3 days. On high sodium intake, baroreceptor reflex studies were performed before and during steady state Ang II infusions. Exogenous Ang II (Hypertensin, CIBA) was infused at two doses, 10.0 and 20.0 ng/kg/min, for 30 minutes each (Treatment A). To separate direct effects of changes in Ang II from effects due changes in baseline arterial pressure, the studies were repeated on a separate day with simultaneous infusion of glyceryl trinitrate adjusted to maintain arterial pressure at the precaptopril levels (Treatment D). Thus, the effects of changing circulating Ang II concentration on the baroreceptor-HR reflex were assessed with four different study treatments, the order of which was systematically varied (see Table 1). Within each treatment baroreceptor reflex experiments were performed according to a fixed time protocol to avoid experimenter bias.

To compare the effects of high and low sodium intake, the following measurements were also made: plasma sodium, potassium, and creatinine concentrations; urine sodium/creatinine ratios; plasma volume and hematocrit; plasma renin activity (Gammacoat plasma renin activity radioimmunoassay kit, Travenol Laboratories, Morton Grove, IL, USA); and plasma Ang II concentration (Ang II radioimmunoassay kit, Buhlmann Laboratories, Basel, Switzerland). Further Ang II measurements were made on plasma samples collected during all manipulations of circulating Ang II.

Effects of high and low sodium intake were compared using analysis of variance or, for plasma volume and plasma electrolytes, the Mann-Whitney test. The effects of Ang II infusion or converting enzyme inhibi-
Results

Effects of Dietary Sodium Intake

Urinary sodium/creatinine ratio was significantly reduced during the low sodium intake (Table 2). Plasma sodium concentration was also reduced, while plasma potassium and creatinine were unchanged. There were no significant changes in plasma volume or hematocrit. Plasma renin activity and plasma Ang II concentration increased significantly during the low sodium intake. There was no significant difference between baseline baroreceptor reflex sensitivity during high and low sodium intake (-1.06 ± 0.08 vs -1.04 ± 0.08 beats/min/mm Hg, mean ± SEM). Baseline SBP and HR were not altered by changes in dietary sodium intake (Tables 3 and 4).

Effects of Exogenous Ang II During High Sodium Intake

Ang II infusion produced similar dose-related elevations in plasma Ang II concentration (p < 0.01) during
Table 3. Effects of Exogenous Ang II During High Sodium Intake

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment A ($n = 4$)</th>
<th>Treatment B ($n = 4$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Baseline</td>
</tr>
<tr>
<td></td>
<td>Ang II (1)</td>
<td>Ang II + GTN (1)</td>
</tr>
<tr>
<td></td>
<td>Ang II (2)</td>
<td>Ang II + GTN (2)</td>
</tr>
<tr>
<td>Plasma Ang II (pg/ml)</td>
<td>15 ± 9</td>
<td>14 ± 5</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>120 ± 5</td>
<td>130 ± 8</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>125 ± 14</td>
<td>122 ± 12</td>
</tr>
<tr>
<td></td>
<td>137 ± 38</td>
<td>115 ± 29</td>
</tr>
<tr>
<td>Baroreceptor reflex slope (beats/min/mm Hg)</td>
<td>-1.16 ± 0.12</td>
<td>-0.97 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>-0.86 ± 0.03</td>
<td>-0.85 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SEM. $r$ = correlation coefficient for linear regression analyses.

Treatment A = high sodium intake with exogenous Ang II; Treatment B = Treatment A plus glyceryl trinitrate (GTN).

1 = Ang II, 10 ng/kg/min; 2 = Ang II, 20 ng/kg/min.

Table 4. Effects of Converting Enzyme Inhibition with Captopril During Low Sodium Intake

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment C ($n = 4$)</th>
<th>Treatment D ($n = 4$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Baseline</td>
</tr>
<tr>
<td></td>
<td>Captopril (1)</td>
<td>Captopril + PHE (1)</td>
</tr>
<tr>
<td></td>
<td>Captopril (2)</td>
<td>Captopril + PHE (2)</td>
</tr>
<tr>
<td>Plasma Ang II (pg/ml)</td>
<td>32 ± 10</td>
<td>32 ± 11</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>123 ± 4</td>
<td>122 ± 8</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>132 ± 18</td>
<td>132 ± 10</td>
</tr>
<tr>
<td></td>
<td>137 ± 16</td>
<td>140 ± 11</td>
</tr>
<tr>
<td>Baroreceptor reflex slope (beats/min/mm Hg)</td>
<td>-1.08 ± 0.13</td>
<td>-1.01 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>-0.84 ± 0.04</td>
<td>-0.88 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>-1.39 ± 0.16</td>
<td>-1.44 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>-0.88 ± 0.04</td>
<td>-0.87 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SEM. $r$ = correlation coefficient for linear regression analyses.

Treatment C = low sodium intake with captopril; Treatment D = Treatment C plus phenylephrine (PHE).

1 = captopril, 1 mg/kg; 2 = captopril, 5 mg/kg.

Treatments A and B (Figure 4; see Table 3). During Treatment A, there were dose-related rises in SBP ($p < 0.01$), without any significant change in HR. Figure 2 shows examples of baroreceptor reflex curves before and during Ang II infusion in a sodium-replete animal. Ang II caused the baroreceptor reflex response to be shifted to the right, with a dose-related fall in sensitivity ($p < 0.01$). With Treatment B, when glyceryl trinitrate was given to oppose the hypertensive action of Ang II, SBP did not change significantly. Under these conditions Ang II still produced a dose-related reduction in baroreceptor reflex sensitivity ($p < 0.01$) that was not significantly different from that seen with Treatment A.

Effects of Converting Enzyme Inhibition During Low Sodium Intake

Converting enzyme inhibition produced similar significant falls in plasma Ang II concentration ($p < 0.01$) during Treatments C and D (Figure 5; see Table 4). During Treatment C, converting enzyme inhibition was associated with small falls in SBP. There was no significant change in HR. Figure 3 shows examples of baroreceptor reflex curves before and after converting enzyme inhibition in a sodium-depleted animal. Converting enzyme inhibition caused the baroreceptor reflex response to be shifted to the left, with a significant increase in sensitivity ($p < 0.05$). During Treatment D, when the hypotensive effect of converting enzyme inhibition was prevented with phenylephrine, SBP did not change from baseline. However, the increase in baroreceptor reflex sensitivity was still seen ($p < 0.05$) and was not significantly different from that elicited by Treatment C.

Discussion

In conscious baboons exogenous Ang II produced a dose-related reduction in baroreceptor reflex sensitivity. Conversely, reducing endogenous Ang II with a converting enzyme inhibitor produced an increase in reflex sensitivity. These effects were seen with small changes in circulating Ang II concentration and were independent of changes in blood volume and arterial pressure.

Previous studies in monkeys, sheep, rabbits, and dogs have suggested this effect of exogenous Ang II. Reports that converting enzyme inhibitors increase baroreceptor reflex sensitivity complement such data. However, the complex integration of mechanisms for blood pressure, sodium homeostasis, and volume homeostasis has made interpretation of previous studies difficult, and a physio-
logical role for Ang II in modulating baroreceptor reflex function therefore remains uncertain. For example, when circulating Ang II concentration is altered, the baroreceptors may be reset in the direction of the resultant blood pressure change. Also, when endogenous Ang II production is altered by changes in dietary sodium, baroreceptor reflex function may be affected by changes in extracellular fluid sodium concentration or by changes in circulatory volume. In some studies only the effects of pharmacological Ang II doses have been examined, and in others the effects of converting enzyme inhibition have not been related to circulating Ang II concentration. Finally, extrapolating animal data to humans may be difficult because of species variation in responses to sodium depletion and converting enzyme inhibition, while in nonhuman primates the use of physical restraint for experiments may alter autonomic function.

In this study, baroreceptor reflex function was assessed in conscious, unrestrained adult baboons in both sodium-replete and sodium-depleted states. The study was designed to separate direct effects of Ang II on baroreceptor reflex sensitivity from indirect effects due to changes in arterial pressure. With all study treatments, circulating Ang II concentration was measured directly. Although there is little available information on Ang II levels in baboons, in this study Ang II was administered at physiological doses that achieved circulating Ang II concentrations within the range previously reported in anesthetized sodium-depleted hamadryas baboons. In the present study of conscious animals, the first Ang II infusion achieved plasma levels similar to those elicited during mild sodium depletion. Plasma volume and hematocrit were measured, and no significant changes were produced by the diets used to alter endogenous Ang II production.

In the sodium-replete state, graded infusions of Ang II produced dose-dependent reductions in sensitivity of the baroreceptor-HR reflex. This effect was independent of Ang II-induced changes in arterial pressure. The findings confirm earlier reports of altered baroreceptor reflex control of HR by exogenous Ang II. Conversely, in the sodium-depleted state, reductions in circulating Ang II by converting enzyme inhibition were associated with increased baroreceptor reflex sensitivity. This increase in reflex sensitivity agrees with reports of the effect of converting enzyme inhibition in humans, but not in the dog. In conscious dogs, sodium depletion per se had no effect on baroreceptor reflex function, but after converting enzyme inhibition the baroreceptor reflex curve was shifted to the left, as we have found. However, in contrast to our study, no effect on baroreceptor reflex sensitivity was seen in the dog. This discrepancy may be due to the different methods used to analyze baroreceptor reflex responses. Hatton et al.
calculated reflex sensitivity from linear regression of heart period on diastolic blood pressure. When heart period is used to construct baroreceptor reflex curves, the responses to rises and falls in arterial pressure are asymmetrical.\(^{32}\) In the study with dogs,\(^{31}\) this problem was overcome by restricting the analysis to a small portion of the baroreceptor reflex curve. In our study with baboons, HR responses to a wide range of arterial pressure changes were obtained, and analysis using HR gave a single regression line describing symmetrical baroreceptor reflex responses to rises and falls in arterial pressure over this wide range. There are no strong reasons for preferring heart period over HR for baroreceptor reflex analysis.\(^{33, 34}\) Studies in anesthetized animals have shown that the input-output relationship for arterial pressure and HR, like that for the baroreceptors themselves, is sigmoidal.\(^{35}\) The use of drug injections in conscious, intact animals makes it difficult to achieve the large changes in arterial pressure necessary to define this sigmoidal relationship, particularly as severe hypotension may cause syncope and high arterial blood pressures may be associated with marked irregularities in HR.\(^{31}\) Moderate changes in arterial pressure around the operating point produce HR responses that are well described by a linear model,\(^{36}\) as shown in this study by the correlation coefficients obtained. The use of a wider range of arterial pressure changes in our study may account for the identification of a significant increase in reflex sensitivity after converting enzyme inhibition, which was not found in dogs,\(^{31}\) but has been reported in humans.\(^{6, 7}\)

This study of intact baboons has not localized the site of action for circulating Ang II on the baroreceptor-HR reflex arc. Ang II is considered unlikely to have any direct effects on baroreceptors, since direct application of Ang II into the wall of the carotid sinus did not produce any systemic cardiovascular response\(^ {37}\) and no cardiovascular effects were seen with intracarotid injections of Ang II at doses that produced pressor responses when given into a vertebral artery.\(^ {38}\) Lumbers et al.\(^ {30}\) measured action potentials in single baroreceptor fibers dissected from carotid sinus nerves in dogs and found no difference in discharge frequency when injections of Ang II or phenylephrine or inflation of an aortic balloon cuff was used to increase arterial pressure, indicating that Ang II had no effect on the baroreceptors or afferent nerve fibers independent of its pressor action. Ang II has no direct chronotropic effect on the heart.\(^ {3}\) Ang II has been reported to have central nervous system actions that affect both sympathetic and parasympathetic outflow.\(^ {39-41}\) Studies in sheep\(^ {12, 29}\) and dogs\(^ {29}\) have shown that pharmacological effects of Ang II on control of HR reflect altered parasympathetic activity. Ang II has been shown to cause a central, dose-dependent reduction in parasympathetic tone in sheep,\(^ {29}\) and there are reports of enhanced parasympathetic activity after converting enzyme inhibition in humans.\(^ {8}\) Studies with vertebral artery infusions of Ang II suggest that the area postrema may be the central site for this effect of Ang II.\(^ {42}\) In addition, Ang II may have a peripheral inhibitory effect on efferent cardiac vagal activity.\(^ {43}\)

Ang II contributes to the interactions between the autonomic nervous system and the renin-aldosterone system in blood pressure control by a variety of mechanisms.\(^ {2, 3, 39, 41}\) This study was designed to examine whether small changes in circulating Ang II have an effect on the baroreceptor reflex sensitivity of intact primates that is independent of its effects on blood pressure. The positive findings of an acute reduction in reflex sensitivity when circulating Ang II was increased, and the converse when Ang II was reduced, suggest a further physiological effect of Ang II as a determinant of baroreceptor reflex sensitivity in the primate.

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