Angiotensin-Converting Enzyme Inhibitor Resets Baroreceptor Reflexes in Conscious Dogs

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SUMMARY The present experiments investigate whether the absence of tachycardia during lowering of blood pressure (BP) with an angiotensin-converting enzyme inhibitor (CEI) in salt-depleted dogs is due to an alteration in the activity of the baroreflex. Baroreflex activity was measured after pharmacological manipulation of BP using intravenous nitroglycerine or phenylephrine, and the heart period (R-R interval) relative to the arterial pressure pulse was recorded. The slope of the relationship between BP and R-R interval is a measure of the sensitivity of the baroreceptor reflex and displacement of the line indicates a change in the setpoint of BP. On normal sodium diet, the sensitivity and setpoint of the baroreflex were unaltered by the nonapeptide CEI given both intravenously and into a lateral cerebral ventricle. During salt depletion, however, intravenously but not centrally administered CEI altered the setpoint of the baroreflex without modifying the sensitivity.

The alteration in the setpoint that occurred following intravenous CEI in the salt-depleted dog could well account for the fact that the fall in BP induced by CEI does not cause reflex tachycardia. These results indicate that circulating but not brain angiotensin II is essential for the maintenance of baroreflex function during sodium depletion and provide further evidence for the important interactions between angiotensin and the autonomic nervous system. (Hypertension 3: 676-681, 1981)

KEY WORDS • sodium depletion • plasma renin activity • converting enzyme inhibitor • angiotensin II • baroreflex function

Blood pressure (BP) is maintained by the autonomic nervous system and by angiotensin, and it has been shown in isolated preparations that the two systems interact. There must be a complex interplay in the maintenance of homeostasis as these systems are brought into action, as, for example, during sodium depletion, which itself activates the renin angiotensin system. The availability of inhibitors of angiotensin II (AII) has made it possible to delineate the role of the two systems under these conditions by examining the characteristics of the hypotensive response to the sudden cessation of AII action. In the sodium-depleted dog, we have shown that the fall in BP was proportional to the prevailing level of plasma renin activity (PRA). With both a converting enzyme inhibitor (CEI synthetic nonapeptide) and Sar\(^1\)-Ala\(^4\) AII, it was noticed that the tachycardia and increase in cardiac output that would normally be expected to accompany a fall in BP did not occur. This has been reported by others in the dog and in man.

A possible explanation for the blunted reflex is an interference with baroreflex function at a central site involving AII, because infusion of AII into a vertebral artery leads to tachycardia in the presence of peripheral angiotensin blockade. When AII is administered centrally it augments the reflex vascular response to changes in BP in the dog. We have recently reported that the sympathetically-mediated vasoconstrictor reflex response to reduction in central blood volume is impaired when these inhibitors are used, suggesting that angiotensin may interact physiologically in a subtle way with the sympathetic nervous system to maintain BP.

To examine further the interaction between the two systems we have investigated the action of angiotensin on reflexes by studying the baroreceptor reflex in conscious dogs to determine whether the absence of tachycardia as CEI lowers BP during sodium depletion could be due to alteration in the function of the baroreflex. The study was designed to investigate whether this action of the inhibitor was peripheral by interfering with circulating AII or a direct central action involving the brain iso-renin angiotensin system.
Methods

Experiments were performed in conscious trained male beagle dogs weighing between 12 and 15 kg. Some 2 to 3 weeks prior to the experiments, indwelling vinyl catheters were implanted in the left common carotid and jugular vein under thiopentone/halothane anesthesia. These catheters were exteriorized at the back of the neck and filled with heparinized saline 5000 IU/ml (Pularin-Boots) when not in use. Indwelling lateral cerebroventricular cannulas were also stereotactically implanted at this time in some animals (coordinates AP 13; L9); the depth was determined by cerebrospinal fluid (CSF) pressure monitoring. The dogs were trained to lie quietly in a padded box for at least 30 minutes prior to the experiment. Aortic BP was measured via a pressure transducer (Bell and Howell 4-422) and displayed continuously on a direct writing oscillograph (Devices M19). An electrocardiogram (ECG) was recorded from limb leads after suitable amplification using a preamplifier (Series 3542). From this the heart period (R-R interval) was measured. Heart rate derived electronically from the pressure pulse was displayed continuously. The PRA was measured in arterial blood by the method of Haber et al. with modification as described previously.

Measurement of Baroreflex Activity

Baroreflex function was assessed by pharmacological manipulation of BP using phenylephrine 10–40 μg/kg i.v. (Sigma) to increase pressure and nitroglycerine 10–40 μg/kg i.v. (McCarthys) to lower it according to the method of Smyth et al. Figure 1 is a diagram of an idealized baroreflex function curve constructed by plotting heart period against arterial pressure at any instant. Because the curve is sigmoid, the linear portion of the curve can be used to study the activity of the reflex. The slope of the line by linear regression analysis is used as an indication of the sensitivity or gain of the reflex. A reduction in this slope may explain why the heart rate does not rise as BP is reduced by CEI. Alternatively, a shift in the line to the left, resulting from a change in the "setpoint" of the BP control to a lower value but with normal sensitivity, would also explain the absence of tachycardia.

In our experiments, when the heart period was plotted against the preceding diastolic pressure on a beat-to-beat basis as the BP increased and decreased with phenylephrine and nitroglycerine, it was clear that the leveling of the baroreflex curve at high pressures was not obtainable. A marked irregularity in heart period, similar to pronounced sinus arrhythmia, associated with the low heart rates, occurred and resulted in a large scatter of data points. Analysis of the data points, however, did not include that part of the curve in which this phenomenon was observed since it was outside the pressure range reported here and so did not influence the measurement of baroreflex activity. Linear regression analysis was carried out on points in the range ± 20 mm Hg of the resting diastolic pressure at the time of measurement, which within each animal showed a highly significant correlation. The sensitivity of the reflex was then determined by the slope of this line; as a measure of setpoint, the diastolic pressure at a fixed heart period of 700 msec was measured (corresponding to 86 beats/min, approximate normal resting heart rate).

\[ \text{Heart period} \]

\[ B.P. \]

\[ \triangle \text{Sensitivity} \]

\[ B.P. \]

\[ \triangle \text{"Set-point"} \]

\[ B.P. \]

FIGURE 1. Diagram of an idealized baroreflex curve (top) showing how a change in sensitivity (center) or a change in setpoint (bottom) could explain how a fall in BP can occur without inducing a reflex tachycardia.
Experimental Protocol

The PRA was elevated by dietary sodium depletion and intermittent use of a diuretic over 6 to 8 days as previously described. In a total of nine dogs, CEI (0.5 mg/kg) (synthetic nonapeptide, obtained from Beckman, structurally identical to SQ 20,881) was administered before (5 dogs) and after 6-8 days (9 dogs) of sodium depletion. Baroreflex function curves were constructed immediately before and 10 to 30 minutes after these doses of CEI at which times steady-state conditions had been restored. Arterial blood samples were taken immediately before CEI treatment for measurement of PRA. In five animals, CEI 0.5 mg/kg was also administered by the intracerebroventricular (i.c.v.) route before or during sodium depletion (Day 6 or 8). These central doses were separated from those given by the i.v. route by at least 24 hours.

Linear regression analysis was used to establish the relationship between heart period (R-R interval) and the changes in diastolic BP. Mean data are given with the standard error (SEM), and Student's paired t test has been used to determine the effect of the inhibitor.

Results

Figure 2 shows baroreflex curves from an individual animal obtained in the sodium replete state prior to and 10-30 minutes after i.v. administration of CEI (0.5 mg/kg), which itself did not lower the BP (table 1). As the BP rose with phenylephrine or fell with nitroglycerine, the R-R interval (msec) was plotted against the preceding diastolic pressure; each point represents a single heart beat. There was no obvious change in baroreflex function after CEI in this animal nor in four others similarly treated; thus, CEI did not affect baroreflex activity in the sodium replete state.

In the same animal during sodium depletion (fig. 3), the baroreflex function curve was seen to be shifted to the left after i.v. administration of the inhibitor, which now lowered the BP in this and eight other animals (table 1). This shift appeared to be a parallel one without a change in slope; this was also the case in the eight other similarly treated animals.

The effect of CEI given centrally (0.5 mg/kg i.c.v.) was also recorded in sodium-deplete animals. There was no change in BP after this dose (table 1). No change in the baroreflex function curve in this (fig. 4) and four other animals was seen nor was there any change in the baroreflex curve when this i.c.v. dose was administered to animals prior to sodium depletion.

The slopes and the setpoints for the combined data from all the animals are shown in table 2. In each individual animal, the correlation coefficient for the relationship between heart period (R-R interval) and the changes in diastolic BP. Mean data are given with the standard error (SEM), and Student's paired t test has been used to determine the effect of the inhibitor.
TABLE 1. Effect of Converting Enzyme Inhibitor (CEI), 0.5 mg/kg, on Resting Hemodynamic Parameters in Conscious Dogs Before and After Sodium Depletion (6-8 Days)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Route</th>
<th>n</th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
<th>HR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium replete</td>
<td>i.v.</td>
<td>5</td>
<td>136 ± 5.8</td>
<td>82 ± 3.0</td>
<td>78 ± 3.9</td>
<td>134 ± 5.1</td>
<td>78 ± 3.0</td>
<td>79 ± 5.3</td>
</tr>
<tr>
<td>Sodium deplete</td>
<td>i.v.</td>
<td>9</td>
<td>141 ± 5.8</td>
<td>87 ± 4.1</td>
<td>91 ± 9.3</td>
<td>124 ± 6.0†</td>
<td>62 ± 2.9†</td>
<td>108 ± 9.1*</td>
</tr>
</tbody>
</table>

i.v. = intravenous administration; i.c.v. = intracerebroventricular administration; n = number of dogs; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate. Data given are mean ± SEM.

*p < 0.01, significantly different from pre-CEI value.
†p < 0.001, significantly different from pre-CEI value.

**FIGURE 3.** Effect of intravenous CEI on the baroreflex curve in a conscious dog following sodium depletion. Phenylephrine and nitroglycerine were injected intravenously to alter BP and the corresponding changes in heart period (R-R interval) were measured. Closed circles = measured before CEI (diastolic pressure at rest = 95 mm Hg). Open circles = measured 10-30 minutes after an intravenous injection of 0.5 mg/kg of CEI (diastolic pressure at rest = 70 mm Hg).

**FIGURE 4.** Effect of intracerebroventricular (ICV) CEI on the baroreflex curve in a conscious dog following sodium depletion. Phenylephrine and nitroglycerine were injected to alter BP, and the corresponding changes in heart period (R-R interval) were measured. Closed circles = measured before CEI (diastolic pressure at rest = 99 mm Hg). Open circles = measured 10-30 minutes after an i.c.v. injection of 0.5 mg/kg of CEI (diastolic pressure at rest = 101 mm Hg).
Discussion

This study provides evidence that an inhibitor of angiotensin-converting enzyme interferes with baroreflex function in the sodium-depleted dog. Sodium depletion itself, which was an effective stimulus for renin release, did not result in a significant change in the function of the baroreflex. In these experiments, nitroglycerine produced a tachycardia of 60–90 beats/min as it lowered the BP in the salt-replete or salt-depleted dog, while CEI for a similar fall in BP in the salt-depleted dog caused only a small tachycardia of approximately 15 beats/min, as we have previously reported.10 Thus, during salt depletion the baroreceptor reflex is intact but CEI fails to elicit the reflex as it lowers BP. Since the inhibitor produced a change in baroreflex control only during sodium depletion, this suggests that angiotensin was involved in the maintenance of normal baroreflex function in the salt-depleted dog. It did this by changing the setpoint of the reflex without changing its sensitivity. This demonstrates an additional role for All in maintaining the BP which is complementary to its action as a direct peripheral vasoconstrictor agent.

CEI administered centrally did not lower the BP nor did it affect baroreflex function, suggesting that the brain isorenin-angiotensin system was not involved in the action. However, a central action involving structures situated outside the blood-brain barrier (e.g., area postrema) is possible since these are associated with medullary areas, in particular the nucleus tractus solitarius, which itself is involved in BP control. In addition, infusion of angiotensin into a vertebral artery leads to tachycardia in the sodium-depleted dog treated with CEI. In this context it was interesting to note that the bradycardia induced by phenylephrine is believed to be predominantly mediated via the vagus nerve, and it has recently been shown that All inhibits vagal efferent nerve activity as it raises BP.11 In another study the angiotensin antagonist Sar^Thr8 All produced a reduction in heart rate which, although not related to angiotensin levels, had a large parasympathetic component. Therefore, CEI, by inhibiting the formation of angiotensin, may enhance vagal influence over the heart most likely at a central rather than peripheral site.

It is likely that an action on the sympathetic nervous system is also involved in the shift of the baroreceptor reflex curve produced by CEI since the tachycardia elicited by nitroglycerine is believed to depend on this system. It is well known that angiotensin can increase BP and heart rate by a central action involving the sympathetic nervous system and CEI could interfere with these actions of All. The observed parallel shift of the whole baroreflex function curve, which might be expected to involve both sympathetic and parasympathetic components in a similar manner, suggests that a more fundamental action of CEI exists on a controlling mechanism that equally affects both systems at some common point. The most likely explanation is that a central site of action is involved which has close association with areas of BP control in the medulla and is sensitive to blood-borne All.

Although we have no direct evidence to determine the site of action of CEI on these reflexes, an action of CEI on baroreceptor input seems unlikely since there is evidence derived from acute experiments that show that All does not appear to modify carotid sinus afferent nerve activity as it increases BP. In other experiments, All nevertheless impaired the reflex vasodepressor and bradycardia effects induced by stimulation of the carotid sinus nerve isolated from its normal sensory input. It must be recognized that these latter experiments involved the acute elevation of angiotensin levels by bolus injection. In our experiments, angiotensin was elevated chronically by sodium depletion, and in view of the observation that baroreflex function appeared normal during sodium depletion, this indicates that chronically elevated angiotensin may be important in the maintenance of the normal setpoint of the baroreceptors themselves in the control of BP without affecting the sensitivity. The present study cannot distinguish between this latter possibility and the other actions of angiotensin already

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**Table 2. Effect of Converting Enzyme Inhibitor (CEI) on Baroreceptor Function in Conscious Dogs Before and After Sodium Depletion**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Route</th>
<th>n</th>
<th>Pre CEI Slope (msec/mm Hg)</th>
<th>Post CEI Slope (msec/mm Hg)</th>
<th>Pre CEI Diastolic pressure (mm Hg) (Heart period 700 msec)</th>
<th>Post CEI Diastolic pressure (mm Hg) (Heart period 700 msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>i.v.</td>
<td>5</td>
<td>20.5 ± 2.3</td>
<td>19.4 ± 2.8</td>
<td>88 ± 3.3</td>
<td>86 ± 5.6</td>
</tr>
<tr>
<td>replete</td>
<td>i.c.v.</td>
<td>5</td>
<td>24.2 ± 2.6</td>
<td>24.4 ± 2.3</td>
<td>91 ± 4.0</td>
<td>88 ± 3.0</td>
</tr>
<tr>
<td>Sodium</td>
<td>i.v.</td>
<td>9</td>
<td>19.7 ± 2.6</td>
<td>23.3 ± 4.3</td>
<td>96 ± 4.7</td>
<td>73 ± 4.5*</td>
</tr>
<tr>
<td>deplete</td>
<td>i.c.v.</td>
<td>5</td>
<td>17.7 ± 2.5</td>
<td>18.9 ± 3.6</td>
<td>95 ± 7.4</td>
<td>95 ± 8.9</td>
</tr>
</tbody>
</table>

The slope of the line in msec/mm Hg is a measure of the sensitivity of the reflex, and the diastolic pressure at a heart period of 700 msec is an indication of the setpoint of the reflex. i.v. = intravenous administration; i.c.v. = intracerebroventricular administration; n = number of dogs. Data given are mean ± SEM. *p < 0.01 significantly different from pre-CEI value.
referred to which, when interfered with, may result in a lack of tachycardia as CEI lowers the BP during sodium depletion.

Our work and that of others therefore shows that angiotensin is involved in the maintenance of normal homeostatic reflex activity during sodium depletion, an action that is complementary to, and as important as, its direct vasocostrictor action. CEI, by unmasking the changes brought about by sodium depletion, then interferes with this reflex, causing a change in the setpoint of BP control without affecting the sensitivity of the reflex, most probably at a central site that is accessible to circulating angiotensin. These effects of CEI may play an important part in the anti-hypertensive action of these agents.

References

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