Angiotensin II modulates cardiovascular autonomic control in the absence of baroreflex loading

J C Vaile, J Fletcher, W A Littler, J H Coote, J N Townend

Abstract

Objective—To investigate the effects of angiotensin II in the absence of baroreflex activation.

Design—Ten healthy male volunteers were studied in a single blind, randomised, crossover study of heart rate variability during intravenous angiotensin II infusion (5–20 ng/kg/min) compared with a control pressor infusion of phenylephrine (0.7–2.8 µg/kg/min). Each infusion was titrated to increase mean blood pressure by 20 mm Hg; sodium nitroprusside was then infused simultaneously to restore blood pressure to baseline values.

Results—During concomitant angiotensin II (AII) and sodium nitroprusside (SNP) infusion, the mean (SD) RR interval (864 (117) ms) was significantly shorter than during phenylephrine (PE) and sodium nitroprusside infusion (1057 (163) ms), and was significantly shorter than at baseline (999 (164) ms), despite comparable levels of blood pressure. Values of high frequency heart rate variability measured in the time and frequency domains were significantly lower during AII/SNP infusion than during PE/SNP; percentage of successive RR interval differences exceeding 50 ms, 30(16)% vs 57 (21)%; root mean square of successive RR interval differences, 63 (39) vs 90 (40) ms; high frequency power 0.48 (0.19) vs 0.66 (0.26) nu.

Conclusions—When the pressor response is controlled by sodium nitroprusside, angiotensin II infusion is associated with tachycardia. Analysis of heart rate variability suggests that this reflects inhibition of cardiac vagal activity.

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Keywords: angiotensin II; heart rate variability; autonomic nervous system; parasympathetic nervous system

There is strong evidence that impaired cardiac autonomic function is associated with an adverse prognosis after myocardial infarction and in heart failure, and animal experiments have shown that angiotensin II has direct effects on both vagal and sympathetic nervous control. Inhibition of cardiac vagal activity by circulating angiotensin II is mediated both centrally and peripherally. There is also evidence of facilitatory effects on sympathetic nervous activity. Most of this work has been performed in anaesthetised animals, making it difficult to draw conclusions which can be applied reliably to humans.

Investigation of the effect of angiotensin II on cardiac autonomic activity in intact conscious animals and in humans is hampered by baroreflex activation caused by its powerful pressor effect. One approach has been to control for the pressor effect by comparing the results of angiotensin II infusion with those resulting from a control pressor agent such as phenylephrine. In dogs, little increase in vagal activity occurred during angiotensin II infusion despite the rise in arterial pressure; in contrast there was a marked increase in vagal activity during control pressor infusion of phenylephrine. In humans, the baroreceptor heart rate reflex is similarly inhibited by angiotensin II. This does not appear to result from facilitation of sympathetic tone, as tritiated noradrenaline kinetic studies showed no significant difference in sympathetic activity during angiotensin II infusion and during an equipressor infusion of phenylephrine. In a previous study using analysis of heart rate variability we showed that the primary cause of the reduced heart rate response to pressor doses of angiotensin II was an inhibitory action on cardiac vagal activity. We postulated that angiotensin II directly inhibits cardiac vagal activity rather than having any specific action on the baroreflex. Such an effect should therefore be demonstrable even in the absence of baroreflex activation.

In this study we investigated the effect of an angiotensin II infusion on cardiac vagal activity in humans while attempting to prevent pressor stimulation of the arterial baroreceptors by simultaneous infusion of the vasodilator sodium nitroprusside. Cardiac vagal activity was determined by analysis of heart rate variability in the time and frequency domains and control experiments were performed using phenylephrine and sodium nitroprusside infusions.

Methods

Ten male subjects with a mean age of 19 years (range 18 to 23 years) were studied in a single
Table 1  Blood pressure, RR interval, and RR variability at baseline, before infusion of either drug

<table>
<thead>
<tr>
<th>Phenylephrine</th>
<th>Angiotensin II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bood pressure (mm Hg)</td>
<td>87 (8)</td>
</tr>
<tr>
<td>RR interval (ms)</td>
<td>966 (142)</td>
</tr>
<tr>
<td>SDNN (ms)</td>
<td>97 (46)</td>
</tr>
<tr>
<td>IQRDN (ms)</td>
<td>118 (37)</td>
</tr>
<tr>
<td>IQRDS (ms)</td>
<td>115 (57)</td>
</tr>
<tr>
<td>RMSSD (ms)</td>
<td>104 (59)</td>
</tr>
<tr>
<td>pNN50 (%)</td>
<td>59 (16)</td>
</tr>
<tr>
<td>Total power (ms²)</td>
<td>6996 (3449)</td>
</tr>
<tr>
<td>LF power-abs (ms²)</td>
<td>1269 (1090)</td>
</tr>
<tr>
<td>LF power (nu)</td>
<td>0.28 (0.24)</td>
</tr>
<tr>
<td>HF power-abs (ms²)</td>
<td>3378 (2181)</td>
</tr>
<tr>
<td>HF power (nu)</td>
<td>0.63 (0.21)</td>
</tr>
</tbody>
</table>

Values are mean (SD). p values not significant for any parameter.

The power of each underlying frequency was calculated using algorithm 16 with a model order between 8 and 30, and assuming 1% of ectopic beats; when ectopic beats were detected before analysis to exclude ectopic and artefact signals. No signal contained more than 1% of ectopic beats; when ectopic beats were deleted the RR interval was replaced with a running mean. The ECG series for analysis were coded so that the investigator performing the analysis was blinded to the vasoactive agent or agents under study. Heart rate variability was assessed off line using both the Lab-View 3.0 software and Statview (Abacus Concepts Inc, San Francisco, California, USA). We used the standard time domain measures of SDNN (standard deviation of RR interval values), RMSSD (root mean square of successive RR interval differences), and pNN50 (percentage of successive RR interval differences exceeding 50 ms). In addition we determined the interquartile differences (75th to 25th centile) of the frequency distributions of the total number of RR intervals (IQRDN) and of successive RR interval differences (IQRDS). As we have reported before, 7 IQRDN and IQRDS are simple indices, which exclude extreme values at each end of the frequency distribution and minimise the influence of any artefact or ectopic activity that may have escaped the editing process.

Frequency domain analysis was performed to determine the power of the underlying component oscillations. Stationarity of the time series was tested by calculation of the mean and variance of the first and last 128 beats of each recording period in order to verify a difference of < 10% in the values for each time series. The mean was subtracted from each point in the RR interval series, and power spectral analysis was performed using the Burg algorithm 16 with a model order between 8 and 12. 17 The power of each underlying frequency
RESULTS AT BASELINE
Baseline values of mean blood pressure, RR interval, and measures of heart variability in
both time and frequency domains were not significantly different when subjects received
angiotensin II or phenylephrine (table 1).

RESULTS DURING PRESSOR INFUSIONS
During the pressor infusions of angiotensin II and phenylephrine, although the mean level of
induced hypertension was equal, the pulse interval responses were widely divergent; during
angiotensin II infusion the mean RR interval was significantly shorter than with phenylephrine
(p < 0.001).

The group mean measures of heart rate variability were not significantly different
(with the exception of LF absolute power) during infusion of angiotensin II or phenylephrine
(table 2), despite the widely divergent heart rate responses. The marked bradycardia
associated with phenylephrine infusion was not accompanied by a significant increase in
measures of cardiac vagal activity. It was apparent that these mean data comprised two
different heart rate variability responses to pressor infusions of phenylephrine. These are
illustrated by the tachograms in fig 1. In the first example (subject a), phasic variation in
heart rate (respiratory sinus arrhythmia) is increased during phenylephrine infusion, a
response which can be interpreted as an increase in cardiac vagal activity. However, in
subject b, although there is a bradycardia, heart rate variability is severely reduced. The
latter paradoxical response occurred in five of the 10 subjects.

Table 2 Blood pressure, RR interval, and RR variability during phenylephrine or
angiotensin II infusion

<table>
<thead>
<tr>
<th>Phenylephrine</th>
<th>Angiotensin II</th>
<th>pvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>110 (8)</td>
<td>107 (4)</td>
</tr>
<tr>
<td>RR interval (ms)</td>
<td>1364 (201)</td>
<td>1019 (159)</td>
</tr>
<tr>
<td>SDNN (ms)</td>
<td>79 (29)</td>
<td>114 (66)</td>
</tr>
<tr>
<td>IQRNN (ms)</td>
<td>138 (119)</td>
<td>162 (145)</td>
</tr>
<tr>
<td>IQRSD (ms)</td>
<td>153 (93)</td>
<td>152 (133)</td>
</tr>
<tr>
<td>RMSSD (ms)</td>
<td>89 (50)</td>
<td>122 (114)</td>
</tr>
<tr>
<td>pNN50 (%)</td>
<td>54 (23)</td>
<td>57 (16)</td>
</tr>
<tr>
<td>Total power (ms²)</td>
<td>11674 (13800)</td>
<td>17902 (20511)</td>
</tr>
<tr>
<td>LF power (nu)</td>
<td>0.17 (0.13)</td>
<td>0.23 (0.11)</td>
</tr>
<tr>
<td>HF power (nu)</td>
<td>0.56 (0.26)</td>
<td>0.60 (0.19)</td>
</tr>
<tr>
<td>HF power-abs (ms²)</td>
<td>5502 (6959)</td>
<td>8673 (14167)</td>
</tr>
</tbody>
</table>

Values are mean (SD).

abs, absolute units; HF, high frequency; IQRNN, interquartile differences in frequency distribu-
tions of the total number of RR intervals; IQRSD, interquartile differences in frequency distribu-
tions of successive RR interval differences ; LF, low frequency; pNN50, percentage of successive
RR interval differences exceeding 50 ms; RMSSD, root mean square of successive RR interval
differences; SDNN, standard deviation of RR interval values.

was quantified by decomposing the total variability signal with the method of
Zetterberg.18 This enables the determination of low frequency (LF) power at ~0.1 Hz (reflect-
ing both sympathetic and vagal activity19) and high frequency (HF) power at the measured
respiratory frequency. Because total power varies greatly between individual subjects, power
was determined in both absolute units and as normalised values. The power in normalised
units was calculated by dividing the absolute power of a given component (area under the
component curve) by the total power minus the 0–0.04 Hz component.

STATISTICAL ANALYSIS
Data for blood pressure, RR interval, and RR variability were tested for normality.
The significance of the differences between groups was determined using Student’s t test
for normally distributed data; otherwise the Wilcoxon signed rank test was used.

Results
RESULTS AT BASELINE
Baseline values of mean blood pressure, RR interval, and measures of heart variability in

![Figure 1](http://heart.bmj.com/)

Figure 1 Typical examples of the two different heart rate variability responses to pressor infusions of phenylephrine, illustrated by the tachograms taken from two individual subjects, at baseline and during a pressor infusion of phenylephrine. In subject a, phasic variation in heart rate (respiratory sinus arrhythmia) is increased during phenylephrine infusion, a response which can be interpreted as an increase in cardiac vagal activity. In subject b, although there is a bradycardia, heart rate variability is severely reduced.
mg/hour, respectively. The most striking result was that the heart rate changes in response to the two pressor agents remained divergent, with a shorter mean RR interval during infusion of angiotensin II and sodium nitroprusside (AII/SNP) than during phenylephrine and sodium nitroprusside (PE/SNP). Compared with baseline, AII/SNP resulted in a slight tachycardia (p < 0.02) while there was no significant difference in heart rate from baseline during PE/SNP (table 3, fig 2). The heart rate variability responses to the two infusions are given in table 3. There was no significant difference in time domain measures of overall RR variability such as SDNN or IQDNN. However, measures of high frequency variation such as IQDSD, RMSSD, and pNN50 were significantly greater during PE/SNP than during AII/SNP infusions. Using frequency domain analysis, total power was not significantly different. There was a trend to greater absolute values for HF power and lower values for LF power during PE/SNP than during AII/SNP. When interpatient variance was reduced by the use of normalised units there was significantly greater high frequency power and lesser low frequency power during PE/SNP than during AII/SNP. Figure 2 shows the heart rate variability of an individual subject at baseline, and the responses to phenylephrine and angiotensin II infusions, during pressor infusion, and during correction for the increase in arterial pressure with sodium nitroprusside. In this subject, variability of successive differences between RR intervals increased in response to phenylephrine but did not change with angiotensin II. When the arterial pressure was returned to control level, the variability was returned toward baseline with phenylephrine and reduced to a value lower than baseline with angiotensin II.

Discussion

MEASUREMENT OF CARDIAC VAGAL ACTIVITY IN MAN

The vagus nerve is not accessible to recording techniques in man, so measurement of cardiac vagal efferent activity is necessarily indirect. Heart rate variability measures of vagal activity

Table 3  Blood pressure, RR interval, and RR variability during infusion of phenylephrine or angiotensin II, each with sodium nitroprusside

<table>
<thead>
<tr>
<th></th>
<th>Phenylephrine</th>
<th>Angiotensin II</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>91 (12)</td>
<td>88 (9)</td>
<td>NS</td>
</tr>
<tr>
<td>RR interval (ms)</td>
<td>1057 (163)</td>
<td>864 (117)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SDNN (ms)</td>
<td>93 (25)</td>
<td>86 (35)</td>
<td>NS</td>
</tr>
<tr>
<td>IQDNN (ms)</td>
<td>178 (94)</td>
<td>129 (62)</td>
<td>NS</td>
</tr>
<tr>
<td>IQSD (ms)</td>
<td>126 (59)</td>
<td>65 (42)</td>
<td>0.004</td>
</tr>
<tr>
<td>RMSSD (ms)</td>
<td>90 (40)</td>
<td>63 (39)</td>
<td>0.04</td>
</tr>
<tr>
<td>pNN50 (%)</td>
<td>57 (21)</td>
<td>30 (16)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total power (ms²)</td>
<td>16 361 (13 341)</td>
<td>10 776 (9 819)</td>
<td>NS</td>
</tr>
<tr>
<td>LF power-abs (ms²)</td>
<td>930 (829)</td>
<td>1748 (1288)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>LF power (nu)</td>
<td>0.15 (0.11)</td>
<td>0.45 (0.21)</td>
<td>0.002</td>
</tr>
<tr>
<td>HF power-abs (ms²)</td>
<td>4717 (3672)</td>
<td>2763 (4201)</td>
<td>NS</td>
</tr>
<tr>
<td>HF power (nu)</td>
<td>0.66 (0.26)</td>
<td>0.48 (0.19)</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

Values are mean (SD). abs, absolute units; HF, high frequency; IQDNN, interquartile differences in frequency distributions of the total number of RR intervals; IQDSD, interquartile differences in frequency distributions of successive RR interval differences; LF, low frequency; pNN50, percentage of successive RR interval differences exceeding 50 ms; RMSSD, root mean square of successive RR interval differences; SDNN, standard deviation of RR interval values.

Figure 2  Results of an individual subject are shown using frequency histograms of successive RR interval differences. Plots (A) and (D) show beat to beat variability at baseline. During pressor infusions, variability increased in response to phenylephrine (B) but did not change with angiotensin II (E). When the arterial pressure was returned to control level by simultaneous infusion of sodium nitroprusside, the variability was returned to baseline with phenylephrine (C) and reduced to below baseline with angiotensin II (F).
Angiotensin II inhibits cardiac vagal tone

Assess the high frequency (“beat to beat”) variability resulting from vagal modulation of heart rate caused by respiratory sinus arrhythmia. This high frequency variability is specifically measured by indices such as the variability of successive intervals between RR intervals (IQDSD), RMSSD, and pNN50 rather than by measures of total variability such as SDNN.

Analysis of heart rate variability in the frequency domain allows determination of the power and frequency of component oscillations. The magnitude of respiratory modulation of heart rate can be measured by determining the power of the component which is synchronous with respiration. This is usually centred at about 0.25 Hz and provides a further index of cardiac vagal tone.

No measure of heart rate variability provides an absolute measure of the activity of vagal (or indeed sympathetic) nervous activity. These measurements do, however, reflect the degree of neural modulation of heart rate.

Heart rate variability responses to pressor infusions

In a previous paper we showed that during a pressor infusion of intravenous angiotensin II, the baroreflex mediated increase in cardiac vagal activity assessed by heart rate variability was significantly lower than during a control infusion of phenylephrine. This confirmed the results of earlier animal studies which were able to use direct neural recording to show inhibition of vagal activity during baroreflex loading. The results arising from the first part of this study are at first sight discrepant, with no significant difference between measures of heart rate variability (with the sole exception of LF absolute power) during pressor infusions of angiotensin II and phenylephrine. We believe that the reason for this lies in the limitation of heart variability as a measure of cardiac vagal activity during extremes of baroreflex loading. The results arising from the first part of this study are at first sight discrepant, with no significant difference between measures of heart rate variability (with the sole exception of LF absolute power) during pressor infusions of angiotensin II and phenylephrine. We believe that the reason for this lies in the limitation of heart variability as a measure of cardiac vagal activity during extremes of baroreflex loading. The power of this component was higher during angiotensin II infusion and we are unable to exclude facilitation by angiotensin II of sympathetic nervous activity in addition to inhibition of vagal activity.

Spectrum of action of angiotensin II

Animal experiments suggest that angiotensin II exerts both peripheral and central inhibitory actions on vagal activity. The central actions appear to be mediated through the circumventricular organs such as the subfornical organ, which contain angiotensin II sensitive neurones and which, lacking a blood–brain barrier, are accessible to circulating angiotensin II. In a series of papers examining the influence of angiotensin II on the control of human sympathetic activity, Goldsmith and colleagues were unable to confirm animal work suggesting tonic central and peripheral presynaptic facilitation of sympathetic activity. However, they did show that the normal reduction in forearm vascular resistance during baroreflex loading was attenuated by angiotensin II, suggesting that the effects of angiotensin II on sympathetic activity in humans are limited to inhibition of baroreflex mediated changes. This does not appear to be the case for cardiac vagal activity, which we have shown to be attenuated even in the absence of baroreflex loading.

Although inhibition of efferent cardiac vagal activity is supported by animal evidence, it is possible that differences in the afferent limb of the baroreflex contributed to our results. We controlled for mean blood pressure because this is the main determinant of baroreceptor discharge, with relatively little effect from other variables (for example, pulse pressure, rate of pressure change). However, we cannot ex-
clude influences from these variables merely because mean blood pressure was unchanged. Equally, it is possible that angiotensin II and phenylephrine exert different actions on the mechanical properties of the baroreceptor. Phenylephrine has been shown to cause vasoconstriction of the carotid sinuses in dogs.\textsuperscript{29} In rabbits, angiotensin II increased the dimensions of the aortic arch, while phenylephrine resulted in vasoconstriction although there were no significant differences in aortic nerve activity.\textsuperscript{26} Lumbers \textit{et al.} also found no significant differences between the effects of angiotensin II and phenylephrine on afferent baroreceptor activity.\textsuperscript{11} We therefore believe that an afferent effect is unlikely to account for the results of our study.

LIMITATIONS

Modulation of autonomic activity by nitric oxide as suggested by recent animal evidence may have exerted a confounding influence on this study. This information was not available at the time our study was designed so the nitric oxide donor sodium nitroprusside rather than a non-endothelium dependent vasodilator was used to counteract the pressor effects of angiotensin II and phenylephrine. Nitric oxide synthase (NOS) is found at both central and peripheral sites involved in the integration of the arterial baroreflex and inhibition of NOS causes an increase in baroreflex sensitivity in conscious rabbits.\textsuperscript{31} In the ferret, the bradycardia resulting from vagal stimulation is attenuated by NOS inhibition suggesting that efferent vagal activity is modulated by NO.\textsuperscript{32} The effects of nitric oxide on autonomic control in humans are unknown and require further investigation, but a small study of the effects of NO inhibition found no evidence of modulation of autonomic activity.\textsuperscript{33,34} In our study the lack of any significant difference in dose of sodium nitroprusside between the phenylephrine and angiotensin II groups probably makes it unlikely that the differences in high frequency heart rate variability measures were the result of modulation of vagal activity by NO from sodium nitroprusside. A second limitation is that we used a sampling rate of 165 Hz, which may have resulted in an error of up to 12 ms per RR interval. However, it would not have introduced any bias and differences in high frequency heart rate variability were demonstrable despite this technical limitation.

CLINICAL IMPLICATIONS

Our results may have important clinical implications for the underlying mechanisms of action of drugs inhibiting the renin-angiotensin system including angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor antagonists. Low cardiac vagal activity measured by heart rate variability and baroreflex sensitivity has been associated with an adverse prognosis after myocardial infarction\textsuperscript{35,36} and in heart failure.\textsuperscript{37} Treatment with ACE inhibitors has been shown to increase heart rate variability and baroreflex sensitivity in both groups of patients,\textsuperscript{38-39} suggesting that at least part of the cause of reduced cardiac vagal activity is the increase in circulating angiotensin II found in these conditions. The reasons why ACE inhibitors reduce mortality in heart failure and after myocardial infarction\textsuperscript{38-40} are not understood but we suggest that an increase in cardiac vagal activity as a result of a reduction in circulating angiotensin II may be an important component mechanism. Cardiac vagal activity appears to exert an antiarrhythmic action in experimental animals by increasing the fibrillatory threshold of ischaemic myocardium.\textsuperscript{41} If an increase in vagal activity associated with ACE inhibitor treatment acts in humans to reduce ventricular arrhythmias and fibrillation, a reduction in mortality from sudden presumed arrhythmic death might be expected. There is now abundant evidence for such an effect from the results of trials of ACE inhibitors in both heart failure\textsuperscript{3} and myocardial infarction.\textsuperscript{1} In view of the results of the ELITE trial,\textsuperscript{5} studies investigating the effects of angiotensin II receptor blockade on cardiac autonomic control are required.

This study was supported by the British Heart Foundation.