Aldosterone blunts the baroreflex response in man

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ABSTRACT

1. Recent animal evidence suggests that aldosterone, like angiotensin II, may possess detrimental autonomic modulating properties. Aldosterone has been shown to impair the baroreflex response in animal models. This study is designed to test the hypothesis that aldosterone directly attenuates the baroreflex in vivo in man.

2. Fourteen healthy male volunteers [mean age (S.D.) 25 (9) years] received intravenous d-aldosterone (12 pmol [min]^{-1} [kg]^{-1}) and 5% dextrose (vehicle) in a double-blind crossover fashion, co-infused with incremental doses of intravenous phenylephrine and sodium nitroprusside. Aldosterone had no significant effect on resting blood pressure, heart rate or baroreflex response to sodium nitroprusside. However, reflex responses to phenylephrine were impaired with aldosterone (P < 0.01) while blood pressure responses were unaltered. Baroreflex sensitivity was significantly blunted in the aldosterone group [8.36 ± 2.19 versus 10.12 ± 2.27 ms/mmHg; P < 0.04].

3. This study confirms previous observations from animal models that aldosterone impairs the baroreflex response. High aldosterone levels may contribute to the baroreflex dysfunction in cardiovascular diseases such as hypertension and heart failure.

INTRODUCTION

It is well established that angiotensin II (ANG II) attenuates baroreflex control of heart rate and sympathetic activity [1,2], and that angiotensin-converting enzyme (ACE) inhibitors are able to improve baroreceptor sensitivity (BRS) [3,4]. However, it is now being appreciated that aldosterone too may influence the baroreflex, irrespective of ANG II.

We recently found that spironolactone improves heart rate variability (as a measure of parasympathetic activity) in patients with chronic heart failure (CHF) [5]. Although it remains inconclusive whether this was a direct effect of autonomic modulation by aldosterone blockade or due to the diuretic effect of spironolactone (which may potentially reduce right atrial stretch and improve heart rate variability by mechanoelectric feedback [6]), there is experimental data to suggest that aldosterone has major direct effects on the autonomic nervous system and the baroreflex. In an animal model, Wang et al. [7] showed that aldosterone infusion directly reduced baroreceptor discharge from the carotid sinus in dogs. However, no direct information has yet been reported on the effects of aldosterone on baroreflex responses in man. As with all animal studies, it is essential to determine if these observations also occur in man, especially since

Key words: aldosterone, autonomic nervous system, baroreceptors, blood pressure, heart failure, heart rate.

Abbreviations: ACE, angiotensin-converting enzyme; ANG II, angiotensin II; BP, blood pressure; BRS, baroreflex sensitivity; CHF, chronic heart failure; HR, heart rate.

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baroreceptor dysfunction is known to play a central regulatory role in the development of cardiac arrhythmias [8–10]. Thus, we have designed this study to test the hypothesis that aldosterone attenuates the baroreflex control of heart rate in vivo in normal man. We have examined the effects of an acute intravenous infusion of aldosterone on the vagal and sympathetic limbs of the autonomic nervous system, by assessment of blood pressure and reflex heart rate responses to phenylephrine, a vasopressor agent, and to sodium nitroprusside, a vasodilator.

METHODS

Subjects

Fourteen normal male volunteers [mean age (S.D.) 25 (9) years] were studied. None had a history of hypertension or cardiac disease, and physical examination, routine haematological and biochemical parameters, and 12-lead ECGs were normal in all subjects. Each subject provided informed consent and the study was approved by the Tayside Committee on Medical Research.

Protocol

The subjects were studied on 2 separate days, at least 72 h apart, in a placebo-controlled, randomized, double-blind, crossover fashion. Subjects were asked to refrain from alcohol, caffeine and cigarettes for 24 h and to fast for 2 h before each study day.

Subjects rested quietly in the supine position throughout the study. Two 18G intravenous cannulae were inserted into forearm veins, one in the right arm for blood sampling and one in the left arm for infusion of either aldosterone or 5% dextrose solution (as vehicle). After 45 min of bed rest, baseline values of blood pressure (BP) and heart rate (HR) were measured non-invasively in triplicate using a semi-automatic sphygmomanometer (Dinamap Vital Signs Monitor 1846; Critikon, Tampa, FL, U.S.A.) with the cuff placed around the subject’s left arm. A 12-lead ECG and venous blood (15 ml) for baseline aldosterone and angiotensin II assays were also obtained.

After this, a continuous infusion of either vehicle or aldosterone (Tayside Pharmaceuticals) in similar volumes was commenced in the left arm. The d-aldosterone was infused at a rate of 12 pmol·min⁻¹·kg⁻¹. After 45 min of infusion, further triplicate recordings of BP, HR and continuous 12-lead ECGs were obtained along with blood samples for aldosterone and angiotensin II assays. The haemodynamic study was then started, i.e. after 90 min of supine bed rest and 45 min after commencement of the infusion. The baroreflex response to a vasopressor agent, phenylephrine, was assessed in the first half of the study. Phenylephrine was given intravenously by infusion into the right forearm. It was administered in stepwise 10-min infusions (0.2–3.6 μg·min⁻¹·kg⁻¹) by use of an infusion pump (IMED, San Diego, CA, U.S.A.). The infusion was stopped when a 35–40 mmHg rise in systolic arterial pressure had been achieved. The average systolic BP, HR and R–R interval obtained from continuous ECG recordings between 8 and 10 min after each infusion dose were recorded.

After completion of these measurements with phenylephrine, HR and BP were allowed to return to baseline values for 30 min before the second phase of the study began. Intravenous sodium nitroprusside was given in stepwise 5-min infusions (0.2–5.2 μg·min⁻¹·kg⁻¹) until a maximum drop in systolic BP of 25 mmHg was achieved. HR, BP and ECG recordings were obtained after 4–5 min of each infusion dose.

BRS assessment

The R–R intervals were plotted against the systolic BP values on a graph, and a computerized curve fit was carried out to establish a linear portion of the line of best fit. Separate linear regression lines were plotted for the responses to phenylephrine and sodium nitroprusside. Traditionally, the slope derived from the linear regression line (dRR/dSBP) obtained from the vasopressor (phenylephrine) half of the haemodynamic study has been used as an index of BRS. As in previous studies [4,11], only regression lines that had a correlation coefficient of $> 0.8$ were used. This method of assessment of the baroreflex using an infusion of phenylephrine has previously been shown to be reproducible [11].

Aldosterone and ANG II assays

Five-millilitre venous blood samples in lithium heparin tubes and 10-ml venous samples in chilled glass tubes containing a solution of 0.05 mol/l o-phenanthroline, 2 g/l neomycin, 0.125 mol/l EDTA (disodium salt) and 2% ethanol, were collected for measurements of aldosterone and ANG II levels respectively. The samples were centrifuged at 4 °C and the plasma was separated and stored at $−20$ °C (aldosterone) and $−70$ °C (ANG II) until assayed. Commercially available radioimmunoassay kits (Sorin Biomedica, Saluggia, Italy, and Nichols Institute Diagnostics B. F., Nieuweweg, The Netherlands) were used for the aldosterone and ANG II assays respectively.

Statistical analysis

All data were analysed using the Statgraphics software package (STSC Softwear Publishing Group, Rockville, MD, U.S.A.). Analysis of variance at each dose increment, using subjects and treatment as within factors, and Bonferroni multiple range tests were performed to determine the significance of the effects of aldosterone on the haemodynamic response to phenylephrine and so-
Aldosterone and human baroreflex

dium nitroprusside. The relationships between R–R intervals and systolic BP were studied by correlation and linear regression analyses; BRS between treatment groups was analysed using the paired Student’s t-test. Differences were considered statistically significant if \( P < 0.05 \).

RESULTS

Baseline measurements (Table 1)

No significant differences in baseline measurements were seen between vehicle and aldosterone infusion days. Aldosterone infusion was painless. A significant rise in plasma aldosterone levels was noted after 45 min of aldosterone infusion compared with vehicle. There was a non-significant decrease in ANG II levels after aldosterone infusion compared with vehicle.

Haemodynamic measurements (Figures 1–3, Table 2)

No significant changes in resting BP and HR recordings were observed during aldosterone infusion compared with vehicle. Systolic BP increased and decreased in a stepwise fashion in response to the phenylephrine and sodium nitroprusside infusions respectively in both groups; no significant differences in BP responses were observed in the aldosterone group compared with vehicle (Figures 1 and 2). Similarly, no significant differences in reflex HR responses to sodium nitroprusside were observed between the two groups. The slopes of the linear regression line in response to sodium nitroprusside \((\Delta RR/\Delta SBP)\) were not significantly affected by aldosterone compared with vehicle \( [8.62 \pm 4.64 \text{ ms/mmHg} \text{ (means \pm S.D.)} \text{ versus } 9.07 \pm 2.11 \text{ ms/mmHg}; \ P = 0.7] \) (Figure 3).

However, reflex HR responses to phenylephrine were significantly impaired in the aldosterone group compared with vehicle \( (P < 0.05) \). BRS was significantly depressed in the aldosterone group \( [8.36 \pm 2.19 \text{ ms/mmHg versus } 10.12 \pm 2.27 \text{ ms/mmHg}; \ P < 0.04] \).

Table 1 Baseline values

Results are expressed as means (S.D.). Statistical significance: * \( P < 0.05 \) compared with vehicle.

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Aldosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>61 (8)</td>
<td>60 (9)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>114 (10)</td>
<td>116 (5)</td>
</tr>
<tr>
<td>R–R interval (ms)</td>
<td>999 (156)</td>
<td>1021 (181)</td>
</tr>
<tr>
<td>Aldosterone levels (pg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>115 (107.8)</td>
<td>106.1 (81.8)</td>
</tr>
<tr>
<td>45 min after start of infusion</td>
<td>72.6 (62.6)</td>
<td>489.8 (83.3)*</td>
</tr>
<tr>
<td>ANG II levels — 45 min after start of infusion (pg/ml)</td>
<td>15.2 (7.6)</td>
<td>10.4 (3.5)</td>
</tr>
</tbody>
</table>

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Figure 3  Relationship between changes in RR interval and systolic blood pressure (SBP)

Values are means ± S.E.M. Separate linear regression lines for responses to phenylephrine and sodium nitroprusside for the two treatments are shown.

DISCUSSION

The present study confirms previous observations from animal models that aldosterone impairs the baroreflex response. The sensitivity of the arterial baroreceptors to a change in BP is an important determinant of the ability of the autonomic nervous system to maintain cardiovascular haemostasis.

In this study, aldosterone has been shown to impair the HR response to phenylephrine. This is in keeping with a previous observation of ours that aldosterone halved the bradycardic response to infused noradrenaline [12]. Although acting primarily on the arterial baroreceptors, it must be borne in mind that any change in afterload by vasopressor agents will also tend to cause a corresponding change in preload and thus influence the cardiopulmonary receptors as well. On the other hand, sodium nitroprusside results in vasodilatation and generalized unloading of arterial and cardiopulmonary baroreceptors leading to increased cardiac sympathetic activity [13,14]. It is interesting to note that aldosterone does not appear to have any significant effect on the reflex tachycardic (or cardiac sympathetic) response to sodium nitroprusside. These data together suggest that aldosterone exerts major effects on the parasympathetic limb of the autonomic nervous system.

With regards to its specific mechanism of action, evidence from animal studies suggests that aldosterone may have a direct action on the arterial baroreceptors. Wang et al. [7,15] not only showed that aldosterone reduces the HR response to changes in BP, but also showed conclusively that aldosterone directly reduces baroreceptor discharge from the carotid sinus of dogs. These effects were seen with both acute and chronic administration of aldosterone. On a cellular level, the mechanism is less certain. Aldosterone may elicit part of its effect on the arterial baroreceptors by stimulation of the Na⁺-K⁺-ATPase activity [7,15] as it is known to be a potent Na⁺-K⁺-ATPase stimulant [16,17]. Depressed baroreceptor function after chronic aldosterone infusion in animal models has been shown to be partially reversed with a bolus injection of the Na⁺-K⁺-ATPase inhibitor, ouabain [15]. Similarly, digoxin, another cardiac glycoside/Na⁺-K⁺-ATPase inhibitor, has also been shown to improve BRS in chronic hyperaldosteronaemic states such as CHF [8,18]. However, such an effect of ouabain was not seen after acute administration of aldosterone [7], suggesting that other mechanisms may be responsible for the acute effects of the hormone. Interestingly, the impaired baroreflex response due to acute administration of aldosterone could be prevented by denudation of the endothelial cells in the carotid sinus area, which has led to the suggestion that aldosterone may stimulate endothelial cells to release an unknown substance that depresses BRS activity.

Although this study was not designed to determine the specific mechanism by which aldosterone impairs the baroreflex, it has important clinical implications as it is the first study as such to extend these observations to man. This study adds to the growing body of evidence

Table 2  Change in haemodynamic parameters in response to phenylephrine infusion

Values are means and 95% confidence intervals (in brackets). Statistical significance: *P < 0.05, †P < 0.01 compared with vehicle. Abbreviations: SBP, systolic blood pressure; HR, heart rate.

<table>
<thead>
<tr>
<th>Dose of phenylephrine (µg·min⁻¹·kg⁻¹)</th>
<th>Vehicle</th>
<th>Aldosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ΔSBP (mmHg)</td>
<td>ΔHR (beats/min)</td>
</tr>
<tr>
<td>0.2</td>
<td>3.5 (0.6–6.4)</td>
<td>3.2 (1.9–4.5)</td>
</tr>
<tr>
<td>0.6</td>
<td>12.5 (8.1–16.9)</td>
<td>8.3 (6.1–10.6)</td>
</tr>
<tr>
<td>1.2</td>
<td>20.5 (14.4–26.6)</td>
<td>13.9 (12.1–15.8)</td>
</tr>
<tr>
<td>2.4</td>
<td>43.1 (36.3–49.8)</td>
<td>19.0 (17.5–20.5)</td>
</tr>
<tr>
<td>3.6</td>
<td>57.2 (51.7–62.7)</td>
<td>22.1 (20.1–24.2)</td>
</tr>
</tbody>
</table>
that aldosterone, like ANG II, has autonomic modulating properties. Aldosterone has been shown to block noradrenaline uptake in the heart in vivo in an animal study [19]. In accordance with this, we recently found that spironolactone, an aldosterone antagonist, increased myocardial noradrenaline uptake [19] and improved HR variability [5] in patients with CHF. Although in our study, aldosterone does not appear to have any significant effect on the reflex sympathetic response to sodium nitroprusside in healthy volunteers, this finding is not necessarily a contradiction to our previous findings since infusing nitroprusside into normal man clearly does not exactly reproduce the activated sympathetic system of patients with CHF, even though nitroprusside is a traditional way of assessing sympatho-activation in man. One important difference between the two is that filling pressures are high in CHF but are likely to be subnormal after nitroprusside unloading in normal man.

There are, however, some limitations to our study worth discussing. First, the study was performed in healthy subjects and not in patient groups. Patients with CHF, for example, have a markedly abnormal and complex haemodynamic and neurohormonal state. Although it would be of interest to assess the effects in these patient groups, they are not ideal for the purpose of this study which was to assess the effects of aldosterone on baroreceptor function in vivo in man. In our study of healthy subjects we were able to isolate the effects of aldosterone on the baroreflex (and thus confirm the data from animal models) while avoiding the many possible confounding factors present in patients with CHF who are characterized by the presence of other circulating neurohormones, impaired vascular tone and endothelial dysfunction.

Secondly, salt intake was not controlled for in this study. Alterations in body salt status may potentially affect the renin–angiotensin–aldosterone axis. However, volunteers were advised to remain on their usual diet throughout the study and, as reflected by the baseline measurements, there were no significant differences in plasma neurohormones between the two study days.

Finally, infusions rather than boluses of pressor and vasodepressor stimuli were used in this study, allowing for baroreceptor ‘resetting’ to occur and hence reducing or dampening any change in baroreflex response. This may mean that small changes in baroreflex response to sodium nitroprusside, for example, may have gone undetected. On the other hand, one might expect, if anything, the blunting effect of aldosterone on baroreflex response to phenylephrine to be amplified if the bolus method had been used instead. BRS measurements by infusion method may not be equivalent to the bolus method, but the infusion method has been shown to be reproducible [11].

Infusions were used in this study because it allowed us to monitor HR and BP changes at steady state non-invasively at each incremental dose which has the advantage that our readings were taken in triplicate, which should minimize random measurement error. In addition, the bolus method requires either invasive intraarterial measurements (more risk and discomfort to the subject using radial artery devices) or non-invasive beat-to-beat analysis using the Finapres, which is not possible at our institute as the Finapres devices are no longer available in the U.K. Furthermore, we were more interested in changes or differences in baroreceptor sensitivities between two treatments rather than in using absolute levels of BRS to compare one population with another.

In summary, this study has established that in man in vivo, aldosterone has a detrimental effect on the parasympathetic component of the baroreflex response. The effects of aldosterone on the autonomic nervous system have important clinical implications. In conditions such as CHF and hypertension, it is well documented that the suppressive effect of long-term ACE inhibitors on aldosterone is weak, variable and unsustained, whether or not ANG II itself remains suppressed [20–22]. It is noteworthy that in our study, the plasma concentration of aldosterone [mean (S.D.) 489.8 (83.3) pg/ml] achieved on the treatment days was similar to those observed in patients with CHF [20].

Baroreflex dysfunction is thought to be a key process contributing to the development of ventricular arrhythmias and mortality in patients with CHF and ischaemic heart disease [8,9,23]. Thus, it follows that if residual aldosterone is partly responsible for the blunting of the baroreflex in these patients, there may well be a therapeutic benefit in considering anti-aldosterone therapy in addition to ACE inhibitors. It must be borne in mind, however, that only the effects of acute aldosterone administration have been examined in this study, and whether these observations also extend to conditions characterized by chronic elevated aldosterone levels remains to be determined. The potential mortality benefits of giving spironolactone in addition to ACE inhibitors are currently being evaluated in the multi-centre RALES study [24]. If the RALES study turns out to be positive, then this study will have highlighted an important possible mechanism for such a beneficial effect on mortality.

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