Aldosterone induces acute endothelial dysfunction \textit{in vivo} in humans: evidence for an aldosterone-induced vasculopathy

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Abstract

Experimental studies have suggested a role for aldosterone and glucocorticoids in the pathogenesis of endothelial dysfunction. We therefore set out to characterize the acute effects of these hormones on vascular function \textit{in vivo} in normal humans. A randomized, placebo-controlled, double-blind crossover study was performed on 16 healthy male volunteers (aged 19–29 years), examining the vascular effects of acute intravenous aldosterone infusion (12 pmol min\(^{-1}\) kg\(^{-1}\) for 4 h) and of oral prednisolone (single 50 mg dose). Peripheral arterial vascular function was assessed by bilateral forearm venous occlusion plethysmography using two parallel study protocols. In the first protocol, eight subjects received, successively, acetylcholine, sodium nitroprusside, noradrenaline, angiotensin I and angiotensin II. The remaining eight subjects received, successively, acetylcholine, sodium nitroprusside, verapamil and noradrenaline. Aldosterone attenuated endothelium-dependent vasodilatation to acetylcholine as compared with either prednisolone or placebo (maximum vasodilatation: placebo, 357±38%; aldosterone, 257±21%; \(P<0.05\)). However, background endothelium-independent vasodilatation was not affected by either aldosterone or prednisolone. There were also no significant changes in vasoconstriction induced by angiotensin or noradrenaline following aldosterone or prednisolone treatment compared with placebo. Blood pressure and baseline blood flow did not differ between any of the study phases. Thus acute short-term systemic administration of aldosterone results in endothelial vasodilator dysfunction in normal men, providing evidence for an aldosterone-induced vasculopathy, which may be particularly relevant not only in heart failure but also in hypertensive patients with high aldosterone/renin ratios.

Introduction

There are a number of indirect observations which suggest that aldosterone might have an adverse effect on the endothelium. Firstly, it has been demonstrated in a tissue culture study that aldosterone inhibits inducible nitric oxide release in response to cytokine stimulation [1]. Secondly, chronic spironolactone therapy in patients with chronic heart failure improves nitric oxide bioactivity and improves endothelial vasodilator dysfunction [2]. However, the latter could have been because spironolactone improved the patient’s general clinical state, which then non-specifically improved endothelial dysfunction. The above results certainly raise the possibility that aldosterone worsens endothelial function but, as yet, there is no direct demonstration that aldosterone can by itself cause endothelial dysfunction \textit{in vivo} in humans. If it did, this could explain the RALES (Randomised Aldactone Evaluation Study) results, where spironolactone improved prognosis, since endothelial

Key words: aldosterone, endothelium, glucocorticoids, nitric oxide, vascular angiotensin-converting enzyme.

Abbreviations: ACE, angiotensin-converting enzyme; EDHF, endothelium-derived hyperpolarizing factor; \(\mathrm{L}-\mathrm{NMMA}\), \(\mathrm{N}^\theta\)-monomethyl-\(\mathrm{l}\)-arginine.

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dysfunction has been linked in many studies to prognosis [3–8]. We therefore set out to assess the acute effects of aldosterone on the endothelium in vivo in humans. We also assessed whether aldosterone acutely alters endothelial angiotensin-converting enzyme (ACE), since indirect data also suggest this possibility [9–11].

Glucocorticoids are other related hormones that have also been shown in both animal and human experimental studies to have potentially important vascular effects. Glucocorticoids inhibit the re-uptake of noradrenaline (norepinephrine) in the peripheral circulation, which could potentiate the adverse vasoconstrictive effects of noradrenaline [12]. Glucocorticoids also increase the gene expression of various components of the human renin/angiotensin/aldosterone system, including ACE and angiotensinogen mRNAs, as well as increasing angiotensin II receptor density and responsiveness [13]. As well as potentiating vasoconstrictor responses, glucocorticoids may also suppress vasodilatory mechanisms. Glucocorticoids appear to reduce the endogenous vasodilatory properties of prostacyclin [14], and they may suppress bradykinin-induced vasodilatation [15]. The above studies are nearly all tissue culture studies or studies in experimental animals. We currently lack any evidence that glucocorticoids can alter vascular function acutely in vivo in humans.

We therefore set out to investigate if acute short-term administration of aldosterone does indeed induce adverse vascular effects in healthy humans in vivo. In addition, the vascular effects of a single dose of glucocorticoid (prednisolone) were also assessed. Characterizing what mineralocorticoids and glucocorticoids do acutely in humans is important for two further reasons. First, both aldosterone and glucocorticoids appear to exert both slow genomic effects and fast non-genomic effects, and it is unclear whether their vascular effects are the former or the latter [16]. The latter effects have an onset within 15 min, while the former have an onset at around 2 h [17]. Secondly, the levels of both aldosterone and glucocorticoids show a strong diurnal variation, increasing at dawn [18]. It is well known that cardiovascular events and deaths peak at around the same time [19]. This raises the possibility that acute vascular effects of aldosterone and/or glucocorticoids could contribute to the diurnal variation in cardiovascular events and death.

**METHODS**

**Study population**

A total of 16 male non-smoking healthy volunteers (aged 19–29 years) gave written informed consent to participate in the study, which had prior approval from the Tayside Committee on Medical Research Ethics. The investigation conformed with the principles outlined in the Declaration of Helsinki. None of the subjects had a history of hypertension, hyperlipidaemia or cardiac disease. In addition, all routine biochemical and haematological parameters, as well as 12-lead ECGs, were normal in all subjects.

**Study experimental design (Figure 1)**

Subjects were studied on three separate visits, at least 7–10 days apart, in a placebo-controlled, double-blind, crossover fashion. Following an 8 h fast, during which alcohol and caffeine-containing beverages were excluded, patients attended a temperature-controlled laboratory (24–26 °C) in our research unit and were asked to lie supine. All study visits were carried out at the same time of day (08:00 hours) to minimize any effect of diurnal fluctuation in vascular function. Subjects were randomly allocated in a blinded fashion to receive (a) intravenous d-aldosterone (Tayside Pharmaceuticals, Dundee, U.K.) at a rate of 12 pmol·min⁻¹·kg⁻¹ for 1 h prior to vascular function assessment (and continued for the 3 h of the vascular function assessment); (b) a single dose of oral prednisolone (50 mg) 6–8 h prior to the vascular function assessment; or (c) placebo oral and intravenous medication. This aldosterone dosing infusion has been shown previously to increase plasma aldosterone levels acutely to over 400 pg/ml [20], levels that are similar to those observed in patients with chronic heart failure [21]. The subjects then underwent profiling of their vascular function, as detailed below. The cumulative dose of aldosterone at the end of the acetylcholine infusion was therefore 900 pmol/kg (≈ 63 nmol for a 70 kg volunteer).

**Endothelial function protocol**

After a period of 20 min of supine rest, subjects underwent cannulation of the non-dominant brachial artery using a 27 gauge steel needle mounted on to a 16 gauge epidural catheter under local anaesthesia. After 30 min of saline infusion, baseline forearm blood flow was measured using the technique of differential forearm venous occlusion plethysmography [22], which has been described by our group in detail previously [23]. When resting forearm blood flows had been established, drugs according to the study infusion protocol (see below) were infused into the study arm using a constant-rate infuser. Forearm blood flow was measured at each baseline, and then during the last 2 min of each drug infusion. Blood pressure was measured in the non-infused (control) arm at the beginning of the study, after each saline washout period, and at the conclusion of the study.

**Drug infusions**

**Protocol I**

Eight subjects received the following vasoactive mediators. First, acetylcholine (Miochol; CIBA Vision)
was infused at 25, 50 and 100 nmol/min, each for 5 min, to produce a cumulative dose–response curve. This was followed by sodium nitroprusside (David Bull Laboratories) at 4.2, 12.6 and 37.8 nmol/min, each for 5 min, and then by noradrenaline (Roche) at 60, 120 and 240 pmol/min for 5 min each. This in turn was followed by angiotensin I (ClinAlfa) at 16, 64 and 256 pmol/min for 7 min each, and finally angiotensin II (ClinAlfa) was infused at 4, 16 and 64 pmol/min for 7 min each. Between the different drugs, the drug infusion apparatus was flushed with saline, and sufficient time was allowed for the forearm blood flow to return to baseline values (approx. 20–30 min).

Acetylcholine was used as an endothelium-dependent vasodilator that exerts a proportion of its effect by stimulation of the endothelial production of NO. Sodium nitroprusside is an endothelium-independent vasodilator that acts by donating NO directly to vascular smooth muscle to cause vasorelaxation. Noradrenaline is a direct endothelium-independent vasoconstrictor. Angiotensin I only exerts its vasoconstrictive effect in this forearm model through its conversion in the vasculature to angiotensin II, and therefore the vasoconstriction elicited by angiotensin I reflects vascular angiotensin I→angiotensin II conversion [24,25].

Protocol 2

The second group of eight subjects received acetylcholine and sodium nitroprusside infusions as in Protocol 1. Then subjects received verapamil (Knoll) at 10, 20 and 40 nmol/min, each for 5 min, followed by noradrenaline (Roche) at 60, 120 and 240 pmol/min for 5 min each. In the forearm model, verapamil is an endothelium-independent and nitric oxide-independent vasodilator that acts via calcium channel blockade.

Statistical analysis

Forearm blood flow values were expressed as ml·min⁻¹·100 ml⁻¹ forearm volume. These blood flows were then converted into the ratio between the increase in blood flow in the infused arm and the blood flow in the control arm, expressed as the percentage change in forearm blood flow from the baseline immediately preceding each drug administration (mean ± S.E.M.), calculated according to the accepted method of Whitney.

Clinical characteristics at the placebo and active treatment study visits were compared using Student’s paired t tests. Statistical analysis of forearm blood flow measurements for individual subjects were compared between treatments using two-way ANOVA with repeated measures, correcting for multiple comparisons for within-group effects. A probability (P) value of < 0.05 was considered significant.

RESULTS

Subject characteristics

No significant differences in basal forearm blood flow were observed between any of the treatment phases (Table 1). In addition, the respective baseline forearm blood flow values preceding each drug infusion were not significantly different between the limbs, indicative of satisfactory drug washout between each infusion phase. There were also no significant differences in baseline blood pressure or heart rate between the two study days. Throughout the study visits, during the infusion of the different vasoactive substances, no significant change from baseline in blood pressure was observed (Table 1).

Forearm vascular blood flow responses to acetylcholine, sodium nitroprusside and verapamil

A significant reduction in endothelium-dependent vasodilatation in response to acetylcholine was seen in the aldosterone treatment group (maximal percentage change in blood flow from baseline: placebo, 357 ± 38%; aldosterone, 200 ± 38%).
Table 1  Biochemical and haemodynamic parameters during each treatment phase
Values are means ± S.D. FBF, forearm blood flow; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; ACh, acetylcholine; SNP, sodium nitroprusside.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo</th>
<th>Prednisolone</th>
<th>Aldosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum potassium (mmol/l)</td>
<td>4.1 ± 0.4</td>
<td>4.0 ± 0.4</td>
<td>4.0 ± 0.2</td>
</tr>
<tr>
<td>Serum urea (mmol/l)</td>
<td>6.4 ± 1.3</td>
<td>7.1 ± 2.7</td>
<td>6.0 ± 1.6</td>
</tr>
<tr>
<td>Serum creatinine (umol/l)</td>
<td>99 ± 13</td>
<td>91 ± 16</td>
<td>92 ± 12</td>
</tr>
<tr>
<td>Serum glucose (mmol/l)</td>
<td>5.4 ± 0.5</td>
<td>5.5 ± 0.4</td>
<td>5.3 ± 0.4</td>
</tr>
<tr>
<td>Baseline heart rate (beats/min)</td>
<td>75 ± 8</td>
<td>74 ± 7</td>
<td>74 ± 8</td>
</tr>
<tr>
<td>Baseline FBF (ml · min⁻¹ · 100 ml⁻¹)</td>
<td>3.8 ± 0.9</td>
<td>3.9 ± 0.8</td>
<td>3.8 ± 1.0</td>
</tr>
<tr>
<td>Baseline SBP (mmHg)</td>
<td>134 ± 6</td>
<td>133 ± 4</td>
<td>132 ± 5</td>
</tr>
<tr>
<td>Baseline DBP (mmHg)</td>
<td>73 ± 7</td>
<td>73 ± 8</td>
<td>74 ± 5</td>
</tr>
<tr>
<td>Baseline MAP (mmHg)</td>
<td>93 ± 5</td>
<td>93 ± 5</td>
<td>93 ± 4</td>
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<tr>
<td>After ACh</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SBP (mmHg)</td>
<td>133 ± 6</td>
<td>133 ± 4</td>
<td>132 ± 5</td>
</tr>
<tr>
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<td>75 ± 3</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>94 ± 4</td>
<td>93 ± 2</td>
<td>94 ± 3</td>
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<tr>
<td>After SNP</td>
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<tr>
<td>SBP (mmHg)</td>
<td>131 ± 8</td>
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<td>132 ± 5</td>
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<tr>
<td>DBP (mmHg)</td>
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<td>73 ± 4</td>
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<td>MAP (mmHg)</td>
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<td>MAP (mmHg)</td>
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<td>After noradrenaline</td>
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<tr>
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<td>131 ± 5</td>
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<tr>
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<td>93 ± 2</td>
<td>92 ± 5</td>
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<td>After angiotensin I</td>
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<tr>
<td>SBP (mmHg)</td>
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<td>134 ± 6</td>
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<tr>
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<td>72 ± 5</td>
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<tr>
<td>MAP (mmHg)</td>
<td>95 ± 5</td>
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<tr>
<td>After angiotensin II</td>
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<tr>
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<td>DBP (mmHg)</td>
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<td>72 ± 3</td>
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<tr>
<td>MAP (mmHg)</td>
<td>93 ± 5</td>
<td>92 ± 4</td>
<td>93 ± 3</td>
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In contrast, neither aldosterone nor prednisolone had any significant effect compared with placebo on responses to sodium nitroprusside [maximum changes in forearm blood flow: placebo, 372 ± 37%; aldosterone, 341 ± 27% (P = 0.52 compared with placebo); prednisolone, 367 ± 40% (P = 0.92 compared with placebo)] (Figure 2).

Similarly, the vasodilatory response to verapamil was not affected by either aldosterone or prednisolone (maximum changes in forearm blood flow: placebo, 219 ± 25%; aldosterone, 244 ± 43%; prednisolone, 225 ± 28%) (Figure 2).
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Figure 3 Forearm blood flow (FBF) responses to the vasoconstrictors noradrenaline (norepinephrine), angiotensin I and angiotensin II after placebo (●), prednisolone (■) or aldosterone (▲) administration.

Forearm blood flow is expressed as the percentage change in the ratio of flow in the infused arm to that in the non-infused arm relative to the baseline period preceding infusate administration. Values are means ± S.E.M.

DISCUSSION

The principal finding of the present study is that the acute short-term systemic administration of aldosterone adversely attenuates endothelial function in normal healthy men. The effect of aldosterone was mainly seen at high doses of acetylcholine, but, for reasons that are unknown, this is commonly the case in studies where acetylcholine infusions are used to assess treatment-induced changes in endothelial function [26]. This provides some evidence for an aldosterone-induced vasculopathy that could be relevant to conditions where hyperaldosteronism is a predominant feature, such as chronic heart failure or certain forms of hypertension. However, the precise mechanism behind this aldosterone-induced endothelial dysfunction is not fully clarified by the present study.

The fact that the responses to acetylcholine could be attenuated after such a short period of aldosterone administration is particularly interesting, since there are well known diurnal changes in endogenous aldosterone, such as the early morning peak (between 06:00 and 10:00 hours). Aldosterone-induced endothelial dysfunction in this early morning period could potentially contribute to the well known peak in cardiovascular events/deaths that also occur at this time of day. We have already shown that the early morning peak in aldosterone levels has a major acute effect on the autonomic nervous system [27].

The lack of any effect on responses to either nitroprusside or verapamil shows that this effect of aldosterone is not a generalized one on all vasodilator responses, but rather a specific attenuation of endothelial function. Also, in the present study, blood pressure and baseline blood flow parameters were not altered by aldosterone, which implies that non-vascular factors or systemic effects per se were not responsible for our observations in forearm vascular tissue.

Before the present study, a lot of experimental data had indirectly suggested that aldosterone may worsen endothelial function directly. As well as the basic experimental data discussed above, aldosterone levels have been shown to correlate closely with arterial compliance in humans, and this may be related in part to aldosterone producing endothelial dysfunction [28]. Also, aldosterone amplifies the response to angiotensin II in tissue culture [9–11], and chronic aldosterone blockade with spironolactone in patients with chronic heart failure already taking ACE inhibitors decreases endothelial ACE activity [2]. Interestingly, in the present study vascular responses to angiotensin I or angiotensin II were not affected by acute aldosterone infusion. This is not unduly surprising, as it is likely that any effects of aldosterone on regulation of the vascular conversion of angiotensin I to angiotensin II take time to develop, because it is a slow genomic effect requiring synthesis of enzyme or receptor. In fact, the present results agree with previous work by our group where we showed that...
changing NO activity acutely by administration of \(N^0\)-monomethyl-L-arginine (L-NMMA) did not influence vascular conversion of angiotensin I in vivo in humans [29].

Vasodilatation caused by cholinergic agents such as acetylcholine is mediated to some extent by endothelial NO synthase, although other indirect mechanisms contribute, such as vascular prostacyclin or endothelium-derived hyperpolarizing factor (EDHF). However, prostanoids have been shown previously not to alter forearm cholinergic vasodilatation in healthy individuals [30], and therefore the attenuation of endothelial vasodilator function by aldosterone is more likely to be related to alterations in stimulated nitric oxide release or EDHF, although we do not have definitive data on the relative effects of aldosterone on the various mediators that contribute to overall endothelial dysfunction.

Rather than trying to dissect out the precise mediators, we chose to study overall endothelial function by the traditional method of using the differential response to acetylcholine compared with nitroprusside (compared with other non-specific vasodilators, e.g. verapamil). After all, this is the technique that is used most widely to link endothelial function to future cardiovascular events [3–6]. Furthermore, the use of L-NMMA may not be that informative here, since there are suggestions that the main effect of aldosterone is to increase cytokines and oxidative stress, which then breaks down formed NO, whereas L-NMMA is a pharmacological tool used to investigate the different process of NO synthesis [31]. This is further complicated by the fact that NO synthase produces superoxide radicals as well as NO, and hence any effect of aldosterone on superoxide radicals might have a secondary stimulatory effect on NO synthase, which would offset any direct inhibitory effect of aldosterone on NO synthase. In other words, data from L-NMMA infusions using the present study protocol might have been hard to interpret whatever the results showed. Another reason not to include L-NMMA infusions in our protocol was a logistical one, in that subjects can only lie still enough for these studies for a limited time span, and we preferred robust data on the traditional measures of endothelial function (using acetylcholine and nitroprusside) rather than small amounts of data on a wide range of different infusions. Furthermore, L-NMMA is undoubtedly a less sensitive technique overall, because the size of the experimental signal is much greater with acetylcholine ( +300 %) than with L-NMMA (often only –30 %), especially since the magnitude of the effect of aldosterone on the 300 % dilatation was only modest anyway, and seen mainly at high acetylcholine doses.

The present study was also designed to elucidate whether any changes in vascular function could be observed following the acute administration of a single high dose of glucocorticoid in normal healthy humans. The rationale for doing this was based on the effects discussed above, plus the fact that glucocorticoids have been shown to reduce endothelial NO activity, although, interestingly, not by inhibiting NO synthase per se, but rather by inhibiting tetrahydrobiopterin, an essential cofactor for the efficient activity of all isoforms of NO synthase [32]. It is worth commenting on why we used oral prednisolone rather than intravenous hydrocortisone. We did this for the same reason that the British Thoracic Society guidelines prefer the former to the latter, which is that intravenous hydrocortisone requires the use of a diluent, which is associated with very unpleasant perianal itching and burning, and we thought this unacceptable in a research study. Furthermore, oral prednisolone and intravenous hydrocortisone are generally considered to be equally effective ways of exploring the effects of glucocorticoids in humans.

In our study, we showed that a single oral dose of 50 mg of prednisolone did not adversely affect any vascular responses. A recent study showed a non-significant trend towards the attenuation of acetylcholine-induced vasodilatation after 2 days of glucocorticoid treatment [33]; this only reached statistical significance after 5 days. This would concur with our own data, which would imply that, although glucocorticoids can produce detrimental effects in endothelial function, these are exerted over a period of time rather than acutely. This suggests that any adverse vascular effect of glucocorticoids is likely to be a slower genomic effect rather than a fast non-genomic effect. Our data also provide no support for the possibility that the early morning increase in cortisol contributes to the early morning increase in cardiovascular events/death, although they do not exclude this possibility. Clearly, this contrasts with aldosterone, whose ability to acutely produce endothelial dysfunction could in theory contribute to the diurnal changes in cardiovascular events and mortality. More work is now required to explore this possibility, especially since we have already documented an acute beneficial effect of spironolactone on sympathovagal balance in the 06:00-10:00 hours time period [27], and many investigators now consider that sympathovagal balance is determined largely by vascular NO [34,35]. All this leads to the credible hypothesis that acute increases in aldosterone in the early morning might adversely affect endothelial function, which then also alters autonomic function, both of which might lead to early morning adverse cardiovascular events.

In conclusion, our results demonstrate that aldosterone can acutely impair endothelial dysfunction in humans in vivo, as suggested by previous experimental data. This could have significant implications in conditions where hyperaldosteronism and endothelial dysfunction occur concomitantly, such as chronic heart failure and in hypertensive patients with high aldosterone/renin ratios [36]. This implies that aldosterone-induced endothelial
dysfunction may be mediated, at least in part, by a fast non-genomic effect. It also opens up the possibility that diurnal changes in endogenous aldosterone levels might contribute to the well known morning peak in cardiovascular events and death.

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REFERENCES


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