Influence of Acute and Chronic Mineralocorticoid Excess on Endothelial Function in Healthy Men

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Abstract—Aldosterone has rapid nongenomic effects in the human vasculature. However, data are not uniform and little is known about chronic effects of aldosterone. Therefore, we investigated acute and chronic effects of elevated aldosterone levels on endothelial function in the forearm vasculature of healthy men. In a first crossover study, the effects of arterial aldosterone infusion in ascending doses (3.3 to 55 pmol/min per 1000 mL forearm volume) on forearm blood flow were investigated in 8 healthy men (26±2 years). In a second study, endothelium-dependent (acetylcholine; 0.08, 0.275, and 2.75 μmol/min per 1000 mL) and endothelium-independent (sodium nitroprusside 0.02 μmol/min per 1000 mL) vasodilation and basal nitric oxide formation (forearm blood flow response to blockade by Nω-monomethyl-L-arginine 8 μmol/min per 1000 mL) were tested in 10 healthy men (age 30±5 years) at baseline, during infusion of 55 pmol/1000 mL per min aldosterone (acute effects), and after 0.3 mg/d oral fludrocortisone for 2 weeks (chronic effects) on separate days. Forearm blood flow was assessed by venous occlusion plethysmography. No change in forearm blood flow was seen with aldosterone infusion alone. Acute coinfusion of aldosterone increased vasodilation to sodium nitroprusside by 93% (P<0.01) and to acetylcholine by 60% (P=0.14). Response to Nω-monomethyl-L-arginine did not change. After 2 weeks of oral fludrocortisone, response to acetylcholine was enhanced by 72% compared with baseline (P=0.03). Additionally, response to Nω-monomethyl-L-arginine was enhanced by 80% compared with baseline (P=0.05). Aldosterone acutely enhances vasodilation to exogenous nitric oxide whereas mineralocorticoid excess for 2 weeks enhances basal nitric oxide bioactivity and improves endothelium-dependent, nitric oxide–mediated vasodilation in the forearm vasculature of healthy men. (Hypertension. 2007;50:1-7.)

Key Words: aldosterone ■ endothelial function ■ mineralocorticoid excess ■ forearm vasculature ■ nitric oxide

Aldosterone has been claimed to lead to endothelial dysfunction (review, see Brown1), a condition related to development of cardiovascular disorders and to poor prognosis.2 However, studies of aldosterone effects on endothelial function led to discrepant findings, which may be related, at least in part, to inhomogeneity of the populations studied. Thus, studies in healthy subjects showed no detrimental effects of aldosterone on endothelial function and no positive effect of aldosterone inhibition,3-5 whereas populations with established cardiovascular diseases showed negative effects of aldosterone and positive effects of spironolactone therapy.5-11 Still, other factors may be of importance as effects of aldosterone on endothelial function are not homogenous even in a healthy population.12 Dosages of aldosterone,3,4,6 concomitant drug use,13 as well as the vascular bed investigated4,14-16 may influence the effects observed.

Furthermore, little is known about chronic endothelial effects of aldosterone that could indicate a primary and direct role of aldosterone in development of cardiovascular diseases. In patients with hyperaldosteronism diminished flow-mediated dilation was found, indicating impaired endothelial function compared with hypertensive patients without elevated aldosterone.11 However, it is not known whether these results represent endothelial dysfunction as the result of a direct aldosterone effect on the vasculature or a secondary effect attributable to more substantial hypertension.

Genomic effects take several days to develop and become relevant. Therefore, if genomic effects play an important role in the development of a postulated aldosterone-mediated endothelial dysfunction, findings in a chronic setting may differ from those in the acute setting. We, therefore, investigated vascular and endothelial effects of chronic mineralocorticoid excess compared with acute aldosterone administration in pathophysiologically relevant doses in a homogenous group of healthy men.
Methods

Study Design
The study was divided into 2 sub-studies:

In study A including 8 volunteers, we investigated the acute effects of aldosterone on forearm blood flow. In study B including 10 volunteers (others than in study A), we investigated the acute and chronic effects of aldosterone on endothelium dependent and independent vasodilatation and basal nitric oxide bioactivity.

The trial was approved by the local ethics committee and was in accordance with institutional guidelines. All volunteers gave written informed consent prior to study inclusion.

Study A: Acute Effect of Different Doses of Aldosterone on Vascular Tone
After baseline measurements, intraarterial infusion of ascending doses of aldosterone (rates of 3.3, 11, 33, 55 pmol/1000 mL per min for 15 minutes each) were performed and compared with saline infusion using similar infusion rates in all 8 volunteers. Thereafter, aldosterone was infused at a dose of 11 pmol/1000 mL per min over 2 hours to control for the stability of a potential effect. Forearm blood flow was measured and intraarterial blood pressure and heart rate were recorded after a resting period of 30 minutes and after each infusion step. During continuous aldosterone infusion, measurements were repeated every 30 minutes.

Study B: Acute and Chronic Effects of Aldosterone on Endothelial Function
Forearm blood flow was measured in response to different vasoactive substances for evaluation of endothelium-dependent (infusion of acetylcholine [Ach]) and endothelium-independent (infusion of sodium nitroprusside [SNP]) vasodilation and blockade of basal nitric oxide (NO) formation using Nω-monomethyl-L-arginine (L-NMMA). Infusions were performed at baseline and repeated during coinfusion of 55 pmol/1000 mL per min aldosterone (acute effects) in random order on 2 different days (single blinded). Subjects were then given oral fludrocortisone (0.3 mg/d) for a period of 2 weeks and measurements were repeated (chronic effects). Forearm blood flow, intraarterial blood pressure and heart rate were recorded before and immediately after each infusion.

Vasoactive Substances
After baseline measurements, the drugs were infused according to the following schedule:

1. Ach: 3 ascending dosages of 0.08, 0.275, and 2.75 μmol/min per 1000 mL forearm volume (=15, 50, and 500 μg) for 5 minutes each, followed by a wash-out period of 30 minutes.
2. NO synthase blocker L-NMMA at a dosage of 8 μmol/min per 1000 mL forearm volume (=2 mg) during 5 minutes.
3. L-arginine at a dosage of 40 μmol/min per 1000 mL forearm volume (=8.5 mg) during 7 minutes to reverse the effects of L-NMMA, followed by a waiting period of 45 minutes.
4. SNP at a dosage of 0.02 μmol/min per 1000 mL forearm volume (=6 μg).

These drug dosages affect only regional, but not systemic blood flow.17

Plethysmography
Forearm volume was measured as previously described.17 A 3F catheter was inserted into the brachial artery of the nondominant arm under local anesthesia for drug administration, blood sampling, and continuous recording of arterial blood pressure. Heart rate was monitored from the continuously recorded ECG during the whole study period.

Venous occlusion technique was used to measure forearm blood flow (FBF) in both arms with a mercury insilastic strain gauge plethysmograph as described previously.17 The strain gauge was placed approximately 5 cm below the elbow on the forearm and coupled to an electronically calibrated plethysmograph (EC4; Hokanson). A blood pressure cuff applied proximal to the elbow was inflated to 40 mm Hg using a rapid cuff inflator (EC10; Hokanson) to occlude venous backflow from both forearms. A cuff around the wrist was inflated to 50 mm Hg above systolic blood pressure at least 1 minute before measurements to interrupt circulation to the hand and to eliminate the influence of arteriovenous shunts. Plethysmograph recordings were analyzed using a digitized board and a suitably programmed computer. The mean value of 4 recordings obtained within 1 minute was taken for statistical analysis. FBF was corrected for infusion rates. Additionally, forearm vascular resistance was calculated by dividing mean arterial pressure by FBF. Forearm vascular resistance (FVR) was calculated by dividing mean arterial pressure, obtained immediately after flow measurements, by FBF and is expressed in arbitrary units (U).

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Drugs were administered on the nondominant (experimental) forearm. FBF measurements on the dominant (control) arm were control measurements for potential systemic drug effects. Control values for the respective intervention were obtained from the FBF in the experimental arm preceding each intervention. Administration of these drugs was performed using constant speed infusion pumps (Perfusor Secura FT) with volume rates between 30 and 90 mL per hour.

Study Volunteers
Healthy nonsmoking male were recruited for the study. Volunteers were asked to refrain from caffeine containing beverages for at least 12 hours and from alcohol or foods containing high levels of vitamin C (eg, fruit juices) for 24 hours before the investigations.

Measurement of Plasma Hormones
Plasma samples drawn from the infusion arm, as well as the contralateral arm were taken into EDTA tubes, centrifugated, and stored at −80°C until analysis. Plasma renin activity was determined using a trapping assay and angiotensin II was measured by RIA technique as previously described.18 Active renin concentration was measured by a commercial kit based on immunoradiometric methodology (CisBio). Aldosterone was analyzed using a commercial kit based on ELISA technique (DRG).

Statistical Analysis
Data are presented as means±SD unless otherwise indicated. Statistical analyses were done using the statistical package SPSS for Windows 14.0. Dose response curves were done by multifactorial general linear model. Because of skewed distribution of FVR values, they were compared using nonparametric testing. Further comparison between groups was done by 2-tailed paired t test or Wilcoxon test, as appropriate, with adjustment of the significance level for multiple comparisons. A probability value of ≤0.05 was considered statistically significant.

Results
Mean age was 26±2 years in study A and 30±5 years in study B, respectively. At rest, mean resting blood pressure was 82±7 mm Hg and mean heart rate 66±14 beats per minute (bpm), respectively, in study A. In study B, mean blood pressure was 84±11 mm Hg and mean heart rate 58±28 bpm, respectively. Hemoglobin levels were within the normal range in all subjects (data not shown). After 2 weeks treatment with oral fludrocortisone, we found a trend toward the expected increase of serum sodium (from 140.2±1.5 mmol/L to 141.4±2.0 mmol/L, P=0.08) and a significant decrease of serum potassium (from 3.86±0.25 mmol/L to 3.48±0.13 mmol/L, P<0.01). No alteration of urinary so-
Dium excretion was found (186±109 mmol versus 181±76 mmol, P=0.76). None of the brachial artery infusions caused any systemic hemodynamic effects and forearm blood flow did not change in the control arm (data not shown). In particular, blood pressure (mean 86±9 mm Hg versus 85±7 mm Hg, P=0.93) and heart rate (57±10 bpm versus 59±9 bpm, P=0.32) did not change with acute infusion of aldosterone.

Study A: Effects of Aldosterone Infusion

Compared with infusion of saline 0.9%, neither brachial artery infusion of ascending dosages nor prolonged infusion of aldosterone caused statistically significant changes of FBF (Figure 1). By dividing aldosterone infusion rate of 55 pmol/min/1000 mL forearm volume by resting forearm blood flow and considering an estimated hematocrit of 40%, an approximate increase in the plasma concentration in the arterial blood of 1.72±0.55 pmol/mL locally was calculated. This corresponds to plasma levels as seen in patients with different disorders such as heart failure or hyperaldosteronism. Systemic concentrations did not change. In fact, arterial (infusion interrupted for ~15 sec for blood sampling) aldosterone concentrations were identical at baseline and at highest infusion rate (0.26±0.07 pmol/mL versus 0.26±0.10 pmol/mL, P=0.99). Venous plasma concentration of the treatment arm increased to 2.14±0.68 pmol/mL at highest dose, which was in the expected range, whereas it remained unchanged in the control arm (0.25±0.11 pmol/mL).

Study B: Effects of Acute and Short Term Aldosterone Administration on Vascular Reactivity

Brachial artery infusion of SNP (Figure 2) caused the expected increase of forearm blood flow. Both acute infusion of aldosterone or 2 weeks treatment with fludrocortisone increased this endothelium-independent vasodilation significantly (P<0.01 and P=0.03, respectively). However, this increase was significantly larger with infusion of aldosterone compared with fludrocortisone (93% versus 51% increase in FBF, P=0.02). SNP decreased FVR at baseline from 24.7±8.0 to 7.2±2.3U, with acute coinfusion of aldosterone from 20.7±7.7 to 4.5±1.3U (P<0.01 compared with baseline), and after 2 weeks of fludrocortisone from 23.3±7.3 to 6.2±2.8U (P=0.13 compared with baseline). The endothelium-dependent vasodilator acetylcholine led to a dose-dependent increase in forearm blood flow (Figure 3). Two weeks of fludrocortisone treatment enhanced the vasodilation induced by Ach significantly (P=0.03). The FVR fell accordingly (baseline: rest 32.1±10.3, Ach0.08 26.4±12.8, Ach0.275 6.1±4.4, Ach2.75 3.2±2.3 U; fludrocortisone 24.9±10.3, 18.3±6.5, 2.9±1.1, 2.1±0.8, P<0.01). The enhancing effect of aldosterone infusion on response to Ach did not reach statistical significance (P=0.14), but the fall in FVR was significantly different to baseline (29.5±13.5, 10.7±6.5, 3.7±2.7, 2.2±0.8 U, P<0.01). There was no statistical difference when comparing the effects of aldosterone with baseline.
Discussion

Brachial artery infusions of aldosterone in (patho-)physiological doses did not affect forearm blood flow, but enhanced the vasodilator response to exogenous NO. It had no significant effect on endothelium-dependent NO-mediated vasodilation or basal NO bioactivity in healthy young men. In the setting of artificially induced mineralocorticoid excess by oral intake of fludrocortisone for 14 days, endothelium-dependent vasodilation of the forearm vasculature was enhanced and possibly also basal NO availability. Therefore, our results argue against a primary role of aldosterone in the development of endothelial dysfunction, at least over a limited time period of 14 days and in the absence of cardiovascular risk factors or endothelial damage.

Although our data are compatible with enhanced stimulated NO bioactivity, we can only speculate on the underlying mechanism(s) of enhanced endothelial function during short-term mineralocorticoid excess. The nongenomic effects of aldosterone entail a change in intracellular calcium via a not yet further classified membrane-receptor and stimulation of phosphatidylinositol 3-kinase (PI-3-K) via the classical mineralocorticoid-receptor. Binding of aldosterone to the mineralocorticoid-receptor leads to stimulation of PI-3-K and subsequent stimulation of endothelial NO-synthase and, thereby, to vasodilation. This pathway, depending on an intact endothelium, can be blocked by spironolactone, but is independent of transcription factors. Liu et al investigated underlying mechanisms of aldosterone actions in aortic ring preparations of normo- and hypertensive rats and in cell cultures. They found an increase in NO-synthase activity within minutes after administration of aldosterone in physiological doses suggesting a nongenomically mediated effect. This increase was mediated by PI-3-K and was absent when aldosterone was administered after pretreatment with spironolactone. They also found an inhibitory effect of aldosterone on phenylephrine-mediated vasoconstriction, an effect that was abolished by L-NMMA, suggesting a central role of NO-synthase. In endothelium denuded preparations, they found no effects of aldosterone. In contrast, in aortic ring preparations of hypertensive rats, an enhancement of phenylephrine-mediated vasoconstriction after aldosterone-application was found. Wehling et al found an increase of intracellular calcium in vascular smooth
muscle cells and vascular endothelial cells after application of aldosterone suggesting a direct vasoconstrictor effect on vascular smooth muscle cells together with an endothelium mediated vasodilation. This suggests that aldosterone effects depend on endothelial function. Thus, with endothelial dysfunction, the vasodilatory effect of aldosterone mediated by the endothelium may be reduced resulting in a vasoconstrictor effect. Compatible with an important role of NO-synthase, brachial artery infusion of aldosterone did not cause vasoconstriction in another study with healthy volunteers, indicating the use of only locally active concentrations of aldosterone for 2 weeks without suppression of renin and angiotensin-II. Importantly, the findings of the acute study, where confounding factors are basically excluded because of unchanged blood pressure, electrolytes, and activation of the renin–angiotensin system, went in parallel with those of the chronic study, making it unlikely that confounding factors explain the findings of 2 week mineralocorticoid excess. Infusion of aldosterone did not lead to suppression of renin and angiotensin-II plasma levels, indicating the use of only locally active concentrations of aldosterone in the acute setting.

We cannot exclude a type-II error because of the relatively small sample size. In addition, only a trend toward enhanced vasodilation with coinfusion of aldosterone and acetylcholine compared with acetylcholine alone was observed. However, a neutral finding still supports the main finding of lacking evidence for aldosterone induced endothelial dysfunction.

The overall vascular effect of aldosterone may also depend on the dose used: Liu et al found vasodilation in physiological doses, but no effect in supraphysiologic doses. In agreement with another study (aldosterone infusion at 28 to 280 pmol/L), we used (patho-) physiological doses of aldosterone and found no significant effect. With lower doses, vasoconstriction was observed, and Schmidt et al using pharmacological doses (1.4 mmol/min) found vasodilation. The reason for these discrepancies is not clear.

Finally, the level of oxidative stress may play a role as NO is easily scavenged by reactive oxygen substrates (ROS). In an unstressed cell, low levels of ROS result in vasodilation by stimulation of PI-3-K and reduction in intracellular calcium levels, whereas high levels of ROS may lead to cell damage and vasoconstriction. Aldosterone leads to formation of ROS via stimulation of NADPH oxidase. This effect can become deleterious in stressed cells and presumably in the presence of high aldosterone levels, where increased formation of ROS can lead to an uncoupling of endothelial NO-synthase, thereby further reducing NO synthesis and enhancing ROS formation. This could be one mechanism why Abiose et al found an improved flow-mediated dilation after treatment with spironolactone in heart failure patients.

The influence of aldosterone antagonism on nongenomic effects is another unsolved issue. Whether eplerenone leads to more complete inhibition of nongenomic aldosterone effects than spironolactone by blocking a not yet defined additional aldosterone receptor remains uncertain. To answer this question, the postulated receptor should be defined first.

Our current as well as previous experimental and clinical data suggest that the effects of aldosterone depend on aldosterone concentration, endothelial NO–mediated vasodilator function, and on the level of oxidative stress.

**Limitations**

Our study was conducted over a time period of 14 days. We cannot exclude a primary role of hyperaldosteronism on the development of endothelial dysfunction over a longer time period, but with a longer period of hyperaldosteronism, it becomes even more difficult to differentiate the role of aldosterone from confounding effects, particularly increase in blood pressure. We cannot exclude that in the setting of mineralocorticoid excess the rise in blood pressure itself caused alteration of responses to vasoactive substances, although worsening of endothelial function rather than improvement would have been expected. Suppression of renin and angiotensin II was seen with mineralocorticoid excess for 2 weeks. This may have counterbalanced mineralocorticoid effects, but we found the expected increase in blood pressure and alterations in serum electrolyte, proving systemic effects of fludrocortisone. It is not possible to exclude that changes in potassium or other neurohumoral systems influenced by mineralocorticoid excess (eg, reduced activity of sympathetic nervous system) may have influenced the findings of this study. However, potassium was not found to influence endothelium dependent vasodilation in healthy subjects and to even improve it in hypertensive subjects. Thus, the reduction in potassium as seen with mineralocorticoid excess for 2 weeks cannot explain the improvement in endothelial function. We cannot exclude a positive effect of the reduction in angiotensin II on endothelial function. However, we cannot think of a setting in human to achieve only locally active concentrations of aldosterone for 2 weeks without suppression of renin and angiotensin-II. Importantly, the findings of the acute study, where confounding factors are basically excluded because of unchanged blood pressure, electrolytes, and activation of the renin–angiotensin system, went in parallel with those of the chronic study, making it unlikely that confounding factors explain the findings of 2 week mineralocorticoid excess. Infusion of aldosterone did not lead to suppression of renin and angiotensin-II plasma levels, indicating the use of only locally active concentrations of aldosterone in the acute setting.
Fludrocortisone was used instead of aldosterone itself to mimic short term mineralocorticoid excess. The dose chosen was to achieve pathophysiologically important levels in a chronic setting, which seems to be the case because of the observed rise in blood pressure and small changes in electrolytes. Still, we did not perform a dose-finding study in the chronic setting, nor did we measure fludrocortisone levels. Further, we did not control for physical activity during the fludrocortisone study, which potentially might have influenced the results, although most likely not substantially.

Finally, we used the forearm model to assess vascular aldosterone effects, and different findings might be possible in other vascular beds as shown by other groups.16,38

**Conclusion**

Mineralocorticoid excess for 2 weeks resulted in improved endothelial NO–mediated vasodilators function, possibly by stimulation of endothelial NO synthase in healthy men. No negative effects of aldosterone, either acutely or chronically, were found.

**Perspectives**

In line with previous findings, our study suggests that nongenomic aldosterone effects are not harmful on the intact vasculature. Even after mineralocorticoid excess for 2 weeks, when both nongenomic and genomic effects can be expected, the effects on the vasculature were not harmful, but even potentially beneficial. These findings argue against a primary role of aldosterone in the development of endothelial dysfunction, and as a consequence, atherosclerosis. However, after development of endothelial dysfunction, the beneficial effects may no longer be present or masked by deleterious effects. Further studies in human are needed to test this concept of preponderance of beneficial and harmful effects of aldosterone on the endothelium subject to the underlying condition and to test the potential of therapeutic interventions in these settings.

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**Disclosures**

None.

**References**

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