Rapid Nongenomic Effects of Aldosterone on Human Forearm Vasculature

Bernhard M.W. Schmidt, Sebastian Oehmer, Christian Delles, Renate Bratke, Markus P. Schneider, Arnfried Klingbeil, Erwin H. Fleischmann, Roland E. Schmieder

Abstract—The impact of aldosterone in cardiovascular disease and hypertension has recently gained new interest. Aldosterone is now suggested to be a far more common cause of hypertension than previously believed and has been linked to myocardial fibrosis, independent of its hypertensive effects. Finally, rapid nongenomic aldosterone effects have been proposed to be important in hypertension, in addition to its genomic effects. Forty-eight healthy male volunteers were examined in a randomized, placebo-controlled, double-blind crossover trial to elucidate the rapid nongenomic, vascular effects of aldosterone in humans. Forearm blood flow was measured by venous occlusion plethysmography. First, aldosterone (500 ng/min) and placebo were infused into the brachial artery for 8 minutes. The volunteers then received ascending doses of acetylcholine, \( \Delta^G \)-monomethyl-L-arginine (L-NMMA), sodium nitroprusside, or phenylephrine. Aldosterone increased forearm blood flow \((P<0.001, \text{ANOVA})\). The maximum effect was an increase in forearm blood flow with aldosterone of 7.9±2.6% compared with 0.1±1.9% with placebo treatment after 8 minutes. With aldosterone, L-NMMA induced a greater vasoconstriction \((P<0.05, \text{ANOVA})\), sodium nitroprusside induced an attenuated vasoconstriction \((P<0.01, \text{ANOVA})\), and phenylephrine induced an exaggerated vasoconstriction \((P<0.01, \text{ANOVA})\) within minutes as compared with placebo. These data suggest that aldosterone acts through rapid nongenomic effects at the endothelium by increasing NO release and at the vascular smooth muscle cells by promoting vasoconstriction. This is consistent with in vitro data showing an increase in intracellular calcium in both cell types. Disturbances of these aldosterone effects on both levels might be important in promoting hypertension. (Hypertension. 2003;42:156-160.)

Key Words: aldosterone ■ hypertension, essential ■ vasculature ■ endothelium

The impact of aldosterone in cardiovascular disease and hypertension has recently gained new interest. Aldosterone is now suggested to be a far more common cause of hypertension than previously believed, accounting for up to 10% in unselected patients. In addition, aldosterone has been linked to myocardial fibrosis and thus hypertensive heart disease, independent of its hypertensive effects (for review, see Schmidt and Schmieder). Finally, besides the classic genomic aldosterone effects, rapid nongenomic aldosterone effects have been proposed to be of importance in human essential hypertension.

Rapid nongenomic aldosterone effects are characterized by their rapid onset of action (within minutes), an insensitivity to inhibitors of transcription (eg, actinomycin D), protein synthesis (eg, cycloheximide), and to antagonists of the type I mineralocorticoid receptor (eg, spironolactone) (for review, see Falkenstein et al).

Nongenomic aldosterone effects have been extensively characterized in vitro. After binding to a putative membrane receptor, a second-messenger cascade involving inositol 1,4,5-triphosphate, diacylglycerol, and protein kinase C is activated. In vascular smooth muscle cells and porcine aortic endothelial cells, a rapid increase of intracellular \( \text{Ca}^{2+} \) was demonstrated. In addition, an increase of intracellular cAMP levels within 1 minute in porcine coronary vascular smooth muscle cells leading to an increased CREB (cAMP-response element binding protein) phosphorylation has been shown. Furthermore, synergistic effects of aldosterone and isoproterenol on CREB phosphorylation exist. For the in vivo situation, these data suggest a vasoconstrictive response (by vascular smooth muscle cells) as well as vasodilative response (by endothelial cells).

In addition to these in vitro data, nongenomic aldosterone effects have been shown in humans. Klein and Henk demonstrated an increase of systemic vascular resistance 5 minutes after injection of 0.5 mg aldosterone in humans.

Recent studies performed with modern invasive and noninvasive methods have confirmed the latter data. In addition to the early vasoconstrictive effect of aldosterone, a pronounced postprandial vasodilation could be demonstrated for this steroid. This diversity of nongenomic cardiovascular aldosterone effects has been confirmed in a study that used...
pretreatment with a β-receptor antagonist and a β-receptor agonist. After pretreatment with the β-receptor antagonist esmolol, aldosterone increased blood pressure; after pretreatment with the β-receptor agonist dobutamine, aldosterone decreased blood pressure.14

Further evidence for this diversity comes from very recent ex vivo studies in rats and rabbits. Barbato et al15 showed that aldosterone decreases vascular resistance in the coronary vasculature through a nongenomic effect, whereas Arami and colleagues16 found vasoconstriction in afferent and efferent renal arterioles.

We conducted this clinical trial to further elucidate the rapid nongenomic, vascular effects of aldosterone in humans and to differentiate between potential endothelial effects and direct action to vascular smooth muscle cells.

Methods

Study Volunteers
Forty-eight healthy male volunteers were enrolled in the study. All volunteers had a medical examination within 2 weeks before inclusion into the study. The examination consisted of medical history, a physical examination, a 12-lead ECG, and determination of clinical laboratory parameters. All subjects gave their written informed consent to participate in the study.

Study Procedures
The study was designed as a randomized, placebo-controlled, double-blind, crossover trial. It was conducted in accordance with the International Conference on Harmonization Guidelines for Good Clinical Practice (ICH-GCP) and the Declaration of Helsinki after approval by the institutional review board of the Friedrich-Alexander University Erlangen-Nuremberg, Germany.

Study Design
All 48 volunteers received aldosterone and placebo on 2 different days in random order at least 1 week apart, according to the crossover design. Twelve volunteers then received either acetylcholine (for testing of endothelium-dependent vasodilation), N\textsuperscript{ω}-monomethyl-L-arginine (L-NMMA, for assessment of basal NO release), sodium nitroprusside (for testing of endothelium-independent vasodilation), or phenylephrine (as direct α\textsuperscript{1}-receptor-mediated vasoconstrictor). Study procedures were finished within 30 minutes to ensure rapid nongenomic origin of the observed effects.

Assessment of Forearm Blood Flow
Forearm blood flow (FBF) and responses to different vasoactive drugs were assessed by forearm plethysmography, as reported previously.17

After baseline measurement during constant flow of normal saline, aldosterone or placebo was administered for 8 minutes. FBF was obtained from an average of measurements recorded for 9 seconds of every 15 seconds during each minute. After 8 minutes, one of the following substances was administered (each dose was infused intra-arterially for 4 minutes): (1) acetylcholine at sequential doses of 3, 12, 24, and 48 μg/min; (2) L-NMMA at sequential doses of 2, 4, 8, and 16 μmol/min; (3) sodium nitroprusside at sequential doses of 1.6, 3.2, 6.4, and 12.8 μg/min; and (4) phenylephrine at sequential doses of 2.5, 5, 10, and 20 μg/min.

Study Drugs
Aldosterone was prepared as mixed micelles. The placebo preparation consisted of mixed micelles only (both from Clinalfa). Acetylcholine and L-NMMA were also from Clinalfa. We used Niprus (Schwarz Pharma) as source of sodium nitroprusside and Neo-Synephrine HCl (Abbott Laboratories) as source of phenylephrine, respectively.

Aldosterone Measurement
Aldosterone was measured by a commercially available test kit (Aldosterone MAIA, BioChem ImmunoSystems). The limit of detection of this assay is 6.0 pg/mL. Interassay variance is 6.4%; intra-assay variance is 5.4%.

Statistical Analysis
All statistical analyses were done with the use of the SPSS software package (SPSS for Windows v10.0, SPSS Inc).

For statistical inference, the percent change from baseline was calculated for each variable and each measurement time point. Baseline value of dose-response curves was the mean of the last 2 measurements of FBF during the preceding aldosterone/placebo infusion.

Analysis of time-response and dose-response curves was done by multifactorial ANOVA with the factors of treatment, period and time, or dose, respectively. Further analysis was conducted with the use of paired t tests. The level of significance was set to P<0.05 (2-tailed). All values are given as mean±SEM.

Results

General Characteristics
All volunteers were healthy according to standard laboratory parameters and physical examination, including resting blood pressure and ECG. The mean age was 25.2±0.5 years; mean height was 181.0±1.0 cm; and mean weight was 72.8±1.3 kg. Resting blood pressure was 122.7±1.3 mm Hg systolic and 77.0±1.5 mm Hg diastolic, at a heart rate of 63.2±1.7 bpm. Sodium and potassium levels were within the normal range (141.2±0.3 mmol/L and 3.93±0.03 mmol/L, respectively).

No systemic effects of aldosterone or the drugs infused occurred as assessed by blood pressure and heart rate during the study (Table).

Effect of Aldosterone Infusion
Aldosterone and placebo were infused for 8 minutes without further intervention. Aldosterone increased FBF (P<0.001, ANOVA). Looking at single time points, this effect was statistically significant first after 4 minutes and then again after 7 and 8 minutes. The maximum effect was an increase in FBF with aldosterone of 7.9±2.6% compared with 0.1±1.9% with placebo treatment after 8 minutes (Figure 1).

Effect of Acetylcholine and L-NMMA
Acetylcholine was infused in ascending doses to test for endothelium-dependent vasodilation. The dose-response curve of acetylcholine was not significantly altered by aldosterone compared with placebo infusion (ANOVA). At the 2 lower acetylcholine doses, there was a slight tendency to an increased vasodilatory response; at higher doses, there was a decreased vasodilation with aldosterone infusion compared with placebo (Figure 2A).

L-NMMA was infused to test for basal nitric oxide bioavailability. Along with aldosterone, L-NMMA induced a greater reduction in FBF (P<0.05, ANOVA) than with placebo. Looking at single doses, this effect was statistically significant with the lowest dose used (2 μmol/min) (P<0.05, paired t test) and diminished with higher doses of L-NMMA (Figure 2B).
Systemic Hemodynamics During the Study Procedure

<table>
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<th>Condition</th>
<th>Parameter</th>
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<th>Placebo</th>
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<td>Diastolic BP</td>
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<tr>
<td></td>
<td>Heart rate</td>
<td>56.6±1.2</td>
<td>56.7±1.1</td>
</tr>
<tr>
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<tr>
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<td>Heart rate</td>
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<td></td>
<td>Heart rate</td>
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<td>Heart rate</td>
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<td>Heart rate</td>
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<tr>
<td></td>
<td>Heart rate</td>
<td>57.5±0.9</td>
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</tr>
</tbody>
</table>

BP indicates blood pressure; aldosterone 8', values after 8 minutes of aldosterone or placebo infusion; end of study, pooled analysis of all BP readings at the highest dose of acetylcholine; and L-NMMA, nitroprusside or phenyl-
ephrine.

No statistically significant changes occurred between aldosterone and placebo treatment.

Effect of Sodium Nitroprusside and Phenylephrine

Sodium nitroprusside was infused to test for endothelium-
independent vasodilation. Compared with placebo, vasodila-
cation caused by sodium nitroprusside was decreased by aldo-
sterone (P<0.01, ANOVA). Looking at single doses, this effect was statistically significant with the use of 6.4 μg/min (P<0.05, paired t test) (Figure 3A).

Phenylephrine causes vasocostriction through α1-
adrenoceptors. This vasocostriction was exaggerated with aldosterone compared with placebo (P<0.01, ANOVA).

Figure 1. Effect of aldosterone versus placebo infusion alone on FBF. Difference between aldosterone and placebo was statistically significant by ANOVA, as shown by inserted probability value. *P<0.05, paired t test.

Figure 2. A, Acetylcholine dose-response curve showing effect of aldosterone or placebo infusion on FBF. B, L-NMMA dose-
response curve showing effect of aldosterone or placebo infu-
sion on FBF. Difference between L-NMMA dose-response curve with aldosterone or placebo was statistically significant by ANOVA, as shown by inserted probability value. *P<0.05, paired t test.

Looking at single doses, this effect was significant with the use of 2.5 and 5 μg/min (P<0.05, paired t test) (Figure 3B).

Aldosterone Levels in the Forearm

Aldosterone levels at baseline were 107±15 and 103±14 pg/mL in the aldosterone and placebo periods, respectively. After aldosterone/placebo infusion, aldosterone concentrations in the forearm were found to be 6535±1014 pg/mL for the aldosterone period versus 97±13 pg/mL for the placebo period.

Discussion

Rapid nongenomic aldosterone effects have repeatedly been shown in humans. Based on in vitro studies, either vasocon-
striction or vasodilation is possible. The increase of intracel-
ular calcium in vascular smooth muscle cells should cause vasocostriction, whereas the increase of intracellular calcium in endothelial cells and the increase of cAMP in vascular smooth muscle cells should cause vasodilation. In

Figure 3. A, Sodium nitroprusside dose-response curve showing effect of aldosterone or placebo infusion on FBF. Difference between nitroprusside dose-response curve with aldosterone or placebo was statistically significant by ANOVA, as shown by inserted probability value. *P<0.05, paired t test. B, Phenyleph-
rine dose-response curve showing effect of aldosterone and placebo infusion on FBF. Difference between phenylephrine dose-
response curve with aldosterone or placebo was statistically significant by ANOVA, as shown by inserted probability value. *P<0.05, paired t test.
studies in humans, a vasoconstrictive response has primarily been observed, as assessed by measurements of total peripheral resistance.\textsuperscript{11–13} However, vasodilatory effects have also been observed.\textsuperscript{13,14} This diversity might in part be explained by interactions with the adrenergic system.\textsuperscript{14} Another explanation for the observation of these differing effects may be a diversity of responses to aldosterone by different vascular beds. This is supported by recent animal experiments. In rat hearts, vasodilation of the coronary arteries was observed with aldosterone,\textsuperscript{15} whereas in afferent and efferent arterioles from rabbit kidney, vasoconstriction could be demonstrated.\textsuperscript{16}

The results obtained by this study are in line with these theoretical considerations: the interventions affecting the vascular smooth muscle cell show a tendency to vasoconstriction; the endothelium-independent vasodilatation by sodium nitroprusside was attenuated by aldosterone; and the $\alpha_1$-receptor–mediated vasoconstriction by phenylephrine was enhanced by aldosterone. This is consistent with an increase in intracellular calcium in vascular smooth muscle cells, which shifts the vascular tone to a vasoconstrictive reaction. The endothelium-dependent vasodilatation with acetylcholine is unchanged. NO, as shown by the sodium nitroprusside infusion, causes a decreased vasodilatory response when aldosterone is present. This would suggest that more NO is released, thus both effects counteract each other. This is confirmed by the L-NMMA infusion, which shows an increased effect with aldosterone reflecting higher activity of the endothelial NO synthase.

The effects modulating the endothelial cell response are dose-dependent: at the 2 lower acetylcholine doses, there is a tendency toward increased vasodilatation. This is consistent with the net effect of pure aldosterone infusion, which is slight vasodilatation. Obviously, with lower acetylcholine doses as well as without acetylcholine, the vasodilatory effect is more pronounced than with higher doses.

The extent to which aldosterone changed FBF in this study is also consistent with previous experiments. Rapid nongenomic aldosterone effects are rather small and thus have been proposed to represent a “fine-tuning system.”\textsuperscript{18} Its physiological function might be the modulation of cardiovascular processes within defined limits. On the other hand, it might act as a sensitizer, as has been shown in vitro for isoproterenol effects.\textsuperscript{10}

The aldosterone plasma concentrations reached in the forearm after intra-arterial application of 500 ng/min aldosterone are supraphysiological but are still far lower than concentrations expected to induce unspecific membrane effects ($\geq 10 \mu$mol/L).\textsuperscript{4} Furthermore, there is evidence for the local production of aldosterone in the rat heart, and local aldosterone levels were estimated to be $\approx$20-fold higher in heart tissue in rats than in plasma.\textsuperscript{19} In addition, in rats there is evidence for aldosterone synthesis in blood vessels.\textsuperscript{20} Recently, enzymes required for aldosterone synthesis were also detected in diseased human hearts.\textsuperscript{21} Thus, tissue levels rather than aldosterone plasma levels might be important when assessing cardiovascular aldosterone actions. Therefore, the high plasma levels observed in this study may actually be representative of pathophysiological or physiological tissue levels, producing the effects shown here. However, further studies in vitro are required to confirm the concept of paracrine secretion of aldosterone in blood vessels and to estimate the “real” aldosterone levels acting on the vessel wall.

**Perspectives**

Based on the data provided in this study, it seems likely that rapid nongenomic aldosterone effects acting on the vasculature might not be harmful in the early stages of hypertension, when no secondary organ damage is present. However, after the occurrence of endothelial dysfunction, rapid nongenomic aldosterone effects promote the disease process by increasing vascular tone. The same might be true in other diseases with impaired endothelial function such as hypercholesterolemia, diabetes mellitus, and heart failure. Moreover, in secondary aldosteronism (as linked to heart failure), the rapid nongenomic effects of aldosterone may be compounded by genomic effects, which have been shown to impair endothelial function, to cause further deleterious vasoconstriction. Additional studies are required to study rapid nongenomic aldosterone effects in patients with impaired endothelial function.

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