Aldosterone Enhances Ischemia-Induced Neovascularization Through Angiotensin II–Dependent Pathway

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Background—We analyzed the role of aldosterone in ischemia-induced neovascularization and the involvement of angiotensin II (Ang II) signaling in this effect.

Methods and Results—Ischemia was induced by right femoral artery ligation in mice treated or not with aldosterone (4.5 μg/day), aldosterone plus spironolactone (aldosterone receptor blocker; 20 mg/kg per day), or aldosterone plus valsartan (angiotensin type 1 [AT1] receptor blocker; 20 mg/kg per day). After 21 days, neovascularization was evaluated by microangiography, capillary density measurement, and laser-Doppler perfusion imaging. Protein level of vascular endothelial growth factor (VEGF) was determined by Western blot analysis in hindlimbs. mRNA levels of renin–angiotensin system components were assessed by reverse transcription–polymerase chain reaction. Angiographic score, capillary number, and foot perfusion were improved in ischemic/nonischemic leg ratio by 1.4-, 1.5-, and 1.4-fold, respectively, in aldosterone-treated mice compared with controls (P <0.05). Aldosterone proangiogenic effect was associated with 2.3-fold increase in VEGF protein content (P <0.05). Treatments with spironolactone or with neutralizing VEGF antibody hampered the proangiogenic effect of aldosterone (P <0.05 versus aldosterone-treated mice). Interestingly, AT1 receptor blockade completely abrogated the aldosterone proangiogenic effect, emphasizing the involvement of Ang II–related pathway in aldosterone-induced vessel growth. In this view, angiotensinogen mRNA content was 2.2-fold increased in aldosterone-treated mice in reference to controls (P <0.05), whereas that of renin, angiotensin-converting enzyme, and AT1 receptor subtype was unaffected. Aldosterone treatment also decreased AT2 mRNA content by 2-fold (P <0.05 versus controls), suggesting that aldosterone may switch the Ang II pathway toward activation of vessel growth.

Conclusions—This study shows for the first time that aldosterone increases neovascularization in the setting of ischemia through activation of Ang II signaling. (Circulation. 2004;109:1933-1937.)

Key Words: aldosterone • neovascularization • ischemia • angiotensin

Thrombotic vessel obstruction of the feeding artery leads to insufficient organ perfusion and ultimately to important loss of function and serious health consequences. Revascularization of ischemic tissues by promoting the growth of new vessels or the maturation of preexisting ones is viewed as a highly promising strategy to counterbalance the deleterious effect of tissue ischemia.1 Therefore, understanding the mechanisms of the neovascularization reaction is of major importance. Neovascularization in the setting of ischemia is a complex process involving numerous factors. Among the known angiogenic factors, vascular endothelial growth factor-A (VEGF-A) has emerged as a central regulator of vessel growth under both physiological and pathological conditions.1 Alternatively, hormones, such as angiotensin II (Ang II), also appear to be involved in the regulation of the neovascularization process. Ang II, through its type I receptor subtype (AT1), activates in vivo angiogenesis2,4 and increases the revascularization reaction in a mice ischemic hindlimb model.4,5

Aldosterone is a mineralocorticoid hormone that acts classically, via the mineralocorticoid receptor, on blood pressure regulation and electrolyte balance. Besides the well-known effect of Ang II in stimulating aldosterone production from the adrenal cortex, a reciprocal interaction has been reported between hormones in extra-adrenal tissues. Activation of cardiac aldosterone production after rat myocardial infarction is mediated by cardiac Ang II.6 Alternatively, aldosterone increases Ang II binding and potentiates Ang II hypertrophic response in cultured cells.7,8 Similarly, aldosterone-salt treatment induces cardiac fibrosis through AT1 receptor activation.9 Finally, aldosterone has been shown to upregulate angiotensin-converting enzyme mRNA expression, suggesting that aldosterone may control local production of Ang II.10 We therefore hypothesized that aldosterone...
may potentiate the proangiogenic effect of Ang II and subsequently may modulate neovascularization in the setting of ischemia.

To test this hypothesis, we assessed the proangiogenic potential of aldosterone administration in a model of operatively induced hindlimb ischemia and analyzed the involvement of the Ang II–dependent pathway in aldosterone-related effects.

Methods

Experimental Protocol
This study was conducted in accordance with both institutional guidelines and those formulated by the European community for experimental animal use (L358-86/609EEC). C57Bl/6 mice (aged 10 weeks; Ifa Creddo, Lyon, France) were anesthetized by isoflurane inhalation, and unilateral hindlimb ischemia was induced by ligation (tied for complete occlusion) on the right femoral artery, as previously described. Mice were then randomly assigned to one of the following groups (n=7): (1) control group: vehicle (1% ethanol in drinking water); (2) mice receiving the mineralocorticoid receptor blocker spironolactone (spironolactone dissolved in ethanol at 25 mg/mL and added to drinking water; mice then received 20 mg/kg per day; Sigma); (3) mice treated with the AT1 receptor blocker valsartan (drinking water, 20 mg/kg per day; Sigma); (4) aldosterone (4.5 μg/d by osmotic minipumps implanted subcutaneously in the back of the mice; model 2004, Alza Corp); (5) aldosterone plus spironolactone; (6) aldosterone plus valsartan; and (7) aldosterone plus a-VEGF neutralizing antibody (10 μg IP twice per week; R&D Systems).

Quantification of Angiogenesis
Twenty-one days after ischemia, vessel density was evaluated by high-definition microangiography, capillary density analysis, and laser-Doppler perfusion imaging experiments, as previously described. VEGF protein expression was determined by Western blot in ischemic and nonischemic legs, as previously described.4,6

Determination of VEGF Protein Expression
VEGF protein expression was determined by Western blot in ischemic and nonischemic legs, as previously described.4,6

Statistical Analysis
Results are expressed as mean±SEM. One-way ANOVA was used to compare each parameter. Post hoc Bonferroni t test comparisons were then performed to identify which group differences account for the significant overall ANOVA. A value of P<0.05 was considered significant.

Results

Effect of Aldosterone on Vessel Density

Angiographic score (Figure 1) showed significant improvement in ischemic/nonischemic leg ratio of 1.4-fold in aldosterone-treated mice compared with controls (0.92±0.07 versus 0.64±0.07; P<0.05). Spironolactone hampered aldosterone-induced increase in the neovascularization process (P<0.05 versus aldosterone-treated mice). Interestingly, the aldosterone proangiogenic effect was also prevented by AT1 receptor blockade or by VEGF neutralizing antibody (P<0.05 versus aldosterone-treated mice).

Capillary Density
In mice treated with aldosterone, the ratio of ischemic to nonischemic leg capillary density was increased 1.5-fold compared with untreated mice (P<0.05) (Figure 1). Mineralocorticoid receptor or AT1 receptor blockade treatments as well as injection of neutralizing VEGF antibody completely abrogated the aldosterone-induced increase in capillary number (P<0.05 versus aldosterone-treated mice).

Laser-Doppler Perfusion Imaging
The ischemic/nonischemic ratio for cutaneous blood flow recovery was increased 1.4-fold in aldosterone-treated mice compared with controls (P<0.05) (Figure 1). Interestingly, spironolactone, valsartan, and neutralizing VEGF antibody reduced aldosterone-induced blood flow recovery (P<0.05 versus aldosterone-treated animals).

Molecular Mechanisms Associated With Aldosterone-Induced Increase in Neovascularization Process

Vascular Endothelial Growth Factor
The aldosterone proangiogenic effect was associated with a 2.3-fold increase in VEGF protein content (P<0.05 versus untreated mice; Figure 1). This rise was prevented by addition of spironolactone (P<0.05 versus aldosterone-treated mice). Interestingly, VEGF protein level returned to control value in mice treated with aldosterone and AT1 receptor blockade.

Renin–Angiotensin System Components
We finally analyzed the effect of aldosterone administration on renin–angiotensin system components mRNA level in ischemic hindlimb (Figure 2). Angiotensinogen mRNA content was increased 2.2-fold in aldosterone-treated mice in reference to controls (P<0.05), whereas that of renin, angiotensin-converting enzyme, and AT1 receptor subtype was unaffected in either group. The increase in angiotensinogen mRNA level was prevented by the addition of spironolactone (P<0.05 versus aldosterone-treated mice). Finally, aldosterone treatment decreased AT2 mRNA content by 2-fold in reference to control (P<0.05). Such a decrease was hampered by spironolactone treatment (P<0.05 versus aldosterone-treated mice).

Discussion
Although accumulating lines of evidence indicate the modulatory function of aldosterone in cardiovascular tissues, little is known about its role in blood vessel growth. In human pathological adrenal cortex, aldosterone-producing adenomas have been shown to express VEGF at a higher level, and tumor vascularization was positively associated with aldosterone. Moreover, the aldosterone receptor blocker spironolactone inhibited basic fibroblast growth factor–induced angiogenesis in vitro and in a rabbit corneal micropocket assay. We extended these previous studies by demonstrating that aldosterone may stimulate vessel growth in an ischemic context. Despite the use of 3 different methods to assess the neovascularization process, it should be noted that we do not measure whether perfusion is adequate (by analyzing areas of hypoxia) in the ischemic muscle of treated animals. Never-
theless, taken together, these studies highlight the putative involvement of aldosterone in the neovascularization process.

Interestingly, aldosterone-induced increase in vessel growth and in VEGF protein content was blocked by AT_1 receptor antagonist, suggesting that the aldosterone proangiogenic effect was mediated by Ang II signaling. Similarly, aldosterone induces cardiac fibrosis through AT_1 receptor activation and participates in Ang II–induced stimulation of cultured rat vascular smooth muscle cell proliferation.9,14 It is also noteworthy that synthesis of Ang II in the ischemic leg might depend on both angiotensin-converting enzyme–dependent and non–angiotensin-converting enzyme–dependent pathways. However, whatever the pathway, the subsequent activation of Ang II signaling may modulate vessel growth in an ischemic context.4,5

In addition, a subtle interaction seems to modulate aldosterone- and Ang II–related actions in ischemic tissue. Indeed, aldosterone improved angiotensinogen mRNA level in ischemic tissue. Angiotensinogen upregulation may be responsible for increased production of Ang II and subsequently for AT_1 receptor activation. Concentrations of angiotensinogen are indeed believed to directly influence renin-angiotensin system activity.15,16 Aldosterone treatment also reduced the AT_2 mRNA level in ischemic tissue. Interestingly, the AT_2 receptor subtype negatively modulates ischemia-induced neovascularization.17 Hence, one can speculate that the decrease in AT_2 gene expression associated with aldosterone treatment might enhance the AT_1 receptor activation–induced Ang II proangiogenic effect.

However, these results do not preclude that, in vivo, aldosterone may activate cellular events other than those related to Ang II and VEGF. Neovascularization appears to also be controlled by monocyte/macrophage accumulation that occurs within the ischemic area.18,19 Recently, aldoste-
Aldosterone has been shown to induce production of monocyte chemoattractant protein-1 and inflammatory cell infiltration in both heart and vessels, suggesting that aldosterone-induced inflammation may also mediate, at least in part, the aldosterone proangiogenic effect. Recent studies demonstrate that postnatal neovascularization does not rely exclusively on sprouting or remodeling of preexisting vessels but also involves bone marrow–derived progenitor cells. We can also speculate that aldosterone may improve mobilization and/or proangiogenic potential of bone marrow–derived cells. In this case, implantation of AT1a−/− mouse–derived mononuclear cells failed to restore blood flow perfusion in ischemic leg, suggesting that the renin–angiotensin–aldosterone system is of importance in the progenitor cell–mediated revascularization reaction.

In conclusion, our study showed for the first time that aldosterone improves postischemic neovascularization through activation of the Ang II–related pathway, thus adding another potential mechanism for aldosterone-related effects in cardiovascular homeostasis.

References
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