Histamine-dependent prolongation by aldosterone of vasoconstriction in isolated small mesenteric arteries of the mouse


Department of Cardiovascular and Renal Research, Institute of Molecular Medicine, University of Southern Denmark, Denmark; Department of Pharmacology and Pharmacy, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong, China; and Department of Clinical Pharmacy, King Saud University, Riyadh, Saudi Arabia

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Schjerning J, Uhrenholt TR, Svenningsen P, Vanhoutte PM, Skott O, Jensen BL, Hansen PB. Histamine-dependent prolongation by aldosterone of vasoconstriction in isolated small mesenteric arteries of the mouse. Am J Physiol Heart Circ Physiol 304: H1094–H1102, 2013. First published February 8, 2013; doi:10.1152/ajpheart.00524.2012.—In arteries, aldosterone counteracts the rapid dilatation (recovery) following depolarization-induced contraction. The hypothesis was tested that this effect of aldosterone depends on cyclooxygenase (COX)-derived products and/or nitric oxide (NO) synthase (NOS) inhibition. Recovery of the response to high K⁺ was observed in mesenteric arteries of wild-type and COX-2−/− mice but was significantly diminished in preparations from endothelial NOS (eNOS)−/− mice. Aldosterone pretreatment inhibited recovery from wild-type and COX-2−/− mice. The NO donor sodium nitroprusside (SNP) restored recovery in arteries from eNOS−/− mice, and this was inhibited by aldosterone. Actinomycin-D abolished the effect of aldosterone, indicating a genomic effect. The effect was blocked by indomethacin and by the COX-1 inhibitor valeryl salicylate but not by NS-398 (10−4 mol/l) or the TP-receptor antagonist S18886 (10−7 mol/l). The effect of aldosterone on recovery in arteries from wild-type mice and the SNP-mediated dilatation in arteries from eNOS−/− mice was inhibited by the histamine H₂ receptor antagonist cimetidine. RT-PCR showed expression of mast cell markers in mouse mesenteric arteries. The adventitia displayed granular cells positive for toluidine blue vital stain. Confocal microscopy of live mast cells showed loss of quinacrine fluorescence and swelling after aldosterone treatment, indicating degranulation. RT-PCR showed expression of mineralocorticoid receptors (MR), which are present both in endothelial and vascular smooth muscle cells (10, 15). However, the vascular effects of aldosterone are complex and include both vasodilator responses through NO formation (19, 37) and vasoconstrictor effects due to intracellular calcium mobilization (3, 23), whereby the net effect probably depends on the level of oxidative stress (32). MR activation has classical genomic effects or causes acute non-genomic responses. Two types of nongenomic effect of aldosterone, mediated by MR, have been reported in the vasculature: vasodilatation through activation of phosphoinositide 3-kinase and endothelial NO synthase (eNOS) (37) and vasoconstriction mediated by stimulation of protein kinase C (3, 4). Both the dilator and constrictor effects of aldosterone can be present within the same blood vessel as illustrated by previous work in renal arteries (37).

The preponderance of constrictor versus dilator responses to aldosterone could be involved in the detrimental vascular effects of the hormone. Indeed, in congestive heart failure aldosterone levels are correlated with 6-mo mortality (33), and inhibition of aldosterone action results in a 30% reduction in mortality rate in patients with heart failure after myocardial infarction (26). These clinical observations underline the substantial adverse effects of aldosterone on cardiovascular function. In particular, in pathophysiological situations characterized by high circulating levels of aldosterone (e.g., acute myocardial infarction or heart failure), the risk of developing spasms of the small arteries would increase if the constrictor effect of aldosterone dominates and NO-mediated recovery is reduced.

The mechanism underlying the blunting by aldosterone of vasodilatation recovery is not clear. The present study was designed to test the hypothesis that the procontractile effect of long-term treatment with aldosterone observed in renal arteries of the rabbit is a more general phenomenon in blood vessels and can be evoked in mouse arteries and that it involves suppression of NO release combined with local production of vasoconstrictor mediators.

METHODS

Animals. Animal care followed the guidelines of National Institutes of Health, and the protocol was approved by the Danish Animal Experiments Inspectorate under the Danish Ministry of Justice. Studies were conducted in male and female C57Bl/6J [wild-type (WT)] mice (Taconic Farms, Ry, Denmark) in eNOS knockout (eNOS−/−) mice.
mice and cyclooxygenase (COX)-2−/− (both strains from Jackson Laboratories). Mice had free access to rodent chow (Altromin, Lage, Germany) and tap water.

Isolation and microperfusion of mesenteric resistance arteries. The mice were euthanatized by a blow to the head followed by decapitation. Secondary mouse mesenteric arteries were dissected and transferred to a thermostated chamber (Warner Instruments, Hamden, CT). The specimen was mounted in a set of glass pipettes and pressurized to 60 mmHg at the inlet and 55 mmHg at the outlet, resulting in perfusion with physiological saline solution (PSS) at a flow rate of 1 to 2 μl/min, which mimics the in vivo conditions. The chamber was mounted on an inverted microscope (Zeiss Axiovert 10, Oberkochen, Germany). All experiments started with a 30-min period of equilibration at 37°C after the perfusion was established, and viability was tested by administration of a high potassium solution (70 mMol/l, high K+*) to the organ chamber. The inclusion criteria were 50% constriction followed by 10% subsequent dilatation (recovery) except in eNOS−/− mice where the NO donor sodium nitroprusside (SNP) was used to simulate recovery. Phentolamine (10−5 mol/l) was added to all solutions to exclude nerve-mediated α-adrenergic effects of depolarization. In all experimental protocols pharmacological inhibitors were added for 30 min followed by 50 min of incubation with aldosterone plus inhibitor. The effect on recovery was tested by adding high K+* on top of aldosterone and the inhibitor. The experiment was concluded by testing the response to high K+* after a washout period. In another series of experiments SNP was added 60 s after starting stimulation with high K+* with and without aldosterone treatment. Also the effect of 30-min treatment with increasing concentrations of histamine on recovery was determined.

Data analysis. The experiments were recorded using a Till Photonics video (Munich, Germany) camera and software. Images were transferred to custom made imaging software [FluoroFix, University of Southern Denmark (1)], and luminal diameter was determined at the most reactive part of the blood vessel. Recovery represents the difference in diameter from maximal constriction to the diameter after dilatation, which was measured 30 s after maximal constriction for preparations from WT and COX-2−/− mice. For preparations from eNOS−/− mice diameter after recovery was measured at the end of the SNP administration.

Reverse transcription-PCR. Mouse peritoneal mast cells were isolated by injection of 5.0 ml isolation buffer containing (in mM) 140 NaCl, 4.7 KCl, 1.2 MgSO4, 1.0 CaCl2, 5.5 glucose, 10 HEPES, and 200 IE/ml heparin into the peritoneal cavity. After gentle massage the cell suspension was collected and centrifuged at 1,000 rpm for 10 min and the supernatant was discarded. Mouse mesenteric arteries were cleaned of adhering connective tissue using small scissors. RNA was isolated from either mesenteric arteries (75–150 ng/μl RNA pr. mouse) or mast cells by TRIzol reagent from Invitrogen (Carlsbad, CA). RNA isolated from mast cells was treated with 3 units/mg RNA of heparinase I (Sigma, St. Louis, MO) and RNasin (1 unit/ml; Promega, Madison, WI) for 2 h at room temperature followed by DNase I digestion (50 units DNase/lμgRNA; Fermentas, Glen Burnie, MD). RNA from mast cells and mesenteric vessels was reverse transcribed using Superscript and oligo (dT). RT-PCR analysis was performed as described using 50 ng cDNA pr. PCR reaction (1). RT-PCR consisted of 35 cycles, and each cycle included incubation at 95°C for 45 s, 60°C for 20 s (MCP2), or 57°C for 45 s (MR) and 72°C for 45 s. Specific primers were used for the MR and the mast cell-specific marker MCP2. Primers were MR (Genbank NM_001083906 sense 5’TTA GCA CAG TGG GGT CCA TT3’ and antisense 5’TGA GGG AAA GGA ACG TCG TG3’ covering 148 base pairs) and MCP2 (Genbank NM_172044 sense 5’GCA CTT TTT CTT CCT GCT G3’ and antisense 5’ TGT GCA GCA GTC ATC ACA AA3’ 170 base pairs). Negative controls included RNA where no reverse transcriptase was added to the reaction (∼RT).

Morphological evaluation of mast cells in mesenteric arteries. Mesenteric arteries with branches and arcade were dissected and fixed by 4% (vol/vol) formaldehyde. To visualize mast cells, vessels were stained in 0.1% toluidine blue for 10 min. Confocal microscopy was used to evaluate morphological changes in mast cells adjacent to mesenteric arteries after aldosterone stimulation. The confocal microscope allowed using blood vessels surrounded by fat and peritoneal membrane. To minimize movements the gut segment was removed. Microdissected, formaldehyde fixed blood vessels and perfused preparations were examined to ensure that mast cells were present after dissection as well. To identify mast cells, the preparations were loaded with the fluorescent dye quinacrine (10−6 M) at 20°C for 10 min (35). The arteries were incubated for 50 min at 37°C followed by stimulation with aldosterone or vehicle for another 50 min at 37°C. Confocal laser-scanning fluorescence microscopy (Olympus FV1000; Olympus Denmark A/S, Ballerup, Denmark) was performed using an Olympus water immersion objective (×60; numerical aperture, 0.9). The scanning area was set to 1,024 × 512 pixels without internal zoom. Excitation was from a diode laser at 405 nm and fluorescence monitored through a 426 nm long-pass filter.

Laser-scanning confocal microscopy with Fluo-4. To determine whether or not aldosterone changes the calcium mobilization in endothelial cells, the intracellular calcium concentration was measured during stimulation with acetycholine. The intracellular calcium concentration was measured at rest and acetycholine stimulation, with or without aldosterone incubation (10−9 mol/l, 50 min). For imaging [Ca2+]i, dynamics, 4 μM Fluo-4-AM (Molecular Probes/Invitrogen; Carlsbad, CA) in PSS with 2.5% dimethylsulfoxide was perfused through the vessel lumen for 45 min to load endothelial and smooth muscle cells. Excess dye was washed off, and each blood vessel was equilibrated in PSS for 45 min to allow for destereification of dye. Confocal laser-scanning fluorescence microscopy was performed using an Olympus water immersion objective (>20; numerical aperture, 0.5). The scanning area was set to 512 × 128 pixels without internal zoom, corresponding to 873 μm × 218 μm. Full-frame imaging was performed at 2 Hz using excitation from an argon laser at 488 nm with fluorescence monitored through a 505 nm long-pass filter. The focal plane of the microscope was positioned along the surface of the artery to visualize endothelial and smooth muscle fluorescence simultaneously. The laser power was adjusted (transmissivity 20%; photomultiplier tube voltage 450–550 V) to obtain images with a mean intensity of 1,500 arbitrary intensity units (range 200–4,095). This ensured fluorescence acquisition over the full dynamic range of Fluo-4 without pixel saturation or excessive photobleaching. Data are presented as the relative fluorescence intensity change (F/F0), where F0 equals the average fluorescence intensity before stimulation (36).

Chemicals. Phentolamine, HEPES, SNP, ACh, Toluidine Blue O, indomethacin, aldosterone, quinacrine, cimetidine, and pyrilamine were from Sigma-Aldrich. Valeryl salicylate and NS398 were from Cayman Chemical (Ann Arbor, MI). S18886 was a kind gift of the Institut de Recherches Servier, Suresnes, France. The use of blockers and concentrations are all based on the specificity described on IUPHAR homepage (IUPHAR-db.org).

Saline solutions. The physiological saline solution (PSS) used contained (in mM) 115 NaCl, 25 NaHCO3, 1.2 MgSO4, 2.5 K-HPO4, 1.3 CaCl2, 5.5 glucose, and 10 HEPES. The high potassium solution contained (in mM) 45 NaCl, 70 KCl, 25 NaHCO3, 1.2 MgSO4, 2.5 K-HPO4, 1.3 CaCl2, 5.5 glucose, and 10 HEPES. The solutions were equilibrated with 5% CO2 in air resulting in a pH = 7.4 with 0.1% and 1% BSA superfusate and perfusate, respectively.

Statistical analysis. Data are presented as means ± SE. Significance of changes was calculated by two-way ANOVA followed by Tukey’s multiple comparison test and Students t-test for comparison of two groups. P values less than 0.05 were considered to indicate statistically significant differences.
RESULTS

Inhibitory effect of aldosterone on recovery. In perfused small mesenteric arteries of the mouse, elevating the extracellular potassium concentration (high K+; 70 mmol/l) caused constriction (maximal after 15 s) followed by partial dilatation after 45 s (recovery; Fig. 1A, control). The average basal diameter of 54 perfused vessels from WT mice was 140 ± 6 μm. After washout of potassium, the diameter returned to basal.

The effect of aldosterone (10−9 mol/l) on K+ -evoked constriction and recovery was assessed in small mesenteric arteries from WT mice. After the initial potassium test (Fig. 1A, control, before) aldosterone (10−9 mol/l) was added to the superfusate for 50 min with no effect on basal diameter (91.9 ± 9.3 μm to 92.5 ± 8.8 μm) or constriction to K+ (from 23.0 ± 2.9% to 27.3 ± 2.7% of initial resting diameter). However, the subsequent dilatation after high potassium (recovery) was inhibited significantly by aldosterone treatment, from 55.6 ± 6.4% to 13.4 ± 4.8% for control and aldosterone, respectively (Fig. 1, A and B). After washout of aldosterone the recovery returned to a level no different from before aldosterone treatment (59.6 ± 3.4%; Fig. 1A, control after). In three experiments without aldosterone incubation the second recovery was no different from the first and third recovery. When normalized to control recovery, aldosterone reduced recovery to 24.4 ± 5.6% (Fig. 2A). Incubation with the transcription inhibitor actinomycin D (10−6 mol/l) alone had no effect on K+ -induced vasoconstriction (data not shown), whereas K+ -induced vasoconstriction was inhibited significantly by preincubation with actinomycin D plus aldosterone (Fig. 1B). There was no significant recovery in the presence of actinomycin D plus aldosterone (Fig. 1B).

Involvement of NO. In mesenteric arteries from eNOS−/− mice, the initial recovery was reduced significantly compared with WT and the blood vessels did not dilate further over 3 min. The NO donor SNP (10−7 mol/l) was added 60 s after start of stimulation with 70 mmol/l K+. SNP dilated the vessel to a similar extent as the secondary dilatation after high potassium seen in the WT mice preparations (Fig. 1C). After incubation with aldosterone (10−9 mol/l), the SNP-induced dilatation was reduced to the same level as in the control situation for arteries of eNOS−/− mouse (Fig. 1C).

Involvement of COX-1. Incubation with the nonselective COX inhibitor indomethacin (10−6 mol/L) had no significant effect by itself on recovery but abolished the aldosterone (10−9 mol/l)-mediated reduction in recovery (Fig. 2B).

The preferential COX-2 inhibitor NS 398 (10−6 mol/l) had no effect on inhibition of recovery by aldosterone (Fig. 2C). Furthermore, aldosterone reduced recovery significantly in small mesenteric arteries from COX-2−/− mice (Fig. 2D), and this response was not significantly different from that observed in preparations from WT mice. Treatment of the latter with the COX-1 selective inhibitor valeryl salicylate (3 × 10−6 mol/l) did not abolish but attenuated significantly the inhibitory effect of aldosterone on recovery (Fig. 2E vs. 2A). The thromboxane A2 receptor antagonist S18886 (Fig. 2F, 10−7 mol/l) had no significant effect on the inhibition of recovery by aldosterone. In all experiments the statistical

Fig. 1. Recovery and aldosterone. A: changes in diameter in small mesenteric arteries from wild-type (WT) mice during stimulation with K+ (70 mM; control before) and after aldosterone (10−9 M, 50 min) and after washout of aldosterone (control after). B: diameter at maximal constriction elicited by K+ (70 mM; black columns) and after recovery (white columns). Control, initial stimulation; Aldo, stimulation after incubation with aldosterone; Aldo + Act D, stimulation after incubation with the transcription inhibitor actinomycin D (10−6 M); and aldosterone. C: changes in diameter in small mesenteric arteries from endothelial nitric oxide synthase (eNOS)−/− mice. Stimulation with K+ followed by sodium nitroprusside (SNP 10−7 M) and SNP + Aldo, K+, and addition of SNP after incubation with aldosterone is shown. D: recovery in small mesenteric arteries from eNOS−/− mice. Control, stimulation with K+: SNP, K+ and addition of SNP after incubation with aldosterone is shown. Data are mean values ± SE (n = 6 in each group). *P < 0.05.
power attained were over 0.92 except for the valeryl salicylate experiment with power of 0.59.

Involvement of histamine. The histamine receptor H1 antagonist pyrilamine (10⁻⁵ mol/l; Fig. 3A) and the selective H3 antagonist thioperamide (10⁻⁷ mol/l; Fig. 3B) did not significantly alter the effect of aldosterone on recovery (Fig. 3, A and B). By contrast, the histamine H2 receptor antagonist cimetidine (10⁻⁴ mol/l; Fig. 3C, n = 6) abolished it. In mesenteric arteries from eNOS⁻/⁻ mice, the inhibition of SNP-induced dilatation by aldosterone was abolished similarly by cimetidine (Fig. 3D, n = 6, compared with Fig. 1C). Statistical power over 0.84 was attained in all experiments. Pretreatment of arteries with increasing concentrations of histamine (10⁻¹¹–10⁻⁸ mol/l) significantly reduced recovery in a manner similar to the effect of aldosterone (Fig. 3E, n = 6).

Histamine-storing cells in the vascular wall. Toluidine blue staining of living arteries showed granular cells within the adventitia that accumulated the vital dye in a way consistent with mast cells (Fig. 4A). Some of the labeled cells were partly degranulated and were metachromatically stained. Furthermore, PCR analysis revealed cDNA products of the expected molecular size for the mast cell-specific marker MCPT2 in isolated mesenteric arteries (Fig. 4B). The presence of granulated cells was corroborated by staining with the fluorescent dye quinacrine in dissected, perfused arteries (Fig. 4C) and in nondissected blood vessels surrounded by fat and peritoneum (Fig. 4, D and E). The putative mast cells located in situ were labeled with quinacrine, and epifluorescence recordings were obtained (Fig. 4D) before and after aldosterone treatment (10⁻⁹ mol/l, 50 min, at 37°C; Fig. 4E). After aldosterone treatment the cells appeared swollen and degranulated as judged by the disappearance of the fluorescence quinacrine signal.

MRs. PCR analysis showed expression of MR RNA in peritoneal mast cells and mesenteric arteries (Fig. 4B).

Intracellular calcium. In perfused mesenteric arteries from WT mice, ACh (10⁻⁶ mol/l) significantly increased fluorescence reflecting increased calcium concentration in the en-
dothelial cells (Fig. 5). After washout, the fluorescence signal returned to baseline value. Aldosterone pretreatment for 50 min did not alter the ACh-induced increase in fluorescence.

DISCUSSION

The current study provides novel evidence that prolonged exposure to aldosterone, at a concentration that mimics the plasma aldosterone levels (1–50 nM) reported in mice (20) and in upright humans receiving a normal or a low-salt diet (17), has a procontractile effect on blood vessels through inhibition of NO-mediated vasodilatation (recovery). This effect depends on mast cell-derived histamine acting on histamine H2 receptors. Furthermore, the inhibitory effect of aldosterone on vascular relaxation involves prostaglandin production through COX-1. In addition, the data show that aldosterone also counteracts vasoconstriction through a nongenomic mechanism, as previously reported in rabbit renal afferent arterioles (37).

The reduced recovery observed in vessels from eNOS−/− mice indicates that recovery is NO-dependent in the mesenteric artery, which is in agreement with pharmacological studies in rabbit renal afferent arterioles (37). Dilatation in response to the NO donor SNP was attenuated by aldosterone in arteries from eNOS−/− mice, indicating an effect of aldosterone, which is distal to the formation of NO (37). Since aldosterone inhibits the effect of SNP, the effect on recovery is likely to attenuate the effect of NO on the target smooth muscle cells.

Chronic aldosterone treatment of Wistar-Kyoto rats and spontaneous hypertensive rats induced endothelial dysfunction and diminished relaxation to ACh (41). This effect was inhibited by blockers of cyclooxygenases and a TP receptor antagonist (SQ29548). In the present study, COX-1-derived prosta-

Fig. 3. Involvement of histamine in the effect of aldosterone on recovery. A: recovery in the initial test (control) and after aldosterone (10−8 M) and the histamine H2 receptor antagonist pyrilamine (10−8 M) (Aldo + Pyr). B: recovery in the initial test (control) and after aldosterone M and histamine receptor H3 antagonist thioperamide (10−7 M) (Aldo + Thiop). C: recovery in the initial test (control) and after aldosterone in the presence of histamine H2 receptor antagonist cimetidine (10−7 M) (Aldo + Cim). D: involvement of histamine in the effect of aldosterone on recovery in small mesenteric arteries from eNOS−/− mice. Stimulation with K+ (70 nM) (control), K+ and addition of SNP after cimetidine (SNP + Cim), and K+ and addition of SNP (10−7 M) after incubation with aldosterone 50 min in the presence of cimetidine (SNP + Cim + Aldo). E: effect of increasing concentrations of histamine (10−11–10−8 M, hist-11- hist-8) on recovery. Recovery in the initial test (stimulation with K+, control) is compared with recovery after preincubation with histamine. Data are mean values ± SE (n = 6). *P < 0.05; **P < 0.01; ***P < 0.001.
glandins appear to be involved in the aldosterone-mediated inhibition of recovery, whereas COX-2 is apparently not essential for the effect. Furthermore, TP receptors appear not to be involved in the effect of aldosterone since 10^{-9} mol/l of the selective TP antagonist S18886 (31) did not prevent the blunting by aldosterone of recovery. The tested inhibitors of COX enzymes were all used in concentrations above their IC_{50} value, and we therefore expect inhibition of the respective enzymes to average more than 50\%.

Histamine is released from mast cells and influences tissue blood flow. However, it can promote both arteriolar dilatation (29, 39) and vasoconstriction (30). In particular, histamine evokes a biphasic response in arterioles from WT mice and a constrictor response in the eNOS^{-/−} mouse (25). Mast cells are present along resistance arteries from the hamster cheek pouch (8), and they influence vascular resistance by releasing histamine and thromboxane A_{2} (31). Mast cells are also present in small mesenteric arteries (17). The aldosterone-induced morphological changes of the mast cells adjacent to mesenteric arteries observed here are similar to the changes seen after stimulation of degranulation by ATP (6) or compound 48/80 (7). In the present study, the fluorescent staining with quinacrine almost disappeared after aldosterone treatment and MRs are expressed in mast cells of the mouse. Furthermore citti-
dine also blocked the effect of aldosterone on the dilatation induced by the NO donor SNP. Taken in conjunction, these observations indicate that aldosterone causes histamine release via activation of MRs in adjacent mast cells and that the released histamine in turn mediates inhibition of recovery. Actinomycin D inhibits the release of histamine from rat peritoneal mast cells evoked by known stimulators (substance P, compound 48/80, and the calcium ionophore A23187) (13). These earlier findings explain why in the present study actinomycin D prevented the inhibition of the aldosterone-mediated effect on recovery. Likewise, prostaglandin E2 potentiates histamine release (40) and indomethacin inhibits degranulation of isolated rat peritoneal mast cells (11,18). The inhibition by indomethacin on the effect of aldosterone on recovery may be
due to a similar effect on the mast cells localized in the vascular wall.

Vascular responses to histamine are mediated through H₁ and H₂ receptor subtypes (12), and the dissected mesenteric arteries contained both histamine H₁ and H₂ receptor mRNA (data not shown). Histamine H₂ receptors are present in cultured smooth muscle cells (28). In human radial and internal thoracic arteries, histamine evokes constriction and its concentration-response curve is shifted to the right by H₂ receptor antagonists (5). In the present study, the H₂ receptor antagonist cimetidine abolished the effect of aldosterone on recovery, whereas the H₁ antagonist pyrilamine (10⁻⁵ mol/l) did not. The histamine receptor antagonists were used in concentrations 10 to 100 times above the pKi (12), and we therefore assume that more than 50% of the receptors are occupied with the antagonist. Cimetidine at the used concentration (10⁻⁴ M) might not be selective toward H₂ over H₁ (12), but the selective H₃ antagonist thioperamide in the concentration used (12) did not prevent the effect of aldosterone. Thus the inhibitory effect of cimetidine can reasonably be attributed to its blocking properties at H₂ receptors. Furthermore, cimetidine blocked not only the inhibitory effect of aldosterone per se on recovery but also that induced by addition of SNP. Thus the procontractile effect of aldosterone leading to blunting of the recovery vasodilatation is likely to occur downstream of the release on NO rather than by interfering with the latter. An increase in the intracellular calcium concentration in the endothelial cells, leading to the release of NO, is a part of the recovery after depolarization-induced vasoconstriction in the renal afferent arteriole (38). The increase in endothelial cell intracellular calcium upon stimulation of the small mesenteric arteries with the muscarinic agonist was not decreased after incubation with aldosterone, indicating that the endothelial cells can be stimulated as under control conditions. These observations confirm that the effect of aldosterone, and of the liberation of histamine that it must cause, is downstream of the endothelial release of NO and on the vascular smooth muscle cells. One possibility to explain the lack of recovery induced by histamine released by degranulating mast cells could be phosphorylation of the smooth muscle cell-specific protein CPI-17 via PKC activation resulting in inhibition of myosin light chain phosphatase (9, 14).

Although the aldosterone effect observed in the present study could be blocked by a histamine receptor antagonist, we cannot rule out that aldosterone also has a direct effect on either the vascular smooth muscle cells or the endothelial cells. McCurley and colleagues (21), using mice with cell specific deletion of MRs, have shown that MRs in smooth muscle cells regulate vascular tone and arterial blood pressure independently of the kidney, but only do so in aged mice. Furthermore, overexpression of MRs in endothelial cells leads to an increased arterial blood pressure (24). The present study was performed in a vascular segment of resistance vessels as they regulate peripheral vascular resistance; however, the response to aldosterone may vary with artery size as arteries of different size play different roles in the vasculature. The present data from mesenteric small arteries suggest an additional indirect new mechanism for aldosterone in the vasculature, whereby the aldosterone-histamine pathway could be involved in the detrimental effects of aldosterone on cardiovascular function.

Inhibition of recovery in arterioles would increase the risk of ischemia/reperfusion injuries or necrosis of the distal tissue. The potential increased incidence of microscopic necroses would lead to accelerated degradation and decline of function of the organs like the kidney or heart.

In summary, the present data indicate that aldosterone, through COX-1, promotes degranulation of adventitial mast cells in small mesenteric arteries. Histamine through vascular H₂ receptors attenuates the NO sensitivity of the smooth muscle cell and antagonizes the normal rapid reopening of the arterial lumen during vasoconstriction. To judge from the measurements of endothelial calcium signals, the stimulus for NO released by the endothelium was not attenuated by aldosterone. Thus aldosterone inhibits NO-dependent recovery in the small mesenteric arteries from the mouse by inducing histamine release. This inhibition of recovery may represent a blood pressure-independent adverse effect of aldosterone, which contributes to the risk of developing vasospasm.

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DISCLOSURES
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AUTHOR CONTRIBUTIONS

REFERENCES
EFFECT OF ALDOSTERONE ON RECOVERY IN MESENTERIC ARTERIES


