Aldosterone and Salt Loading Independently Exacerbate the Exercise Pressor Reflex in Rats

Masaki Mizuno, Ryan M. Downey, Jere H. Mitchell, Richard J. Auchus, Scott A. Smith, Wanpen Vongpatanasin

Abstract—The sympathetic and pressor responses to exercise are exaggerated in hypertension. Evidence suggests that an overactive exercise pressor reflex (EPR) contributes to this abnormal responsiveness. The mechanisms underlying this EPR overactivity are poorly understood. An increasing body of evidence suggests that aldosterone and excessive salt intake play a role in regulating resting sympathetic activity and blood pressure in hypertension. Therefore, each is a good candidate for the generation of EPR dysfunction in this disease. The purpose of this study was to examine whether excessive salt intake and chronic administration of aldosterone potentiate EPR function. Changes in mean arterial pressure and renal sympathetic nerve activity induced by EPR stimulation were examined in vehicle and aldosterone-treated (4 weeks via osmotic mini-pump) Sprague-Dawley rats given either water or saline (elevated salt load) to drink. When compared with vehicle/water-treated rats, stimulation of the EPR by muscle contraction evoked significantly greater increases in mean arterial pressure in vehicle/saline, aldosterone/water, and aldosterone/saline-treated animals (14±3, 29±3, 37±6, and 44±7 mmHg/kg, respectively; P<0.01). A similar renal sympathetic nerve activity response profile was likewise produced (39±11%, 87±15%, 110±20%, and 151±25%/kg, respectively; P<0.01). The pressor and sympathetic responses to the individual activation of the mechanically and chemically sensitive components of the EPR were also augmented by both saline and aldosterone. These data provide the first direct evidence that both aldosterone and high salt intake elicit EPR overactivity. As such, each represents a potential mechanism by which sympathetic activity and blood pressure are augmented during exercise in hypertension. (Hypertension. 2015;66:00-00. DOI: 10.1161/HYPERTENSIONAHA.115.05810.)

Key Words: aldosterone ■ exercise ■ hypertension ■ salt ■ sympathetic nervous system

The cardiovascular response to exercise is abnormally exaggerated in hypertensive patients and characterized by augmented increases in arterial blood pressure (BP), heart rate (HR), and sympathetic nerve activity (SNA).① Because such responses have been shown to be associated with elevated risks for myocardial ischemia, myocardial infarction, cardiac arrest, and stroke during and after physical activity, elucidating the cause of the cardiovascular hyperexcitability is clinically as well as physiologically important.② Afferent signals from working skeletal muscle are an important source of neural input to the brain stem during exercise and contribute significantly to the regulation of sympathetic outflow as well as the cardiovascular system during physical activity.③ These contraction-induced signals, which comprise the skeletal muscle exercise pressor reflex (EPR), are generated by stimulation of group III (predominantly mechanically sensitive Aβ fibers associated with the muscle mechanoreflex) and IV (primarily chemically sensitive C fibers associated with the muscle metaboreflex) skeletal muscle afferents.④ It has been extensively demonstrated in several animal models of human hypertension that the EPR is overactive in the disease contributing significantly to the exaggerated increases in SNA and BP that manifest during exercise.⑤⑥ That being stated, the mechanisms underlying the pathogenesis of EPR dysfunction in hypertension have not been fully established.

Aldosterone is well known to contribute to the development of hypertension. Circulating aldosterone penetrates the blood–brain barrier at concentrations paralleling those found in plasma.③⑦ Aldosterone has been shown to act centrally stimulating the sympathetic nervous system.⑧⑨ Earlier studies demonstrated that direct infusion of aldosterone into the cerebral ventricles causes a sustained increase in BP and renal SNA (RSNA) in rats and dogs.⑧⑨⑩⑾ As such, aldosterone represents a potential mechanistic candidate for the generation of EPR overactivity.

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Like aldosterone, high salt intake has also been shown to activate the sympathetic nervous system by increasing sodium concentrations in cerebrospinal fluid and neural tissue. Moreover, a recent study suggests that increased salt intake augments EPR function in rats establishing this mechanism as an additional potential candidate for the generation of muscle reflex overactivity in hypertension. Importantly, it has been suggested that the central pressor action of aldosterone is observed only in the presence of sodium excess or in salt-sensitive animals. As such, if aldosterone does indeed augment EPR activity, its action may be amplified by the presence of increased sodium.

Therefore, this study was designed to test the hypotheses that (1) chronic systemic administration of aldosterone potentiates EPR function and (2) aldosterone-induced EPR overactivity is exacerbated by concomitant salt loading. In addition, studies were performed to confirm and support reports that increased sodium intake alone elicits EPR dysfunction. To test these hypotheses, we examined cardiovascular and sympathetic responses to activation of the EPR, as well as its individual mechanically and chemically sensitive components, in vehicle and aldosterone-treated Sprague-Dawley rats given either water or saline to drink.

Methods

Complete description of the Materials and Methods is provided in the online-only Data Supplement.

Animal Models

Experiments were performed on 46 male Sprague-Dawley rats (11–12 weeks, 310–350 g). Under isoflurane anesthesia, osmotic mini-pumps (2ML4; ALZET) were implanted subcutaneously for 28 days to deliver 250 μg/kg per day doses of aldosterone (n=23) or vehicle (n=23). The animals in each group were assigned to drink normal water or 0.9% NaCl saline (vehicle/water; n=11; vehicle/saline; n=12; aldosterone/water; n=12; and aldosterone/saline; n=11). The procedures outlined were approved by the Institutional Animal Care and Use Committee. All studies were conducted in accordance with the US Department of Health and Human Services NIH Guide for the Care and Use of Laboratory Animals.

Experimental Protocols

Mean arterial pressure (MAP), HR, and RSNA were continuously measured at rest and during stimulation of either the EPR, the muscle mechanoreflex, or muscle metaboreflex.

### Results

#### Morphometric Characteristics and Plasma Aldosterone

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<td>Plasma aldosterone concentration, ng/100 mL</td>
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<td>44±117*††</td>
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</table>

Values are mean±SEM. Saline: 0.9% NaCl.

*P<0.05 compared with vehicle/water.

†P<0.05 compared with vehicle/saline.

‡P<0.05 compared with aldosterone/water.
Importantly, the effects of aldosterone on the EPR-induced rise in MAP, HR, and RSNA were not altered by sodium intake (all interaction $P$ value $>0.1$).

High salt intake alone as well as aldosterone alone significantly enhanced the changes in MAP, HR, and RSNA in response to stimulation of the muscle mechanoreflex during passive muscle stretch (Figure 3; aldosterone effect: $P<0.01$ and saline effect: $P<0.05$). The effects of aldosterone on the mechanoreflex-induced HR response, but not the MAP or RSNA responses, were potentiated by high sodium intake.

High salt intake alone and aldosterone alone exacerbated the cardiovascular response to stimulation of the muscle metaboreflex during capsaicin administration (Figure 4). The effects of aldosterone administration on the metaboreflex-induced elevation in MAP, HR, and RSNA were not altered by concomitant sodium intake (all interaction $P$ value $>0.1$).

Additional results are reported in the online-only Data Supplement.

**Discussion**

The major new findings from this investigation were (1) chronic aldosterone administration significantly augmented EPR activity, (2) aldosterone-induced EPR dysfunction was mediated by both the mechanically and chemically sensitive components of the EPR, and (3) the deleterious actions of aldosterone on EPR activity were not appreciably amplified by concomitant high sodium intake. In agreement with a previous report, it was also demonstrated that salt loading alone significantly decreased body mass (Table), while likewise reducing the developed peak tension during muscle contraction (Table S2 in the online-only Data Supplement). The EPR overactivity generated in aldosterone-treated animals might be explained by sensitization of the muscle reflexes as a result of saline/aldosterone-induced muscle atrophy. However, tempest to conclude, this would not explain the EPR dysfunction that manifested in rats treated only with aldosterone. Aldosterone alone is also known to induce cardiomyopathy. Previous studies in rats and patients with congestive heart failure have demonstrated enhanced EPR activity via selective mechanoreflex sensitization. In our study, chronic aldosterone administration induced left ventricular hypertrophy as evidenced by increased heart weight/body weight ratios as well as heart weight/tibial length ratios. However, the lung weight in aldosterone-treated rats was not increased suggesting that congestive heart failure had not developed. Thus, although the hypertrophic cardiomyopathy induced by aldosterone administration may contribute to the generation of EPR dysfunction to some extent, it seems unlikely to be the primary cause. Other possibilities include aldosterone-induced impairments in baroreflex-mediated inhibition of SNA (chronic intracerebroventricular infusion of aldosterone has been shown to inhibit arterial baroreflex control of RSNA and HR) and direct modulation by aldosterone of central sympathoexcitatory pathways.

In our study, high salt intake alone significantly augmented the MAP, HR, and RSNA responses to activation of the EPR (Figure 2). These findings are consistent with a recent report demonstrating that increased dietary salt showing that aldosterone alters sympathetic and cardiovascular responses to activation of the EPR during physical stress. Interestingly, the EPR overactivity demonstrated was mediated by both functional components of the reflex: the muscle mechanoreflex and metaboreflex. The mechanisms underlying aldosterone-induced enhancements in mechanoreflex and metaboreflex function remain unknown. However, it has been reported that aldosterone with salt loading for 4 weeks results in muscle atrophy. It has also been demonstrated that the pressor response to passive stretch is significantly greater in atrophied muscle than in healthy muscle. In the current investigation, aldosterone administration with high salt intake significantly decreased body mass (Table), while likewise reducing the developed peak tension during muscle contraction (Table S2 in the online-only Data Supplement). The EPR overactivity generated in aldosterone-treated animals might be explained by sensitization of the muscle reflexes as a result of saline/aldosterone-induced muscle atrophy. However, tempest to conclude, this would not explain the EPR dysfunction that manifested in rats treated only with aldosterone. Aldosterone alone is also known to induce cardiomyopathy. Previous studies in rats and patients with congestive heart failure have demonstrated enhanced EPR activity via selective mechanoreflex sensitization. In our study, chronic aldosterone administration induced left ventricular hypertrophy as evidenced by increased heart weight/body weight ratios as well as heart weight/tibial length ratios. However, the lung weight in aldosterone-treated rats was not increased suggesting that congestive heart failure had not developed. Thus, although the hypertrophic cardiomyopathy induced by aldosterone administration may contribute to the generation of EPR dysfunction to some extent, it seems unlikely to be the primary cause. Other possibilities include aldosterone-induced impairments in baroreflex-mediated inhibition of SNA (chronic intracerebroventricular infusion of aldosterone has been shown to inhibit arterial baroreflex control of RSNA and HR) and direct modulation by aldosterone of central sympathoexcitatory pathways.

In our study, high salt intake alone significantly augmented the MAP, HR, and RSNA responses to activation of the EPR (Figure 2). These findings are consistent with a recent report demonstrating that increased dietary salt
intake enhances pressor and cardioaccelerator responses to muscle contraction in rats. Our data extended these previous findings by demonstrating that EPR dysfunction in saline-treated rats is mediated by both mechanoreflex and metaboreflex overactivity. Accumulating reports indicate that salt intake does not alter vasoconstrictor responses to sympathetic stimulation in rats. Thus, it is logical to postulate that salt-induced EPR overactivity results from the sensitization of brain stem circuits in the muscle reflex pathway. Sensitization of these circuits could cause the abnormally large elevations in sympathetic output observed during muscle reflex activation. However plausible, the exact downstream mechanisms underlying such changes remain undetermined. High salt intake has been shown to increase the concentration of sodium in cerebral spinal fluid and brain tissue which could evoke central sympathetic activation via stimulation of epithelial sodium channels. Specifically, it has been suggested that sodium acts on neurons within the organum vasculosum of the lamina terminalis to enhance the responsiveness of sympathetic motor neurons emanating from the brain stem circuits.
from the rostral ventrolateral medulla (RVLM) within the brain stem. Continued research in this area is needed to determine whether these pathways underlie high salt intake–induced EPR dysfunction.

In the current investigation, the excitatory effect of aldosterone on EPR activity was not dependent on the level of salt intake. Although it has been suggested that the hypertensive and sympathoexcitatory action of aldosterone requires the presence of excess sodium, we found a strong independent action of aldosterone on EPR activity that was not appreciably affected by increased salt ingestion. This suggests that aldosterone and salt loading may accentuate EPR function through similar pathways. The RVLM, an established cardiovascular regulatory nuclei within the brain stem, is a good candidate for the mediation of this EPR overactivity. Increased dietary salt intake enhances sympathetic responsiveness to stimulation of the RVLM. Furthermore, a neurophysiological study has clearly demonstrated that aldosterone activates RVLM neurons through mineralocorticoid receptors as well as epithelial sodium channels. Thus, it is plausible that aldosterone and salt loading alter neural activity in the RVLM through mineralocorticoid receptor and epithelial sodium channels. The paraventricular nucleus of the hypothalamus, known to be an important contributor to autonomic cardiovascular control, is another reasonable candidate. It comprises 70% of the preganglionic fibers by-passing the RVLM and likewise expresses mineralocorticoid receptor. Because the precollicular decerebration performed in the current study removes the hypothalamus, the paraventricular nucleus was unlikely to contribute substantially to the abnormal EPR function observed in the present investigation. However, we cannot exclude the possibility that chronic administration of aldosterone could have induced permanent alterations in the function of brain stem neurons before removal of the paraventricular nucleus contributing to the results reported. Although speculative in nature, it is tempting to suggest that sodium and aldosterone affect these cardiovascular regulatory regions of the brain and in this manner independently alter EPR function.

It should be noted, in the current study, that concomitant high salt intake did not further augment the increases in baseline systolic arterial pressure induced by aldosterone. This finding is inconsistent with a previous study using rats in which the effects of intracerebroventricular as well as subcutaneous aldosterone administration on BP were examined. The discrepancy may be partly explained by the difference in experimental designs between studies. For example, in the current study, a higher dose of aldosterone was used and the kidneys were not removed. Nevertheless, the findings clearly support the contention that aldosterone and high salt intake alter EPR function and may do so via similar mechanisms.

Perspectives

Excessive BP elevation during exercise has been shown to contribute to impaired exercise tolerance in hypertensive patients even in the absence of coronary artery disease or left ventricular dysfunction. Furthermore, numerous epidemiological studies have demonstrated that exercise BP predicts the development of left ventricular hypertrophy, stroke, myocardial infarction, and death independent of resting BP. Our study in rodents suggests a potential role for both aldosterone and dietary salt intake in modulating the sympathetically mediated BP response to exercise via alterations in reflexes originating from the skeletal muscle. Based on these findings, further studies in humans are needed to confirm if salt restriction and mineralocorticoid receptor blockade constitute 2 independent effective strategies in attenuating the augmented pressor response to exercise in hypertension.

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We thank Martha Romero and Julius Lamar, Jr, for their expert technical assistance. In addition, we thank the UT Southwestern O’Brien Kidney Research Core Center for providing materials and support for the measurement of noninvasive blood pressure in conscious rats.

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Novelty and Significance

**What Is New?**

- Although the sympathetically mediated cardiovascular response to physical activity in hypertensive patients is heightened as compared with healthy subjects, the underlying mechanisms are poorly understood. Accumulating evidence suggests that aldosterone and high dietary salt intake play a role in regulating baseline sympathetic outflow in hypertension. However, whether each contributes to the generation of the excessive sympathetic and cardiovascular responses to exercise in this disease remains unknown.

**What Is Relevant?**

- The findings indicate that controlling salt intake and treating excessive levels of aldosterone may improve blood pressure regulation during exercise in hypertension.

Summary

The present findings demonstrate that systemic administration of aldosterone and high dietary salt intake independently potentiate the function of the skeletal muscle exercise pressor reflex; a reflex which regulates, in part, the rise in sympathetic nerve activity and blood pressure during physical exertion and is known to be overactive in hypertension.
Aldosterone and Salt Loading Independently Exacerbate the Exercise Pressor Reflex in Rats

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Aldosterone and Salt Loading Independently Exacerbate the Exercise Pressor Reflex in Rats

Masaki Mizuno\textsuperscript{1,2}, Ryan M. Downey\textsuperscript{2}, Jere H. Mitchell\textsuperscript{2}, Richard J. Auchus\textsuperscript{4}, Scott A. Smith\textsuperscript{1,2}, Wanpen Vongpatanasin\textsuperscript{2,3}

\textsuperscript{1}Departments of Health Care Sciences, \textsuperscript{2}Internal Medicine and \textsuperscript{3}Hypertension Section, Cardiology Division, University of Texas Southwestern Medical Center, Dallas, Texas, USA; \textsuperscript{4}Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan, USA

Running Title: Aldosterone and Abnormal Muscle Reflex Function
**SUPPLEMENTARY MATERIALS AND METHODS**

*Animal Care:* Animals were housed in standard rodent cages on 12 h light dark cycles and were given food and water ad libitum.

*Measurement of Blood Pressure in Conscious Animals.* In a subset of animals in each group (n = 5/group), systolic arterial pressure (SAP) was assessed by tail cuff using a CODA blood pressure system (Kent Scientific) for 4 weeks after high salt intake and/or aldosterone administration as well as in controls. Animals were acclimated to the measurement procedure and trained for 2 weeks prior to data collection by being placed in a restraining chamber and inflating the blood pressure cuff several times.

*Acute General Surgical Procedures.* As described previously, rats were anesthetized with 1–4% isoflurane in oxygen and intubated for mechanical ventilation. Arterial blood pressure was continuously measured by a pressure transducer connected to a left carotid arterial catheter. To obtain electrocardiograph (ECG) recordings, needle electrodes were placed on the back of the animal. Heart rate (HR) was calculated from the time between successive R waves. To measure RSNA, a branch of the renal nerve was exposed and attached to a pair of stainless steel wire electrodes. The nerve and electrodes were covered with silicone glue for insulation and fixation. The pre-amplified nerve signal was band-pass filtered at 150–1000 Hz then full-wave rectified and low-pass filtered with a cutoff frequency of 30 Hz. Animals were held in a stereotaxic head unit and then, for rendering the animals insentient, a pre-collicular decerebrate procedure was performed. Immediately after the decerebration, isoflurane anesthesia was discontinued.

*Surgical Procedures for Activation of Skeletal Muscle Reflexes.* A laminectomy exposing the lower lumber portions of the spinal cord (L2–L6) was performed as described previously. The L4 and L5 ventral roots were carefully isolated and sectioned. The cut peripheral ends of roots were placed on bipolar electrodes. The gastrocnemius, plantaris and soleus muscle of the right hindlimb were isolated. The calcaneal bone of the right hindlimb was cut and the Achilles’ tendon connected to a force transducer for the measurement of muscle tension. To allow the injection of chemicals into the arterial supply of the right leg, the circulation of the hindlimb was surgically isolated. Briefly, a catheter was placed in the left common iliac artery with the catheter tip advanced to the bifurcation of the abdominal aorta. This allowed injection of chemicals directly into the right common iliac artery without occluding the circulation of the right hindlimb. In addition, a reversible ligature was placed around the common iliac vein emptying the right hindlimb.

*Stimulation of the mechanically-sensitive component of the EPR.* To evoke a mechanical stimulus similar to that elicited during muscle contraction, the triceps surae muscles of the right hindlimb were passively stretched for 30 sec using a rack-and-pinion system. Care was taken to manually generate the same pattern and magnitude of muscle tension developed during static contractions. This maneuver was used to preferentially activate mechanically-sensitive afferent fibers associated with the muscle mechanoreflex.

*Stimulation of the chemically-sensitive component of the EPR.* Selective activation of chemically-sensitive afferent fibers innervating skeletal muscle was achieved by administering graded concentrations of capsaicin into the arterial supply of the right hindlimb (0.1, 0.3, and 1.0 μg/100 μL). Capsaicin was injected into the right common iliac artery while a reversible ligature placed around the
right common iliac vein was occluded for 2 min. This maneuver was used to preferentially activate afferent fibers associated with the muscle metaboreflex.

**End Experiment Procedures.** To confirm that SNA signals were recorded from postganglionic renal sympathetic fibers, an intravenous infusion of hexamethonium bromide (60 mg kg\(^{-1}\)) was given to abolish SNA at the conclusion of all experiments. RSNA background noise was determined 30 min after intravenous injection of saturated potassium chloride (4 M, 2 ml/kg) which was given to humanely kill the insentient decerebrated animal. In all animals, the heart and lungs were excised and weighed. Additionally, the tibia was harvested and measured.

**Data Acquisition and Statistical Analyses**

MAP, HR, RSNA and contractile force data were acquired, recorded, and analyzed using data acquisition software (LabChart, ADInstruments) for the Powerlab analog-to-digital convertor (Powerlab8/30, ADInstruments) at a 1-kHz sampling rate. To analyze RSNA, full-wave rectified signals of RSNA as well as background noise signals were obtained. The noise signal component, which was defined as the signal recorded postmortem, was subtracted from rectified RSNA. To quantify RSNA responses to muscle contraction, basal measurements were obtained by taking the mean value of 30 s of baseline data immediately prior to the maneuver. This mean was considered 100 % of basal RSNA. Subsequently, relative changes in RSNA (\(\Delta\)RSNA, %) from this baseline were evaluated. Data sets of 1 s averages for MAP, HR, RSNA and hindlimb tension were analyzed. Baseline values for all variables were determined by evaluating 30 s of recorded data before a muscle contraction. The peak response of each variable was defined as the greatest change from baseline elicited by contraction.

**SUPPLEMENTARY RESULTS**

Table S1 demonstrates the baseline hemodynamics during 1% isoflurane anesthesia or after decerebration. Four weeks of aldosterone administration significantly decreased HR in aldosterone treated rats compared to vehicle treated animals (aldosterone effect: \(P < 0.01\)). There were no significant differences in basal MAP during 1% isoflurane anesthesia or after decerebration between groups. Further, the baseline signal to noise ratio for RSNA was not different among groups.

Table S2 summarizes the peak tensions developed during muscle contraction and passive muscle stretch maneuvers. Developed peak tensions were not different among groups with the exception of being lower in aldosterone/saline treated rats compared to vehicle/water treated animals.

**SUPPLEMENTARY DISCUSSION**

*Analytical and Methodological Considerations*

Plasma sodium concentration as well as osmolality was not assessed in the present investigation. However, earlier studies clearly indicated that rats drinking 0.9% NaCl for 2 weeks displayed significant increases in plasma sodium concentration and osmolality at night.\(^4\) In the saline/vehicle group, SAP in the conscious state did not differ from water/vehicle animals (Fig. 1). In addition, there were no differences in resting MAP, HR or RSNA under both anesthesia and after decerebration among groups (Table S1). These
findings are consistent with previous reports demonstrating no differences in baseline hemodynamics and SNA or plasma/blood volume in similarly treated animals\textsuperscript{4,5}.

Aldosterone administration with saline loading significantly decreased developed peak tension during muscle contraction and passive stretch as compared to all other groups (Table S2). Since the magnitudes of the sympathetic and cardiovascular responses to activation of the EPR are positively and significantly associated with developed tension\textsuperscript{2,6,7}, the responses were normalized due to the aforementioned difference\textsuperscript{2,7}. For completeness, a similar response profile for MAP and RSNA during EPR stimulation was likewise observed when analyzing data using absolute values (not shown).

**SUPPLEMENTARY REFERENCE**

## Table S1. Baseline hemodynamics

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<td>1% Isoflurane anesthesia</td>
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<td>MAP (mmHg)</td>
<td>104 ± 5</td>
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<td>HR (beats min⁻¹)</td>
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<td>MAP (mmHg)</td>
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<td>HR (beats min⁻¹)</td>
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<td>Baseline signal to noise ratio for RSNA</td>
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Values are means ± S.E.M. * P < 0.05 compared to vehicle/water. † P < 0.05 compared to vehicle/saline. ‡ P < 0.05 compared to aldosterone/water. Saline: 0.9% NaCl.
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Values are means ± S.E.M. * $P < 0.05$ compared to vehicle/water. Saline: 0.9% NaCl.