Aldosterone impairs baroreflex sensitivity in healthy adults

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Monahan KD, Leuenberger UA, Ray CA. Aldosterone impairs baroreflex sensitivity in healthy adults. Am J Physiol Heart Circ Physiol 292: H190–H197, 2007. First published August 18, 2006; doi:10.1152/ajpheart.00622.2006.—Animal studies suggest that acute and chronic aldosterone administration impairs baroreceptor/baroreflex responses. We tested the hypothesis that aldosterone impairs baroreflex control of cardiac period [cardiovagal baroreflex sensitivity (BRS)] and muscle sympathetic nerve activity (MSNA, sympathetic BRS) in humans. Twenty-six young (25 ± 1 yr old, mean ± SE) adults were examined in this study. BRS was determined by using the modified Oxford technique (bolus infusion of nitroprusside, followed 60 s later by bolus infusion of phenylephrine) in triplicate before (Pre) and 30-min after (Post) beginning aldosterone (experimental, 12 pmol·kg−1·min−1; n = 10 subjects) or saline infusion (control; n = 10). BRS was quantified from the R-R interval-systolic blood pressure (BP) (cardiovagal BRS) and MSNA-diastolic BP (sympathetic BRS) relations. Aldosterone infusion increased serum aldosterone levels approximately fourfold (P < 0.05) and decreased (P < 0.05) cardio- vagal (19.0 ± 2.3 vs. 15.6 ± 1.7 ms/mmHg Pre and Post, respectively) and sympathetic BRS [−4.4 ± 0.4 vs. −3.0 ± 0.4 arbitrary units (AU)-beat−1·mmHg−1]. In contrast, neither cardiovagal (19.3 ± 3.3 vs. 20.2 ± 3.3 ms/mmHg) nor sympathetic BRS (−3.8 ± 0.5 vs. −3.6 ± 0.5 AU·beat−1·mmHg−1) were altered (Pre vs. Post) in the control group. BP, heart rate, and MSNA at rest were similar in experimental and control subjects before and after the intervention. Additionally, neural and cardiovascular responses to a cold pressor test and ischemic handgrip to fatigue were unaffected by aldosterone infusion (n = 6 subjects). These data provide direct experimental support for the concept that aldosterone impairs baroreflex function (cardiovagal and sympathetic BRS) in humans. Therefore, aldosterone may be an important determinant/modulator of baroreflex function in humans.

of tonic cardiovagal modulation (high-frequency heart rate variability) increase in healthy young adults (10) and in patients with congestive heart failure (15) after administration of an aldosterone blocker. Collectively, these data suggest that aldosterone may modulate the cardiac arm of the baroreflex in both health and disease. The effect of aldosterone on baroreflex control of efferent sympathetic nervous system outflow is less clear (13). However, because this arm of the baroreflex is thought to be critical in the context of acute systemic BP control, its examination and function are of biomedical and clinical significance.

In the present study we tested the hypothesis that aldosterone impairs both cardiovagal and sympathetic baroreflex sensitivity (BRS) in humans. To address this hypothesis, BRS was determined before (low aldosterone state) and during aldosterone infusion (high aldosterone state) in healthy young adults. Aldosterone was infused at quantities previously shown to increase plasma levels (35) to those observed in several disease states (primary aldosteronism and congestive heart failure) (5, 14, 17, 29).

METHODS

Subjects

A total of 26 young (18–35 yr old) subjects were studied. All were healthy, normotensive (BP < 140/90 mmHg), nonsmokers, and nonobese (body-mass index < 30 kg/m2). The Institutional Review Board at the Pennsylvania State University College of Medicine approved the experimental protocol. Written informed consent was obtained from all subjects before testing.

Measurements

Subjects were studied supine and abstained from food (4 h) and caffeine (12 h) before testing. BP and heart rate. Resting BP was determined by using a semi-automated device (Dinamap). Beat-to-beat BP was measured by using a Finapres (Ohmeda). Heart rate was determined from the ECG. MSNA. Microneurography was used to obtain direct intraneural multifiber recordings of muscle sympathetic nerve activity (MSNA) from the peroneal nerve (30). Raw nerve recordings were amplified, filtered (700–2,000 Hz), full-wave rectified, and integrated (0.1 s time constant) to obtain mean voltage neurograms. Cardiovagal and sympathetic BRS. Cardiovagal and sympathetic BRS were assessed by using the modified Oxford technique (6, 21), as our group (18, 19) has recently described. Briefly, a bolus of nitroprusside was infused intravenously, followed 60 s later by a bolus of phenylephrine. Dosages were chosen to elicit an ~15 mmHg reduction (nitroprusside) and subsequent increase (phenylephrine) in BP from resting levels (18, 19). Data acquisition began 3 min before nitroprusside infusion and continued for 3 min after. Drug doses were...
the same within a given subject for all trials before (Pre) and after (Post) drug intervention.

**Isometric handgrip to fatigue.** After a 3-min baseline period, subjects performed isometric handgrip (IHG) at 30% of their maximal voluntary contraction to fatigue. Maximal voluntary contraction force was assessed before beginning the protocol. IHG was stopped when target force could not be maintained for >3 s despite strong verbal encouragement.

**Cold pressor test.** After a 3-min baseline period, subjects submersed their hand up to the wrist in a bucket of ice water for 2 min.

**Blood samples.** Venous blood samples were obtained at baseline (before making Pre measurements) and immediately before the first and last BRS trial made in the Post period (~30 and ~75 min after beginning the intervention (see Experimental Protocol)). After being collected, plasma or serum was frozen at ~80°C until assayed. Measurements included aldosterone (RIA Diagnostic Products), angiotensin II (RIA Alpco Diagnostics), and potassium (Bayer Rapid Lab Blood-Gas Analyzer, model 865).

**Experimental Protocol**

**Protocol 1: effect of aldosterone on cardiovagal and sympathetic BRS.** Twenty subjects were studied in this randomized, double-blinded, placebo-controlled protocol. Three BRS trials were performed at baseline. After these baseline (Pre) measurements were obtained, subjects randomly received an intravenous infusion of either saline (control group) or aldosterone (experimental group) [12 pmol·kg⁻¹·min⁻¹, Clinalfa AG (35)]. Thirty minutes after beginning this infusion and while the infusion continued, BRS trials were repeated in triplicate (Post 1, Post 2, and Post 3). BP and heart rate at rest were determined in triplicate before beginning the 3-min baseline data collection for the first trial in the Pre condition and before each trial in the Post condition (Post 1, Post 2, and Post 3). MSNA at rest was determined as the mean value over the 3-min baseline period before the first trial in the Pre condition and before each trial in the Post condition (Post 1, Post 2, and Post 3). Consecutive BRS trials were separated by at least 15 min. Previously, we have established that this period of time between consecutive trials allows BP and heart rate at rest to return to resting levels and for reproducible measures of BRS to be made (19). Because aldosterone was infused relative to body weight, the absolute quantity of aldosterone infused over the course of the study varied between subjects but ranged from ~20–40 μg.

**Protocol 2: effect of aldosterone on physiological responses to IHG and cold pressor test.** Six subjects were studied in this protocol. Protocol 2 was identical to protocol 1, except, instead of assessing BRS in triplicate before and after beginning the aldosterone infusion, subjects performed a single IHG trial to fatigue and a cold pressor test (CPT). These tests were separated by at least 15 min. All subjects tested in this protocol received the aldosterone infusion (12 pmol·kg⁻¹·min⁻¹).

**Data Analysis**

Physiological data were digitally recorded (MacLab 8e, ADInstruments) at 400 Hz.

MSNA at rest was quantified as bursts per minute and as the sum of the area under individual bursts per minute [in arbitrary units (AU)/min]. Neurograms were calibrated by assigning the tallest burst at rest an amplitude of 1,000 and by setting the baseline to zero.

Cardiovagal BRS is measured in all subjects Pre and Post (experimental, n = 10; and control subjects, n = 10). However, due to difficulties in obtaining or maintaining an appropriate MSNA recording site, sympathetic BRS was not measured in all subjects (experimental, n = 8; and control subjects, n = 8). At baseline (Pre) cardiovagal and sympathetic BRS were similar in both experimental and control subjects (Fig. 2). Aldosterone infusion decreased (P < 0.05) cardiovagal (19.0 ± 2.3 vs. 15.6 ± 1.7 ms/mmHg for Pre and Post, respectively; Fig. 2) and sympathetic BRS (−4.4 ± 0.4 vs. −3.0 ± 0.4 AU·beat⁻¹·mmHg⁻¹; Fig. 2). In contrast, neither cardiovagal (19.3 ± 3.3 vs. 20.2 ± 3.3 ms/mmHg; Fig. 2) nor sympathetic BRS (−3.8 ± 0.5 vs. −3.6 ± 0.5 AU·beat⁻¹·mmHg⁻¹; Fig. 2) were changed in the control group Pre to Post. There was a significant group by intervention interaction for both cardiovagal (P < 0.01) and sympathetic BRS (P < 0.001). Responses in individual subjects are presented in Fig. 3 to illustrate the consistency of responses. Responses to aldosterone infusion were similar in men and women (data not shown). Therefore, the results from both men
and women have been pooled and are presented as a single group throughout.

Changes in BP induced by the bolus infusions of nitroprusside and phenylephrine were similar across all trials Pre and Post in both control and experimental subjects. Specifically, nitroprusside decreased systolic BP similarly from baseline levels in control (Δ18 ± 2 and Δ19 ± 2 mmHg for Pre and Post trials, respectively) and experimental (Δ18 ± 3 and Δ18 ± 2 mmHg) subjects, and phenylephrine increased systolic BP similarly in control (Δ20 ± 3 and Δ20 ± 2 mmHg) and experimental groups.

Fig. 1. Representative data from a baroreflex trial. A: continuous beat-by-beat recording of the systolic blood pressure (BP), R-R interval, and muscle sympathetic nerve activity (MSNA). A bolus infusion of nitroprusside occurs at minute 0, followed by a bolus infusion of phenylephrine at minute 1. These infusions elicit characteristic decreases and subsequent increases in BP that elicit baroreflex-mediated changes in R-R interval and MSNA. B: regressions between R-R interval and systolic BP (left) and MSNA and diastolic BP (right) during changes in BP. The linear slopes of these relations are used to quantify cardiovagal and sympathetic baroreflex sensitivity (BRS). AU, arbitrary units.

Table 1. Subject characteristics: protocol 1

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Values are means ± SE; n, number of subjects. BMI, body-mass index.
Pre- and experimental and control groups. All values are means ± SE. Pre and Post, before and after drug intervention, respectively; BP, blood pressure; MSNA, muscle sympathetic nerve activity.

*P < 0.05, main effect (intervention); †P < 0.05, interaction (group × intervention).

Effect of Aldosterone on Responses to IHG and CPT

Neural (MSNA) and cardiovascular responses to CPT and IHG to fatigue are presented in Fig. 4. Responses to both physiological stressors were similar in both the low (Pre) and high (Post) aldosterone state.

DISCUSSION

The results from this randomized, double-blinded, and placebo-controlled study indicate that aldosterone infusion decreases both cardiovagal and sympathetic BRS in healthy young adults, without influencing responses to isometric exercise or thermal stress. These data suggest that aldosterone may contribute mechanistically to baroreflex dysfunction in disease states associated with aldosterone excess (e.g., primary aldosteronism and congestive heart failure).

Aldosterone classically is viewed as a hormone important in fluid and electrolyte balance. However, in addition to these effects, a growing body of experimental evidence suggests aldosterone exerts extra renal effects on cardiovascular and autonomic nervous system function (26, 27). The earliest indications that aldosterone may influence autonomic nervous system function came from data in patients with primary aldosteronism and congestive heart failure. Moreover, the hypertensive overshoot and bradycardic response to postural change was blunted in primary aldosteronism despite persistent decreases in BP (1, 2, 24). The fact that adrenalectomy restored normal responses in these individuals (1, 2, 24) suggested that aldosterone may induce baroreflex dysfunction. Most, but not all (20), subse-
sequent studies (31, 32) support the concept that cardiovagal BRS is depressed in primary aldosteronism. However, the role of aldosterone per se in these responses cannot be determined, as other possible modulators of autonomic function, such as hypertension and hypokalemia, tend to occur in primary aldosteronism. Additionally, no studies to date have examined reflex control of sympathetic outflow in primary aldosteronism. However, the lack of BP overshoot during phase IV of Valsalva’s maneuver, which is dependent on peripheral vasoconstriction generated during phase II of the maneuver, strongly suggests dysfunction in reflex control of sympathetic outflow when aldosterone levels are high (8).

Studies designed to more directly examine the effect of aldosterone on the cardiac arm of the baroreflex have been performed by acutely infusing aldosterone or administering a mineralcorticoid receptor antagonist (10, 13, 22, 35). In contrast to the previously mentioned studies in primary aldosteronism, interpretation of these findings is not confounded by concomitant changes in other physiological measures, such as resting BP. Consistent with our findings, most (10, 22, 35), but not all (13), of these prior studies provided experimental support for the concept that aldosterone impairs cardiovagal control. These previous studies (10, 22, 35) were important, but they failed to determine the possible detrimental effect of aldosterone on baroreflex control of sympathetic outflow. As changes in cardiovagal and sympathetic BRS can occur independent of each other in humans (7, 16), we cannot assume that sympathetic BRS is depressed by aldosterone.

Only one previous study in humans has examined the effects of aldosterone on baroreflex control of MSNA. In contrast to our finding of decreased sympathetic BRS after raising circulating aldosterone levels, Heindl et al. (13) showed no effect. Moreover, in contrast to the present and previous findings (22, 35), Heindl et al. (13) also did not observe a reduction in cardiovagal BRS with aldosterone injection. We believe there are at least several important differences between our study and the study of Heindl et al., which may explain the discrepant results. First, in the study by Heindl et al., two separate infusions of aldosterone (50 µg each) occurred ~6 and 1 h before BRS was assessed. In our study continuous aldosterone infusion began just 30 min before BRS measurements. Thus our results may be more indicative of the acute effects of aldosterone. Second, it is possible that the lower circulating levels of aldosterone achieved by Heindl et al. explain their negative findings. Aldosterone levels in the study of Heindl et al. were ~0.15 on a control day and ~0.52 nmol/l on the aldosterone infusion day. In contrast, we achieved higher aldosterone levels during our infusion (0.85 nmol/l), despite baseline levels similar to those of Heindl et al. (0.18 nmol/l). Thus it is possible that greater increases in circulating aldosterone are required to impair cardiovagal and sympathetic BRS. Finally, Heindl et al. used stepwise steady-state increases in BP to assess BRS, unlike the dynamic changes in BP that we induced with the modified Oxford technique. It is possible that with this former approach, baroreflex resetting may have occurred and obscured the ability to observe aldosterone-induced changes (8, 28).

In addition to these previous human studies, animal studies (33, 34) have demonstrated that acute and chronic aldosterone administration impairs baroreflex/mineralocorticoid receptor function. Baroreceptor dysfunction induced by perfusion of aldosterone through the isolated carotid sinus is abolished by locally administered spironolactone or by carotid endothelial denudation (34). Other animal data have indicated that central mineralocorticoid receptors are important in the context of autonomic control (11). Without performing studies in which we applied a mineralocorticoid receptor antagonist, we are unable to determine whether the baroreflex dysfunction we observed in the present study was mediated via the mineralocorticoid receptor. Moreover, we are unable to determine at what level of the baroreflex arc that aldosterone impaired BRS. These issues merit further investigation.
Even small increases in central venous pressure can decrease sympathetic BRS in humans, without effecting cardiovagal BRS (4). These decreases in sympathetic BRS are of similar magnitude to those measured in the present study. Although our short-term infusion of aldosterone is unlikely to have changed circulating blood volume, we cannot exclude that central venous pressure was not increased, possibly via a direct vasoconstrictor effect of aldosterone (23). However, we do not believe that this occurred since we observed no tendency for heart rate or MSNA to decrease at rest during the aldosterone infusion.

Several limitations are associated with the present study. First, our measures of baroreflex control of sympathetic outflow can only include discussion of the effects of aldosterone on sympathetic outflow directed toward skeletal muscle (i.e., MSNA). Second, the aldosterone infusion used in the present study was acute (<2 h). It was not practical to infuse aldosterone for longer periods of time while trying to maintain adequate MSNA recording sites. Therefore, we cannot determine whether similar changes in baroreflex function would occur during more prolonged periods of aldosterone excess as occurs in disease states such as congestive heart failure. It is possible that they may not. However, data in dogs suggest similar detrimental effects of aldosterone on baroreceptor/baroreflex function in the setting of both the acute and chronic administration (33, 34). Third, we are unable to determine at what region aldosterone influenced baroreflex function. It is possible that aldosterone influenced baroreflex function at the
level of the baroreceptors or via a central effect. Fourth, we cannot determine whether the reported detrimental effects of aldosterone on BRS would have been observed had other methods to assess baroreflex function been used, such as steady-state stepwise infusions of nitroprusside and/or phenylephrine. Lastly, it was not the aim of this study to determine possible sex-related differences in the response to aldosterone infusion. In our small comparison of five men and five women, we were unable to observe any sex-related differences in response to aldosterone infusion appear warranted.

Our findings may have important physiological and clinical implications. In humans with primary aldosteronism or congestive heart failure, circulating levels of aldosterone are elevated (17, 29) and baroreflex dysfunction occurs (9, 12, 31, 32). Presently, it has not been determined whether these two events are mechanistically linked. The fact that aldosterone infusion in the present study resulted in circulating levels of aldosterone similar to those observed in patients with congestive heart failure and primary aldosteronism (5, 14, 17, 29) and baroreflex impairment suggests that they may be. More definitive evidence into the role of chronic aldosterone excess in abnormal baroreflex function in pathological conditions (i.e., congestive heart failure) may be obtained by assessing baroreflex function before and after blocking the effects of aldosterone via a mineralocorticoid receptor antagonist such as spironolactone. Longer-term studies could also address possible escape from the effects of the antagonists. If supported, these studies could provide important mechanistic insight into the role of aldosterone in mediating detrimental changes in autonomic nervous system function in these conditions.

In conclusion, the present findings provide the first direct experimental support for the concept that aldosterone impairs sympathetic BRS in humans. These findings suggest that aldosterone may be a determinant of baroreflex dysfunction in disease states associated with aldosterone excess. The specific mechanism(s) underlying the detrimental effects of aldosterone on baroreflex function are unknown but warrant further investigation. ACKNOWLEDGMENTS

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GRANTS

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