Pharmacology

Acute Aldosterone Antagonism
Improves Cardiac Vagal Control in Humans

Janine Fletcher, BSc (Hons) PhD,* Ashesh N. Buch, MBChB, MRCP,†
Helen C. Routledge, BSc, MBChB, MRCP,† Saqib Chowdhary, MBBS PhD, MRCP,†
John H. Coote, PhD, DSc,* Jonathan N. Townend, MBChB, MD, FRCP†

Birmingham, United Kingdom

OBJECTIVES	We have examined the acute effects (<45 min) of aldosterone antagonism on heart rate variability and baroreflex sensitivity, markers of cardiac vagal control, in 13 healthy subjects.
BACKGROUND	Evidence for the beneficial effects of aldosterone antagonists comes from studies showing increased survival rates following their addition to standard heart failure therapy. Many mechanisms have been suggested for this action, including effects upon the autonomic nervous system.
METHODS	Heart rate variability and baroreflex sensitivity were examined 30 min following the administration of potassium canrenoate (intravenous) (aldosterone antagonist) or saline (control).
RESULTS	Active treatment reduced resting heart rate (−6 ± 1 beats/min [mean ± standard error mean]) compared to control (0 ± 1 beat/min) (p < 0.001) and increased measures of high frequency (HF) heart rate variability. Root mean square of successive RR interval differences increased by 21 ± 5 ms versus −6 ± 5 ms control (p < 0.001); HF power increased by 1,369 ± 674 ms^2 with aldosterone antagonism compared to −255 ± 431 ms^2 following saline infusion (p < 0.01). Baroreflex sensitivity (alpha-HF) was increased after active treatment (+4 ± 2 ms/mm Hg vs. 0 ± 1 ms/mm Hg control [p < 0.05]). No changes in plasma potassium levels were observed.
CONCLUSIONS	These results provide evidence that aldosterone antagonists acutely improve cardiac vagal control, irrespective of any diuretic effects, and may in part explain their beneficial effects in treatment of heart failure. (J Am Coll Cardiol 2004;43:1270−5) © 2004 by the American College of Cardiology Foundation

The adverse effects of activation of the renin-angiotensin-aldosterone system in congestive heart failure are evidenced by the beneficial prognostic effects of blockade of the system with angiotensin-converting enzyme inhibitors (1–3). Angiotensin II has traditionally been viewed as the major "culprit" effector of this system, exerting potentially deleterious cardiovascular effects (4–6). However, this octapeptide also stimulates the release of aldosterone (7), which appears to play an important role in the pathophysiology of heart failure. In patients with severe heart failure who were already established with angiotensin-converting enzyme inhibitor therapy, the RALES study showed that inhibition of aldosterone with spironolactone reduces the risk of death by 30% compared to placebo (8). The beneficial effects of aldosterone inhibition have also been confirmed in patients with impaired left ventricular function after myocardial infarction (9). There are a number of potential mechanisms by which aldosterone might contribute to mortality in heart failure, including the retention of sodium, loss of magnesium and potassium, the promotion of left ventricular hypertrophy and fibrosis, vascular fibrosis, and adverse

effects on endothelial function (10–17). In addition, aldosterone exerts adverse effects on cardiac autonomic control, including a reduction in the sensitivity of the human baroreceptor reflex (18). The independent relationship between impaired cardiac autonomic control and prognosis in disease states such as heart failure suggests that high levels of sympathetic activity and reduced vagal control may exert direct deleterious effects (19). Clinical studies have demonstrated that in heart failure patients, chronic treatment with spironolactone improves cardiac autonomic control measured by heart rate variability (11,20), providing a further possible explanation for the prognostic effects of this treatment. These studies could not, however, exclude the possibility that the improved heart rate variability was secondary to the beneficial hemodynamic effects of spironolactone (diuresis and decreased volume load) rather than any direct neuronal or receptor-mediated effects.

We have investigated the effects of acute aldosterone receptor blockade upon cardiac vagal control in healthy subjects by measuring heart rate variability and baroreflex sensitivity responses to a single intravenous dose of potassium canrenoate.

METHODS

Subjects. Thirteen healthy male volunteers age 18 to 34 (mean 27) years were studied. All subjects were in good

---

Abbreviations and Acronyms

HF	=	high frequency
LF	=	low frequency
MR	=	mineralocorticoid receptors
NO	=	nitric oxide
pNN50	=	percentage of successive RR interval differences exceeding 50 ms
RMSSD	=	root mean square of successive RR interval differences
SDNN	=	standard deviation of RR interval values

---

health with no history of cardiovascular or other disease, and all were normotensive (supine cuff blood pressure measurement <140/90 mm Hg during an initial screening visit). No subject was taking any medication. This study was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association and was approved by the South Birmingham Local Research Ethics Committee. Written informed consent was obtained before study entry.

Experimental protocol and study design. The study used a random-order, double-blind, placebo-controlled crossover protocol. Subjects were studied at the same time of day on two separate occasions with at least one week between studies. Experiments took place in a quiet, temperature-controlled autonomic function laboratory. The ambient temperature of the laboratory was maintained at 24 ± 1°C. Subjects were instructed to abstain from food or drink for at least 2 h before the experiment and from alcoholic or caffeinated beverages for 24 h. At an initial acclimatization visit, subjects were trained to breathe at a fixed rate using an audio signal that was adjusted to suit each individual's respiratory frequency.

Subjects were studied in the semisupine position on a couch. Venous cannulae were inserted into an antecubital vein for collection of blood samples and for drug administration. A standard three-lead electrocardiogram signal was amplified, processed (HF signal noise filter >500 Hz), and digitized at 500 Hz (National Instruments Corporation, analogue-to-digital converter board, Austin, Texas). A continuous arterial pressure signal was obtained using the Portapres device (TNO Biomedical Instrumentation, Amsterdam, the Netherlands) and was similarly digitized. In addition, brachial artery pressure was measured by intermittent cuff sphygmomanometry using an automated oscillometric system (Omron 711, Vernon Hills, Illinois, mean of 3 recordings). Respiratory movement was recorded from the amplified output of a strain gauge attached to an elastic strap around the chest. All digitized signals were displayed on the screen of a personal computer running LabVIEW 5.0 software (National Instruments Corporation). Subjects were rested for a 30-min period before the collection of data to allow hemodynamic stabilization. Two baseline recordings of at least 256 consecutive RR intervals together with blood pressure and respiratory measurements were acquired at fixed rate respiration.

Subjects were randomly assigned to intravenous infusions of the aldosterone antagonist potassium canrenoate (200 mg in 100 ml N-saline) (Roche, Welwyn Garden City, Hertfordshire, U.K.) or N-saline (100 ml) given over 5 min during the first of two studies. The other agent was given during a second study 7 to 14 days later.

After 30 min, when the active metabolite of potassium canrenoate, canrenone, is at its highest plasma concentration (21), two further recordings of electrocardiogram, blood pressure, and respiratory movements during fixed-rate respiration were taken.

Venous blood samples were collected at baseline (before infusion) and 30 min after infusion, and were analyzed using a blood gas analyzer (IL 1640 system, Instrumentation Laboratory, Lexington, Massachusetts) in order to determine plasma potassium concentration.

Data analysis. All data were analyzed by an investigator who remained blinded to the study agent. The data segments were reviewed before heart rate variability analysis and if necessary edited to exclude ectopic and artifact signals. Data segments containing >1% of ectopic beats were discarded. RR intervals before and after any ectopic beat were replaced by interpolation from the previous and following sinus RR intervals. Heart rate variability data were analyzed off-line using both the LabVIEW 5.0 software and Microsoft Excel X (Microsoft Corporation, Seattle, Washington). Each RR interval was assigned a value for systolic, diastolic, and average blood pressure, and a value for respiratory movement.

The RR intervals were analyzed in both the time and frequency domain as described previously (22). In brief, standard time domain measures of SDNN (standard deviation of RR interval values), RMSSD (root mean square of successive RR interval differences), and pNN50 (percentage of successive RR interval differences exceeding 50 ms) were calculated. SDNN expresses overall variability, whereas RMSSD and pNN50 assess high frequency or beat-to-beat variability, which is mediated primarily by the vagus nerve (23–25).

Power spectral analysis was performed on stationary time series using autoregressive modeling. The model order was selected to minimize the Akaike information criterion. The power at each underlying frequency was quantified by decomposing the total variability signal according to the method of Zetterburg. Powers at low frequency (LF, centered at ≈0.1 Hz) and at high frequency (HF, corresponding to the observed respiratory frequency) were thus determined. Power was expressed in absolute and normalised units (nU) (power/total power >0.04 Hz) (26).

To assess baroreflex sensitivity by cross-spectral analysis, the recordings of RR intervals and systolic blood pressure were interpolated (with a cubic spline) and then resampled at 1 Hz to produce a uniform time base. A cross spectrum of 256 samples was then analysed by fast Fourier transforms with the use of a Hanning window on successive overlapping records of 64 samples each. Baroreflex sensitivity was

Table 1. RR Interval, Mean Arterial Pressure Before, RR Interval Variability, and Baroreflex Sensitivity (Alpha-Index) at Baseline (Before Infusion of Saline or Active Treatment With Potassium Canrenoate [K-Can]) (n = 13)

Index	Control Baseline	Active Baseline	Significance
RR interval (ms)	932 ± 35	941 ± 37	NS
MAP (mm Hg)	90 ± 3	92 ± 3	NS
Pulse pressure (mm Hg)	63 ± 3	62 ± 3	NS
SDNN (ms)	76 ± 10	69 ± 7	NS
RMSSD (ms)	76 ± 14	64 ± 8	NS
pNN50 (%)	39 ± 6	35 ± 6	NS
LF power (ms^2)	1,232 ± 337	855 ± 155	NS
LF power (% nU)	27 ± 5	24 ± 4	NS
HF power (ms^2)	3,370 ± 1,001	2,921 ± 654	NS
HF power (% nU)	60 ± 5	65 ± 4	NS
Total power (ms^2)	6,963 ± 1,855	5,354 ± 961	NS
Alpha-HF (ms/mm Hg)	19.2 ± 2.3	20.3 ± 2.5	NS
Alpha-LF (ms/mm Hg)	12.8 ± 1.8	11.3 ± 1.3	NS

Values are mean ± SEM.
HF = high frequency; LF = low frequency; MAP = mean arterial pressure; NS = no significance between control baseline and active baseline values; pNN50 = percentage of successive RR interval differences exceeding 50 ms, RMSSD = root mean square of successive RR interval differences; SDNN = standard deviation of RR interval values.

Table 2. Change in RR Interval, Mean Arterial Pressure (MAP), RR Interval Variability, and Baroreflex Sensitivity (Alpha-Index) Following Control Infusion of Saline or Active Treatment With Potassium Canrenoate (K-Can) (n = 13)

Index	Δ Saline	Δ K-Can	p Value
RR Interval (ms)	+6 ± 12	+941 ± 21	0.002
MAP (mm Hg)	+7 ± 2	+2 ± 2	0.090
Pulse pressure (mm Hg)	−1 ± 3	+2 ± 3	0.4
SDNN (ms)	−3 ± 5	+18 ± 5	0.003
RMSSD (ms)	+2 ± 5	+20 ± 6	0.005
PNN50 (%)	0 ± 4	+15 ± 4	0.003
LF power (ms^2)	+219 ± 184	+337 ± 282	0.657
LF power (% nU)	+3 ± 2	−4 ± 4	0.137
HF power (ms^2)	+44 ± 424	+1,354 ± 762	0.040
HF power (% nU)	−3 ± 3	+3 ± 4	0.180
Total power (ms^2)	+397 ± 749	+2,466 ± 1,158	0.134
Alpha-HF (ms/mm Hg)	0 ± 1	+4 ± 1	0.034
Alpha-LF (ms/mm Hg)	+2 ± 1	+3 ± 1	0.108

Values are mean ± SEM.
Abbreviations as in Table 1.

determined by measurement of the alpha-index: the transfer function of variability in the systolic pressure signal to the variability in the RR interval. The alpha-index was calculated as the square root of the ratio of RR interval power to systolic spectral power in both the HF (alpha-HF) and LF (alpha-LF) bands. Data segments were accepted if a coherence of 0.5 or greater was achieved between RR interval and systolic blood pressure traces. Because alpha-HF is determined as a frequency at which sympathetic influences are not effective, this index represents gain in the vagal limb of the baroreflex (27).

Duplicate recordings were made at baseline and following infusion of either the vehicle control, or the active treatment. Results were calculated as the mean of the two recordings.

Statistical analysis. Changes in the parameters from baseline values to post infusion were determined for potassium canrenoate and for saline. Data were tested for normality of distribution and the significance of the differences between groups were determined using a two-tailed paired Student t test for normally distributed data; otherwise the Wilcoxon signed-rank test was used. Statistical significance was assumed at the 5% level (p < 0.05). Values are expressed as mean ± standard error mean.

RESULTS

The frequency of metronomic breathing was within the range 0.18 to 0.22 Hz. The baseline values before administration of saline or potassium canrenoate for RR interval, mean arterial pressure, measures of RR interval variability, and baroreflex sensitivity were not significantly different (Table 1). Infusion of potassium canrenoate had no significant effect on baseline mean arterial pressure compared to saline infusion (Table 2), but was associated with a significant increase in RR interval duration (Table 2).

Heart rate variability assessed by SDNN was significantly increased after aldosterone receptor blockade compared to saline (Table 2). Measures of HF vagally mediated heart rate variability in both the time and frequency domains were also significantly increased following potassium canrenoate infusion compared to saline. In the time domain both RMSSD and pNN50 were elevated by aldosterone receptor blockade compared with control (Table 2). Power spectral analysis of the RR intervals revealed that with potassium canrenoate, HF power was significantly increased, whereas LF power remained unchanged (Fig. 1, Table 2).

Cross-spectral analysis of RR interval and systolic blood pressure variability was possible with all data sets (i.e., coherence was >0.05). Alpha-HF was greater following aldosterone receptor blockade compared with saline infusion, whereas alpha-LF remained unchanged (Table 2).

Baseline values for potassium concentrations were not statistically different (4.4 ± 0.5 mmol/l pre-saline; 4.4 ± 0.2 mmol/l pre-potassium canrenoate). The mean after infusion of saline (4.4 ± 0.5 mmol/l) and potassium canrenoate (4.4 ± 0.2 mmol/l) remained unchanged.

DISCUSSION

Acute administration of the aldosterone antagonist potassium canrenoate results in an increase in HF measures of heart rate variability and an increase in baroreflex sensitivity, suggesting that aldosterone exerts a tonic inhibitory effect on cardiac vagal control. Several studies have demonstrated that the diuretic response to potassium canrenoate does not occur until at least 2 h after intravenous administration (28,29), so it is unlikely that these results can be explained by any change in circulating volume. Similarly, the results cannot be explained by changes in blood pressure or potassium concentration, or by chronic effects on vascular fibrosis and compliance at the arterial baroreceptors.
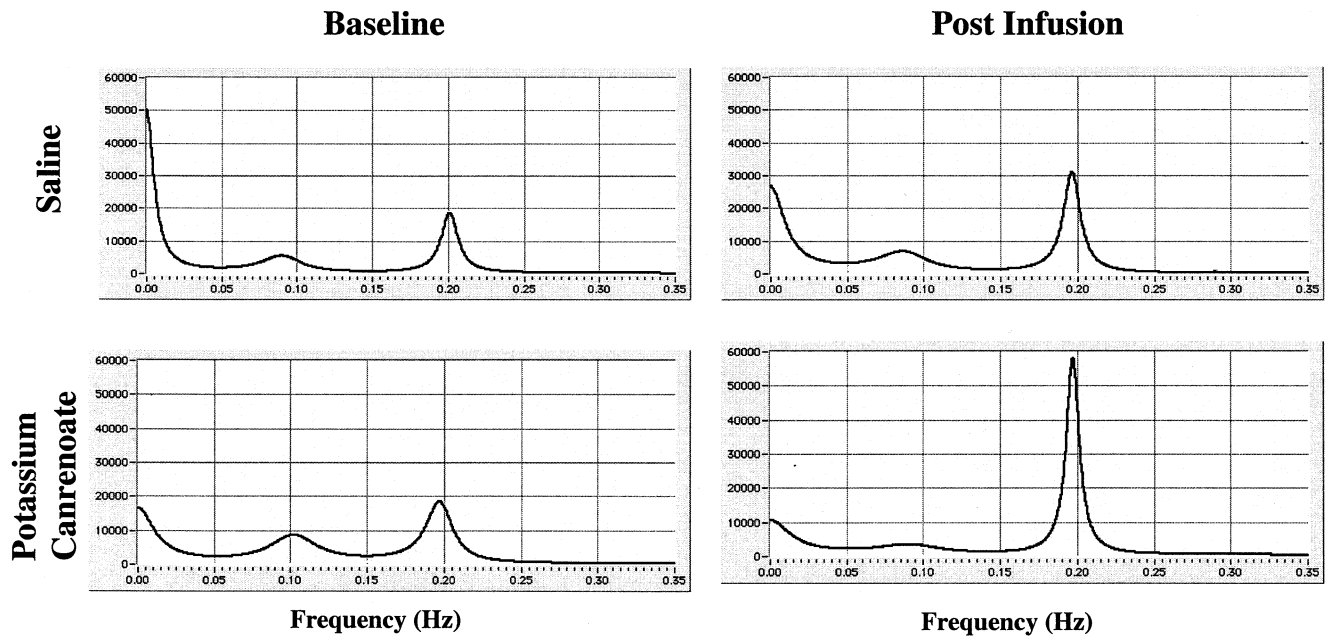
Figure 1. Power spectral density plots showing the distribution of power (ms^2) against frequency (Hz). Representative spectral plots from a subject at baseline and following saline or potassium canrenoate infusion, demonstrating the increase in high-frequency power (~0.2 Hz) after active treatment with no significant change in low-frequency power (~0.1 Hz) compared to control.

The acute autonomic effects of aldosterone inhibition that we have demonstrated are consistent with the findings of human study in which infusion of aldosterone caused an immediate reduction in baroreflex sensitivity (18). One possible mechanism of action of aldosterone could be a direct effect on the arterial baroreceptors themselves. In dogs, local infusion of aldosterone into the isolated carotid sinus caused a reduction in baroreceptor discharge, an effect that was abolished by the aldosterone antagonist spironolactone (30). Denudation of the carotid sinus endothelium also abolished the action of aldosterone. The authors concluded that aldosterone exerts inhibitory effects on the baroreceptor by a mechanism dependent on endothelial cell function. More recently, an adverse effect of aldosterone on endothelial function consistent with a reduced bioavailability of nitric oxide (NO) has been confirmed (31,32). Our findings might therefore be explained by improved baroreceptor function as a result of inhibition of the actions of aldosterone on vascular endothelial cell function.

A second explanation for our results is that aldosterone exerts a tonic inhibitory effect on peripheral or central cardiac vagal neuronal activity. Traditionally, mineralocorticoid receptors (MR) (cytoplasmic type I receptors) were thought to be located exclusively in the distal tubule of the kidneys (33), but numerous studies have identified MRs within the central nervous system as well as in myocardium and blood vessels. Centrally administered aldosterone has been shown to increase blood pressure in rats, suggesting a central neurogenic action (34). Furthermore, direct administration of spironolactone into the central nervous system of rats with chronic heart failure has been shown to improve cardiac autonomic control by reducing sympathetic outflow

and improving the arterial baroreflex (35). These data suggest that activation of mineralocorticoid receptors in the central nervous system plays an important role in the impaired cardiac autonomic function seen in heart failure and supports a central rather than peripheral nervous mechanism for the acute effects of potassium canrenoate.

Like other mineralocorticoids, aldosterone has long been held to exert its actions at a genomic level, affecting the transcription of messenger ribonucleic acid and subsequently protein synthesis. Over the last decade however, convincing evidence of a non-genomic, or direct effect of aldosterone has become available (36). These nongenomic effects cause a rapid (<5 min following intravenous aldosterone administration) rise in systemic vascular resistance, which is not due to sodium or water retention (37). Until recently it was the consensus that these nongenomic effects are insensitive not only to inhibitors of transcription, but also to the classical mineralocorticoid receptor antagonists including canrenone. A recent study, however, has demonstrated that RU28318, a weak competitor of the classic MR, blocks rapid effects of aldosterone. As this compound is structurally similar to the active metabolites of spironolactone and canrenoate, it is therefore plausible that canrenone in our study was blocking rapid nongenomic effects of aldosterone (38) upon the autonomic nervous system. A rapid effect of aldosterone within the autonomic nervous system might also be mediated by an alteration in the NO pathway. The documented effects of aldosterone on the bioavailability of endothelial NO leads us to speculate that it may also reduce the synthesis and or bioavailability of neuronal NO. In our previous studies we have demonstrated a powerful facilitatory effect of NO on human cardiac

autonomic control, a result consistent with a large body of published work that supports a vagotonic role for NO (39). Thus, a further mechanism by which aldosterone may exert an acute inhibitory action on cardiac vagal control may be by reducing the bioavailability of NO within the cardiac autonomic nervous system.

Finally, aldosterone is also known to exert a weak adrenergic action. In healthy humans aldosterone blunted the blood pressure-lowering effect of a beta-blocking agent, suggesting that an acute nongenomic influence on adrenergic activity (40). It is thus also plausible that any inhibitory influence of aldosterone on cardiac vagal activity might not occur via the well-described inhibitory action of the sympathetic nervous system on cardiac vagal activity (41,42). Conversely, vagal enhancement by aldosterone receptor antagonists might in part result from reduced sympathetic activity.

In conclusion, this study demonstrates that acute treatment with an aldosterone antagonist improves cardiac vagal control in healthy volunteers. The improved cardiac vagal tone that we have demonstrated in healthy volunteers may be of clinical significance in patients with heart failure who, even at an early stage of their disease, have profound autonomic dysfunction (43) and in whom levels of aldosterone are often elevated despite treatment with angiotensin-converting enzyme inhibitors. Patients who demonstrate aldosterone "escape" from angiotensin-converting enzyme inhibitor therapy are known to have a particularly poor prognosis (44), and the adverse actions of aldosterone on cardiac autonomic control may contribute to this. The clinical significance of the relationship between activation of the renin-angiotensin system and impaired cardiac autonomic control was reconfirmed recently by the demonstration that plasma renin is an important determinant of cardiac vagal control in hypertension (45). Our results suggest that treatment with aldosterone antagonists improves cardiac autonomic control via rapid nongenomic effects that are most likely exerted within the central nervous system.

Study limitations. Although intra-arterial blood pressure measurement is the most accurate means of beat to beat blood pressure monitoring, the "Portapres" that we employed uses infrared plethysmography and a fast-acting servo to "volume clamp" finger arteries and has been found to provide accurate beat-to-beat blood pressure measurement (46). Intra-arterial recording was not employed in order to minimize patient discomfort and associated adverse autonomic effects. For similar reasons we did not use a urinary catheter. Although a small diuresis in response to potassium canrenoate cannot therefore be excluded, we feel that any such effect, if present, was probably small.

Finally, we did not demonstrate directly the aldosterone receptor antagonism of potassium canrenoate in this study. The single dose used has, however, been shown to result in effective aldosterone antagonism in healthy humans (21,47).

Reprint requests and correspondence: Dr. Ashesh N. Buch, Research Fellow in Cardiology, Department of Cardiovascular Medicine, Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH, United Kingdom. E-mail: asheshbuch@hotmail.com.

REFERENCES

1. The CONSENSUS Trial Study Group. Effects of enalapril on mortality in severe congestive heart failure. Results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS). N Engl J Med 1987;316:1429–35.
2. Cohn JN, Johnson G, Ziesche S, et al. A comparison of enalapril with hydralazine-isosorbide dinitrate in the treatment of chronic congestive heart failure. N Engl J Med 1991;325:303–10.
3. The SOLVD Investigators. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. N Engl J Med 1991;325:293–302.
4. Townend JN. Angiotensin II as a modulator of cardiovascular autonomic control. Cardiologia 1996;41:217–25.
5. Vaile JC, Fletcher J, Littler WA, Coote JH, Townend JN. Angiotensin II modulates cardiovascular autonomic control in the absence of baroreflex loading. Heart 1998;80:127–33.
6. Gavras I, Gavras H. Angiotensin II as a cardiovascular risk factor. J Hum Hypertens 2002;16:S2–6.
7. Peach MJ. Renin-angiotensin system: biochemistry and mechanisms of action. Physiol Rev 1977;57:313–70.
8. Pitt B, Zannad F, Remme WJ, et al. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. N Engl J Med 1999;341:709–17.
9. Pitt B, Remme W, Zannad F, et al. Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. N Engl J Med 2003;348:1309–21.
10. Barr CS, Lang CC, Hanson J, Arnott M, Kennedy N, Struthers AD. Effects of adding spironolactone to an angiotensin-converting enzyme inhibitor in chronic congestive heart failure secondary to coronary artery disease. Am J Cardiol 1995;76:1259–65.
11. MacFadyen RJ, Barr CS, Struthers AD. Aldosterone blockade reduces vascular collagen turnover, improves heart rate variability and reduces early morning rise in heart rate in heart failure patients. Cardiovasc Res 1997;35:30–4.
12. Schlaich MP, Schobel HP, Hilgers K, Schmieder RE. Impact of aldosterone on left ventricular structure and function in young normotensive and mildly hypertensive subjects. Am J Cardiol 2000;85:1199–206.
13. Duprez DA, De Buyzere ML, Rietzschel ER, et al. Inverse relationship between aldosterone and large artery compliance in chronically treated heart failure patients. Eur Heart J 1998;19:1371–6.
14. Hayashi M, Tsutamoto T, Wada A, et al. Relationship between transcardiac extraction of aldosterone and left ventricular remodeling in patients with first acute myocardial infarction: extracting aldosterone through the heart promotes ventricular remodeling after acute myocardial infarction. J Am Coll Cardiol 2001;38:1375–82.
15. Tsutamoto T, Wada A, Maeda K, et al. Spironolactone inhibits the transcardiac extraction of aldosterone in patients with congestive heart failure. J Am Coll Cardiol 2000;36:838–44.
16. Mizuno Y, Yoshimura M, Yasue H, et al. Aldosterone production is activated in failing ventricle in humans. Circulation 2001;103:72–7.
17. Farquharson CA, Struthers AD. Aldosterone induces acute endothelial dysfunction in vivo in humans: evidence for an aldosterone-induced vasculopathy. Clin Sci (Lond) 2002;103:425–31.
18. Yee KM, Struthers AD. Aldosterone blunts the baroreflex response in man. Clin Sci (Lond) 1998;95:687–92.
19. Nolan J, Batin PD, Andrews R, et al. Prospective study of heart rate variability and mortality in chronic heart failure: results of the United Kingdom heart failure evaluation and assessment of risk trial (UK-heart). Circulation 1998;98:1510–6.
20. Yee KM, Pringle SD, Struthers AD. Circadian variation in the effects of aldosterone blockade on heart rate variability and QT dispersion in congestive heart failure. J Am Coll Cardiol 2001;37:1800–7.

21. Krause W, Karras J, Seifert W. Pharmacokinetics of canrenone after oral administration of spironolactone and intravenous injection of canrenoate-K in healthy man. Eur J Clin Pharmacol 1983;25:449–53.
22. Chowdhary S, Vaile JC, Fletcher J, Ross HF, Coote JH, Townend JN. Nitric oxide and cardiac autonomic control in humans. Hypertension 2000;36:264–9.
23. Fouad FM, Tarazi RC, Ferrario CM, Fighaly S, Alicandri C. Assessment of parasympathetic control of heart rate by a noninvasive method. Am J Physiol 1984;246:H838–42.
24. Katona PG, Jih F. Respiratory sinus arrhythmia: noninvasive measure of parasympathetic cardiac control. J Appl Physiol 1975;39:801–5.
25. Kollai M, Koizumi K. Reciprocal and non-reciprocal action of the vagal and sympathetic nerves innervating the heart. J Auton Nerv Syst 1979;1:33–52.
26. Pagani M, Lombardi F, Guzzetti S, et al. Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. Circ Res 1986;59:178–93.
27. DeBoer RW, Karemaker JM, vanMontfrans GA. Determination of baroreflex sensitivity by spectral analysis of spontaneous blood-pressure and heart-rate fluctuations in man. In: Lown B, Malliani A, Prosdocimi M, editors. Neural Mechanisms and Cardiovascular Disease. Padova: Liviana Press, 1986:303–15.
28. Ramsay LE, Shelton JR, Tidd MJ. The pharmacodynamics of single doses of prorenoate potasssium and spironolactone in fludrocortisone treated normal subjects. Br J Clin Pharmacol 1976;3:475–82.
29. Casals-Stenzel J, Schmalbach J, Losert W. Improved method for comparative evaluation of aldosterone antagonists in healthy man. Eur J Clin Pharmacol 1977;12:247–55.
30. Wang W, McClain JM, Zucker IH. Aldosterone reduces baroreceptor discharge in the dog. Hypertension 1992;19:270–7.
31. Bauersachs J, Heck M, Fraccarollo D, et al. Addition of spironolactone to angiotensin-converting enzyme inhibition in heart failure improves endothelial vasomotor dysfunction: role of vascular superoxide anion formation and endothelial nitric oxide synthase expression. J Am Coll Cardiol 2002;39:351–8.
32. Farquharson CA, Struthers AD. Spironolactone increases nitric oxide bioactivity, improves endothelial vasodilator dysfunction, and suppresses vascular angiotensin I/angiotensin II conversion in patients with chronic heart failure. Circulation 2000;101:594–7.
33. Vander A, Malvin R, Wilde W, Lapides J, Sullivan L, McMurray V. Effects of adrenalectomy and aldosterone on proximal and distal tubular sodium absorption. Proc Soc Exp Biol Med 1958;99:323–5.
34. Gomez-Sanchez EP. Central hypertensive effects of aldosterone. Front Neuroendocrinol 1997;18:440–62.
35. Francis J, Weiss RM, Wei SG, et al. Central mineralocorticoid receptor blockade improves volume regulation and reduces sympathetic drive in heart failure. Am J Physiol Heart Circ Physiol 2001;281:H2241–51.
36. Losel RM, Feuring M, Falkenstein E, Wehling M. Nongenomic effects of aldosterone: cellular aspects and clinical implications. Steroids 2002;67:493–8.
37. Schmidt BM, Montealegre A, Janson CP, et al. Short term cardiovascular effects of aldosterone in healthy male volunteers. J Clin Endocrinol Metab 1999;84:3528–33.
38. Alzamora R, Michea L, Marusic ET. Role of 11beta-hydroxysteroid dehydrogenase in nongenomic aldosterone effects in human arteries. Hypertension 2000;35:1099–104.
39. Chowdhary S, Townend JN. Role of nitric oxide in the regulation of cardiovascular autonomic control. Clin Sci 1999;97:5–17.
40. Schmidt BM, Georgens AC, Martin N, et al. Interaction of rapid nongenomic cardiovascular aldosterone effects with the adrenergic system. J Clin Endocrinol Metab 2001;86:761–7.
41. Potter EK. Prolonged non-adrenergic inhibition of cardiac vagal action following sympathetic stimulation: neuromodulation by neuropeptide Y? Neurosci Lett 1985;54:117–21.
42. Wetzel GT, Goldstein D, Brown JH. Acetylcholine release from rat atria can be regulated through an alpha 1-adrenergic receptor. Circ Res 1985;56:763–6.
43. Francis GS, Benedict C, Johnstone DE, et al. Comparison of neuroendocrine activation in patients with left ventricular dysfunction with and without congestive heart failure. A substudy of the Studies of Left Ventricular Dysfunction (SOLVD). Circulation 1990;82:1724–9.
44. Lee AF, MacFadyen RJ, Struthers AD. Neurohormonal reactivation in heart failure patients on chronic ACE inhibitor therapy: a longitudinal study. Eur J Heart Fail 1999;1:401–6.
45. Virtanen R, Jula A, Kuusela T, Helenius H, Voipio-Pulkki LM. Reduced heart rate variability in hypertension: associations with lifestyle factors and plasma renin activity. J Hum Hypertens 2003;17:171–9.
46. Imholz BP, Langewouters GJ, van Montfrans GA, et al. Feasibility of ambulatory, continuous 24-hour finger arterial pressure recording. Hypertension 1993;21:65–73.
47. van Buren M, Boer P, Koomans HA. Effects of acute mineralocorticoid and glucocorticoid receptor blockade on the excretion of an acute potassium load in healthy humans. J Clin Endocrinol Metab 1993;77:902–9.