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Paper

Keywords:
angiotensin II, dietary sodium, angiotensin type 1 receptor antagonism, angiotensin-converting enzyme inhibition, cardiomyocyte contraction, inotropy

Combined renin-angiotensin system blockade and dietary sodium restriction impairs cardiomyocyte contractility

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Abstract

Introduction

Blockade of the renin-angiotensin system (RAS) by combined angiotensin-converting enzyme inhibitor and angiotensin type 1 receptor (AT_1) antagonist treatment with reduced dietary sodium intake produces suppression of cardiac growth and regression of cardiac hypertrophy. The purpose of this study was to investigate whether cardiac growth suppression by combined RAS blockade under conditions of dietary sodium restriction is associated with cardiomyocyte atrophy and contractile dysfunction and whether this intervention modifies cardiomyocyte inotropic responsiveness to angiotensin II (Ang II).

Methods

Rats were fed a high (4% w/w) or low (0.2% w/w) NaCl diet for six days. Both groups were then given a combined intraperitoneal injection of perindopril (6 mg/kg/day) and losartan (10 mg/kg/day) with maintained dietary treatment for another seven days. At the end of the treatment period, animals were anaesthetised and their hearts were removed and weighed. Left ventricular cardiomyocytes were isolated by enzymatic dissociation and cell dimensions were evaluated. A line scan camera and digital imaging technique were used to assess cardiomyocyte contraction and inotropic responses to exogenous Ang II (10^{-10} to 10^{-6} M).

Results

Dietary treatment alone had no effect on body growth, whereas combined RAS blockade suppressed somatic growth in the low sodium (LS) group, compared with the high sodium (HS) group. This growth suppression in the LS group was also evident in the heart at the organ and cellular level. Studies of cardiomyocyte contraction showed that myocytes from the LS group exhibited contractile instability and depression of contractile performance. Compared with the HS group, myocytes from the LS group showed a significant reduction in maximum cell shortening (6.40±0.17 *vs.* 7.32±0.16% resting length, $p<0.05$), and maximum rate of shortening (3.85±0.14 *vs.* 4.29±0.11 cell length/ms, $p<0.05$). Myocytes of the HS group exhibited negative inotropic responses to Ang II at all concentrations tested, with a significant reduction in maximum cell shortening by 11–16% after 12 minutes peptide exposure ($p<0.05$ *vs.* non-treated control). In comparison, Ang II elicited both positive and negative responses in myocytes from the LS group, with a predominant negative inotropic effect.

Conclusions

This study provides evidence that combined RAS blockade treatment under restricted sodium intake conditions can impair cardiomyocyte contractile function in association with cardiomyocyte growth suppression. Chronic RAS blockade qualitatively alters the intrinsic inotropic status and responsiveness of ventricular cardiomyocytes, and this shift is further modulated by dietary sodium intake conditions.

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Introduction

The renin-angiotensin system (RAS) is implicated in the pathophysiology of cardiovascular diseases, such as hypertension, cardiac hypertrophy, and heart failure.[1] The beneficial effects of RAS suppression by treatment with either angiotensin II converting enzyme (ACE) inhibitors or angiotensin type 1 receptor (AT_1) antagonist in cardiovascular disorders have been extensively demonstrated.[2-5] ACE inhibitors (ACE-I) and AT_1 antagonists have been widely used in the treatment of hypertension.[6] These pharmacological agents have also been shown to regress cardiac hypertrophy and to reduce the extent of cardiac contractile dysfunction in experimental heart failure.[2,5]

The observation that chronic administration of ACE-Is to hypertensive patients leads to a lesser degree of angiotensin II (Ang II) suppression, suggests a partial escape of ACE inhibition with long-term treatment.[7] Clinical and experimental studies have found that long-term treatment with ACE-Is is associated with an increase in plasma renin activity and plasma angiotensin I (Ang I) levels.[8,9] Under these circumstances, Ang II formation by enzymatic processes other than ACE, such as chymase,[10] may become important. AT_1 antagonist treatment is also observed to produce an increase in plasma Ang II levels,[8,9] which may partially override the blockade at receptor sites. Therefore, complete blockade of the RAS is unlikely to be achieved by monotherapy with either agent. Experimentally, dual target treatment using combined ACE-I with AT_1 antagonism, has been shown to produce more effective inhibition of the RAS.[9] Moreover, combined administration of an ACE-I

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and AT_1 antagonist is more effective than either agent alone in the treatment of hypertension, cardiac hypertrophy, and heart failure.[3,4,11] Thus, the combination of ACE-I with AT_1 antagonism is a promising therapeutic approach for cardiac and vascular disorders.

The RAS is also important in sodium homeostasis, and variation in sodium intake can modify the operation of this system. Low sodium intake activates renin secretion, whereas high sodium intake suppresses enzyme secretion.[12,13] In Ang II-induced cardiac hypertrophy, salt loading can further increase heart size, indicating that sodium intake and Ang II exert additive effects on cardiac growth.[14] There is clinical and experimental evidence that high sodium intake overcomes the blocking effects of ACE-I in lowering blood pressure and regressing cardiac hypertrophy.[15,16] Therefore, sodium intake is a determinant of the efficacy of RAS suppression. The means by which sodium intake modulates the blockade of RAS is not well understood.

Recent studies have focused on the influence of sodium intake and combined RAS blockade on cardiac growth. Griffiths *et al.*[17] showed that the combination of an ACE-I and AT_1 antagonist with low sodium intake caused a reduction in heart weight in normotensive rats, and that this effect was reversed by high sodium intake. Moreover, in hypertensive rats this co-treatment elicited a more pronounced antihypertrophic effect in a sodium-depleted state, but not in sodium loading.[18] It seems that sodium depletion may amplify the effect of RAS blockade on cardiac growth. There is, however, no information about how this manipulation may affect cardiac function. Thus, we hypothesised that chronic RAS blockade with sodium depletion may cause an alteration in cardiac function associated with growth suppression, which may be modulated by salt loading.

The purpose of this study was to assess the functional characteristics of cardiomyocytes from normotensive rats treated *in vivo* with both an ACE-I and an AT_1 antagonist, together with either high or low sodium intake. It has been previously established that physiological concentrations of Ang II exert a positive inotropic effect on cardiomyocytes,[19] suggesting a role for this endogenous mediator in the regulation of normal cardiac contractility. Thus, we evaluated the additional hypothesis that chronic combined RAS blockade with contrasting dietary sodium regimes is able to alter cardiomyocyte inotropic responses to Ang II. Basal and Ang II-stimulated cardiomyocyte contractile function was assessed using enzymatically-dissociated single left ventricular cardiomyocytes.

Materials and methods

Animals and treatments

Experiments were conducted following the recommendations of the Australian Code of Practice for the Care and Use of Animals Scientific Purposes and with the approval of the University of Melbourne Animal Experimentation Ethics Committee. Male Sprague-Dawley rats aged 10–11 weeks old were obtained from the Biological Research Facility at the University of Melbourne. The animals were divided into two groups to receive different diet and drug regimens. One group of rats received a high sodium diet (4% w/w) and the other was given a low sodium diet (0.2% w/w). Both diets were prepared identically as previously described.[17,20] Animals were allowed to establish feeding on each diet for six days, then both groups were given RAS blockade treatment by combining intraperitoneal (i.p.) injection of an ACE-I (perindopril 6 mg/kg/day) and an AT_1 antagonist (losartan 10 mg/kg/day). All rats were maintained on the diet with drug treatment for eight days, and body weight was determined daily before drug injection. At the end of the treatments, the animals were anaesthetised by i.p. injection of pentobarbitone sodium, 60 mg/kg, (Rhone Merieux). The hearts were removed and weighed before preparation of cardiomyocytes.

Cardiomyocyte isolation and dimension measurement

Left ventricular cardiomyocytes were isolated by an enzymatic dissociation procedure as described previously.[21] In brief, the isolated hearts were immediately mounted on a Langendorff apparatus and retrogradely perfused through the aorta with bicarbonate-buffered Krebs solution at 37°C. Then, collagenase type 2 (Worthington) was added to the final recycling perfusate to achieve 0.5 mg/ml. The left ventricles were minced and the tissue fragments incubated in the same enzymatic buffer for 20 minutes. Dissociated myocytes were filtered, washed and re-suspended in HEPES buffered solution containing trypsin inhibitor (Type 1-S from soybean; Sigma). An aliquot of myocyte suspension was added to a glass-base chamber mounted on the stage of an inverted microscope, and the myocytes were allowed to settle and adhere to the bottom of the chamber. An eyepiece graticule was used to measure the length and width of randomly selected myocytes (50–100 cells per heart) with clear striation and rod-shaped morphology. The eyepiece graticule was calibrated using a stage grati-cule viewed with a x40 objective. Mean cell length and width were calculated for each animal and treated as a single observation for comparison between groups.

Measurement of cardiomyocyte contraction

After dimension measurement was completed, myocytes were superfused with oxygenated HEPES buffer of the following composition (mM): 118 NaCl, 4.8 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25.0 Na-HEPES, 11.0 glucose and 1.0 $CaCl_2$ with a final pH of 7.4. The cell superfusion system, as described previously,[22] maintained the chamber temperature at 36±0.5°C and the flow rate of the superfusate at 2 ml/minute. Cells were paced to contract at 3 Hz using field electrode stimulation.

Cardiomyocyte contraction was determined by measurement of changes in cell length, using a line scan camera and digital imaging system, as

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described previously.[21,23] The system allowed cell length scanning every 1.088 ms during each contraction, with scanning synchronised to commence with the stimulus delivery to the cell. The cell length changes during each contractile cycle were analysed by custom-designed software to obtain a number of contractile parameters. The following contractile parameters were defined. Maximum of cell shortening (%S) was expressed as a percentage of change in cell length relative to diastolic cell length (L_0). Time at maximum shortening (T_M) was the time at which %S was obtained. The excitation-contraction latency and contractile cycle time were designated T_0 and T_F respectively. Maximum rate of cell shortening (MRS) and lengthening (MRL) were calculated as rate of cell length changes during shortening and lengthening. The times at which MRS and MRL were recorded were designated T_{MRS} and T_{MRL} respectively.

Evaluation of basal contraction and inotropic responses

Cardiomyocytes were superfused with HEPES buffer containing 0.25% bovine-serum albumin (BSA; Sigma) for 3–5 minutes to achieve steady state. Data acquisition was monitored and basal contraction was recorded for 3 minutes. The mean basal contractile parameters of cardiomyocytes from rats treated with high or low sodium with RAS blockade were calculated and compared. After the basal contraction from each myocyte was determined, myocytes were then exposed to a single concentration of Ang II for 12 minutes. The responses to Ang II were evaluated over a concentration range of 10^{-10}-10^{-8} M. A separate group of myocytes was superfused with HEPES buffer plus BSA vehicle as a time control. A total of 65 cells was allocated to experimental groups as follows: Control (HS, n=8; LS, n=8); Ang II 10^{-8} M (HS, n=8; LS, n=8); Ang II 10^{-9} M (HS, n=9; LS, n=8); Ang II 10^{-10} M (HS, n=8; LS, n=8). The contractile parameters from each cell were averaged during the test period at 1-minute intervals. The average parameter values from each interval were then expressed as a percentage of the average value of basal contraction for each cell to obtain normalised parameter values. Mean averaged normalised contractile parameter values were calculated for each experimental group.

Statistics

All data are presented as mean ± standard error of mean (SEM). Comparison between two groups was made by unpaired Student's *t*-test. Multiple comparisons between groups were performed using one-way ANOVA, with post-hoc analysis by Tukey HSD as applicable. A value of $p<0.05$ was considered significant.

Results

Animal growth and heart growth

At the commencement of HS and LS diet treatments, the animal body weights were comparable (359±9 *vs.* 360±10 g), and the extent of weight gain during the initial six-day dietary treatments were also similar for the two groups, averaging about 6.5% over this period (Figure 1). These observations confirm that HS and LS diet treatments alone do not differentially influence somatic growth. When the pharmacological RAS blockade treatments were superimposed on the dietary treatments, a marked difference in the growth rate of the LS treatment group was evident. Over treatment days 7–14, a very modest body weight increase of slightly over 1% (relative to pre-drug treatment weight) was recorded for the LS group, whilst the HS group maintained a growth rate similar to that seen prior to the pharmacological intervention (Figure 1). This result suggests that RAS blockade suppresses body growth under conditions in which sodium intake is restricted, but appears to have no growth-suppressing effect at higher sodium intake levels.

Figure 1 Percentage weight gain of rats on high sodium (HS) or low sodium (LS) diet, comparing initial weight *vs.* weight at day 6, and on dietary treatment with combined RAS blockade (RASB), comparing weight at day 6 *vs.* day 14. (HS, n=18; LS, n=18; HS+RASB, n=18, LS+RASB, n=18 animals). *p<0.05 *vs.* HS+RASB.

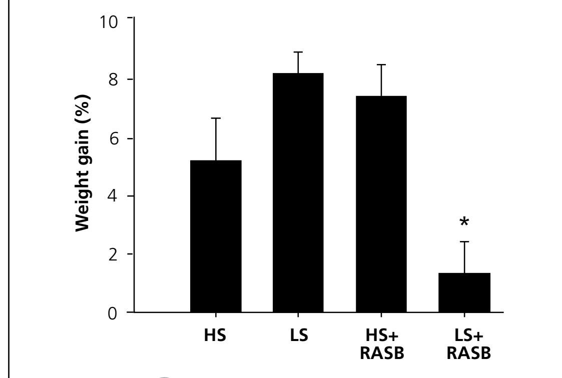

Figure 2 shows the effects of the diet and drug treatments on wet heart weight (HW) and heart weight to body weight ratio (HW/BW) measured at the completion of the 14-day treatment period. Post RAS blockade, both the HW and HW/BW ratio of the LS group were significantly lower than the HS group. Whilst the reduced somatic growth rate observed in the LS group would contribute to the reduced cardiac weight index measured, the finding that non-normalised HW was also reduced in this group indicates that RAS blockade also exerted a specific cardiac growth suppression effect.

The cellular dimensional data confirm this interpretation. The mean cell length and width of cardiomyocytes from rats on the reduced salt diet with RAS blockade were significantly less than those of high salt group, as shown in Figure 3. The relative stunting effect of RAS blockade on cell width growth in the LS group appeared to be slightly more accentuated than the effect on cell length growth. Thus, RAS blockade suppresses cardiac growth in animals on reduced sodium diet, and this effect is observable at the level of the whole heart and at the cellular level.

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Figure 2 Heart weight (HW) (**A**) and heart weight/body weight ratio (HW/BW) (**B**) of rats on combined RAS blockade treatment with high sodium (HS+RASB, n=23) or low sodium diet (LS+RASB, n=23). *p<0.05 *vs.* HS+RASB.

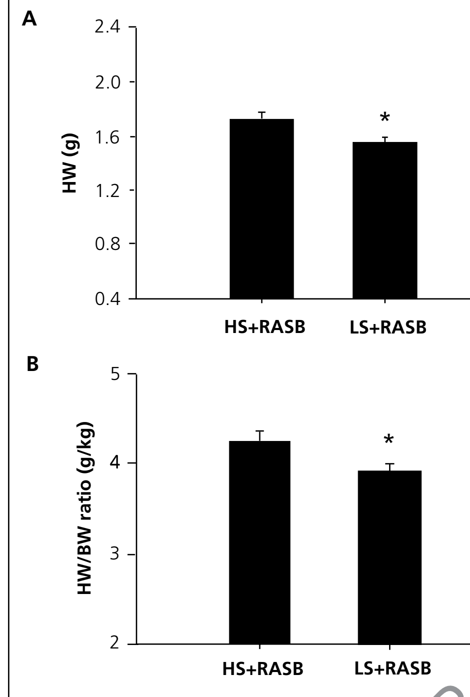
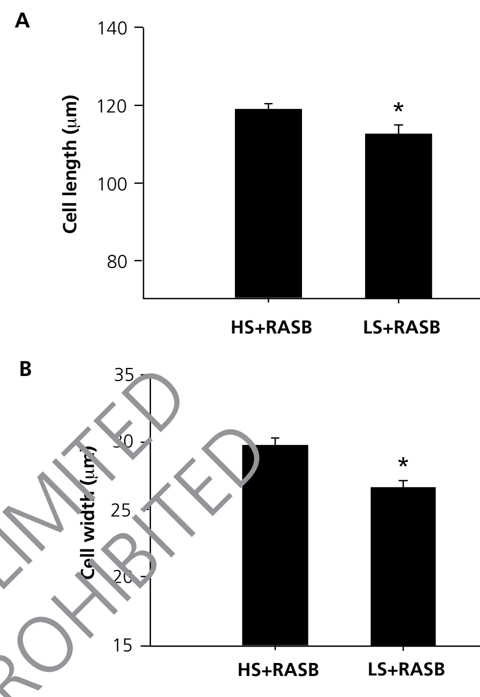


Figure 3 Mean length (**A**) and width (**B**) of cardiomyocytes isolated from rats on combined RAS blockade treatment with high sodium (HS+RASB, n=7) or low sodium diet (LS+RASB, n=7). Each n value represents the average of 50–100 myocytes per heart. *p<0.05 *vs.* HS+RASB.



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Basal contractile performance of cardiomyocytes

Data describing the basal contractile function of ventricular myocytes from rats on the HS and LS diets with RAS blockade are presented in Table 1. In general, the myocytes from the LS group exhibited a higher incidence of contractile instability than cells from the HS group. Data from myocytes observed to have unstable contractile pattern in the initial 5 minutes of the recording were excluded from the analysis. Thus, the averaged data from all cells in the LS group may overestimate the basal contractile function. To control for an effect of cell length *per se*, cells selected for this contraction study were size-matched as confirmed by the similarity of the values for resting cell length (L_0) of myocytes from the HS and LS groups (Table 1). The myocytes from the LS group exhibited a significantly lower basal %S and slower rate of shortening (MRS), compared with those of the HS group, indicative of a reduced contractility in the myocytes from the sodium restricted group. The rate of lengthening (MRL) was also somewhat reduced in the LS myocyte group, but this decline in relaxation rate did not attain statistical significance. No difference in the timing of the events early in the contractile cycle of HS and LS myocytes could be detected, but the final cycle completion time was significantly earlier for the myocytes from the LS diet group. Overall, myocytes from rats treated with RAS blockers and low sodium diet exhibit a depressed basal contractile performance and an abbreviated contractile cycle.

Table 1 Basal contractile parameter values of cardiomyocytes from rats on combined RAS blockade treatment with high sodium (HS+RASB) or low sodium diet (LS+RASB).

Contractile parameters	HS+RASB (n=50)	LS+RASB (n=51)
L_0 (µm)	111.74±2.35	110.68±2.03
%S (%)	7.32±0.16	6.40±0.17*
T_0 (ms)	9.89±0.37	10.23±0.34
T_M (ms)	40.43±0.99	40.26±1.02
T_F (ms)	96.76±2.27	89.52±2.76*
MRS (L_0/ms)	4.29±0.11	3.85±0.14*
T_{MRS} (ms)	12.26±0.51	12.73±0.46
MRL (L_0/ms)	4.03±0.12	3.74±0.16
T_{MRL} (ms)	56.21±1.54	58.03±1.74

n = number of cells. Values are mean±SEM. *p<0.05 *vs*. HS+RASB

Cardiomyocyte response to angiotensin II

The mean time course of inotropic responses to exogenous Ang II of myocytes from LS- and HS-RAS treatment groups is presented in Figure 4. In myocytes from the HS group, at all concentrations tested, Ang II caused a reduction in myocyte contraction in a time-dependent manner. At the end of the 12-minute Ang II exposure period, myocytes of

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Figure 4 Time course of the effect of exogenous Ang II on maximum cell shortening (%S) on cardiomyocytes from rats on combined RAS blockade treatment with high sodium (HS+RASB) or low sodium diet (LS+RASB). (○) time control (no Ang II), (●) Ang II 10^{-10} M, (▼) Ang II 10^{-9} M, (▽) Ang II 10^{-8} M.

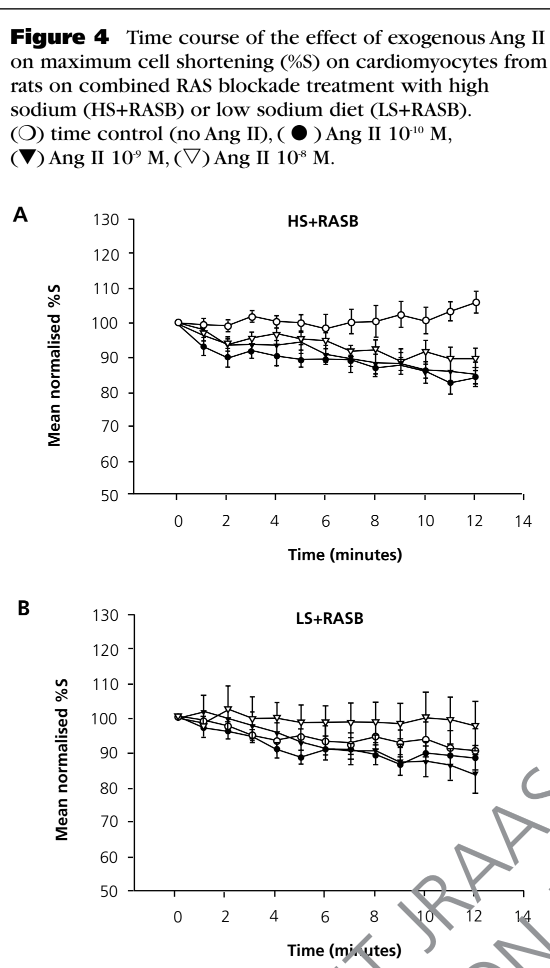
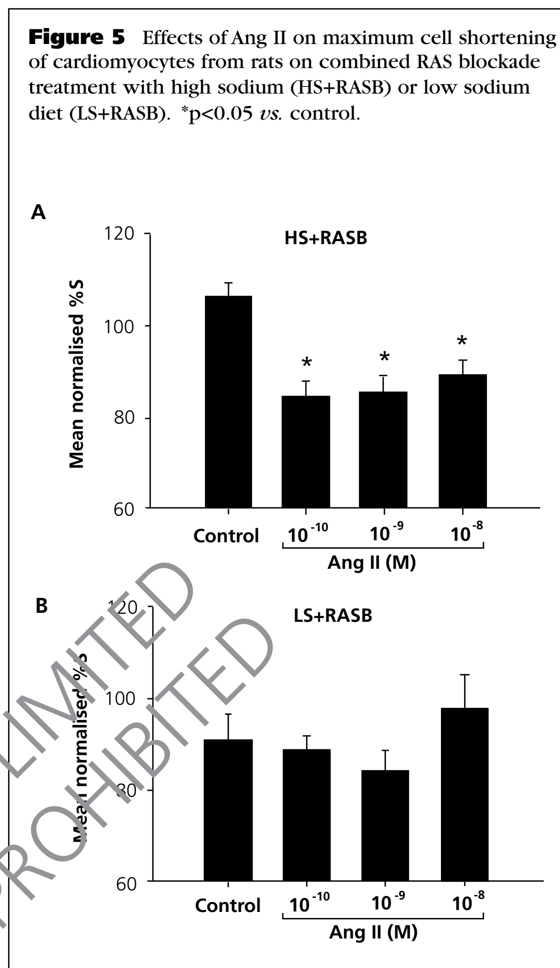


Figure 5 Effects of Ang II on maximum cell shortening of cardiomyocytes from rats on combined RAS blockade treatment with high sodium (HS+RASB) or low sodium diet (LS+RASB). *$p<0.05$ *vs.* control.



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the HS group showed significantly reduced %S compared with time control non-Ang II exposed myocytes. Contraction magnitude was suppressed to 84%, 85% and 89% of pre-Ang II exposure performance levels at Ang II concentrations of 10^{-10}, 10^{-9} and 10^{-8}M respectively (Figure 5A). There was no significant dose-effect of Ang II on myocyte contraction.

Ang II elicited a more complex range of responses from individual myocytes of the LS treatment group. It was found that Ang II exerted both positive and negative inotropic effects on myocytes in this group, with the overall proportion of positive to negative responses of 6:18 (and one cell exhibiting a biphasic response). The mean data, showing overall time-dependent Ang II-associated reduction in performance of this myocyte group somewhat obscures this variability of response. The performance of myocytes from the LS group, even in the absence of exogenous Ang II exposure, was less robust than the HS time control myocytes, with a 10% deterioration of performance evident over the 12-minute recording period (Figure 4B). Interestingly, at the highest concentration of Ang II tested, there appeared to be a suggestion of an overall positive (albeit not significant) inotropic response. Overall, comparisons of mean data at 12 minutes failed to yield any significant Ang II-induced differences in maximal myocyte shortening in this LS treatment group. (Figure 5B).

Discussion

This study demonstrates that combined ACE-I and AT_1-receptor blockade with restricted sodium intake (0.2%) suppresses somatic growth in normotensive rats and induces relative cardiac atrophy associated with cardiomyocyte growth stunting. These anti-growth effects can be reversed by elevated sodium intake (4.0%), even with maintained RAS blockade. In both sodium deplete and replete states, combined RAS blockade is associated with impaired cardiomyocyte contractility, and an inversion of the normally positive inotropic response of myocytes to exogenous Ang II. Thus chronic RAS blockade qualitatively alters the intrinsic inotropic status and responsiveness of ventricular cardiomyocytes, and this shift is further modulated by *in vivo* sodium intake conditions.

The level of dietary sodium restriction used in this study was not extreme (0.2% or 1.57 mmol/kg/day). Standard rat chow sodium levels can be up to about 0.6%. In humans, a normal *ad libitum* dietary sodium intake is about 2.9 mmol/kg/day and a sodium-reduced diet is designed to maintain sodium intake at approximately 0.7 mmol/kg/day.[24] Thus, the sodium restricted diet used in this study is equivalent to an intermediate level of sodium intake in humans and is thus of clinical relevance. In contrast, the high sodium diet used in this study (4% or 31.5 mmol/kg/day), represents a substantial elevation of sodium.

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RAS blockade, sodium, and growth

The appearance of somatic growth retardation in animals on LS intake with RAS blockade is consistent with previous studies in normotensive and hypertensive rats treated with similar dietary regimes.[17,18] The growth retardation in LS diet animals was observed in parallel with poor appetite and a decline in general activity level during combined drug treatment. The body growth suppression is not due to gross depletion of sodium, since rats on the same treatments show a rather small negative sodium balance.[17] These observations suggest that RAS blockade may affect central nervous system control of food intake (through an undefined mechanism) – an effect which is relieved by high sodium intake.

The antigrowth effect of RAS blockade in the salt restricted state, but not in salt loading, can also be observed in the heart at organ and cellular level. It has been shown that combined ACE-I and AT_1 antagonist treatment with LS intake causes a profound increase in plasma renin and depletion of angiotensinogen.[17,18] It would be expected that operation of the intra-cardiac RAS might be modified in the same manner as the systemic RAS during combined drug treatment with salt restricted intake. In the heart, renin sequestered from the circulation[25] would contribute to enhanced Ang I production. In circumstances of pharmacologically-suppressed ACE activity, Ang II formation is likely dependent on local chymase activity,[26] with locally-produced Ang II selectively acting on AT_2-receptor subtypes in the presence of chronic AT_1-receptor blockade. AT_2-receptor activation in the heart has been linked with antigrowth and apoptotic outcomes.[27,28] There is evidence that combined ACE-I and AT_1 antagonist treatment up-regulates myocardial AT_2 receptors,[29] implying that the effects of AT_2 activation may be amplified in rats on combined RAS blockade with LS intake. Decreased heart size in these animals may also be partly due to a reduction in cardiac haemodynamic loading. It has been demonstrated that both systolic and diastolic blood pressure are decreased in rats on LS diet with combined drug treatment.[17,18] The effects of unloading and RAS-mediated atrophic influence would be expected to be additive.

The mechanism by which sodium repletion may 'rescue' the RAS-blocked sodium deplete heart is unclear. It seems unlikely that this is a haemodynamically-mediated effect, as previous work has shown that a HS diet is able to increase cardiac mass independently of arterial pressure change.[30] Griffiths *et al.*[17] also demonstrated that the haematocrits of rats on HS and LS diets with combined drug treatment were comparable, inferring that plasma volumes were relatively normal. HS diet may overcome RAS blockade by a direct trophic influence on the heart. *In vitro* studies suggest that elevated extracellular Na^+ can induce an increased rate of protein synthesis and cell growth responses in myocardial fibroblast cultures[31] Other early work suggests that an increase in Na^+-H^+ exchanger activity might mediate HS-induced cardiac growth.[32] Further investigation of these possibilities is required.

Altered myocyte contractility and responses to angiotensin II

Cardiomyocytes from the LS treatment group exhibited a higher incidence of contractile instability, reduced maximal cell shortening, and slower rate of cell shortening when compared with those from the HS treatment group. The findings suggest that there is an impairment of basal myocyte contractile function when *in vivo* sodium restriction is combined with RAS blockade. Thus, we find that depressed basal contractility is associated with cardiac growth suppression. This observation is consistent with a recent study reporting that, during cardiac atrophy associated with chronic unloading *in vivo*, there is evidence of a progressive decline in myocyte contractile function.[33] Suppressed contractile function in the unloaded heart has been previously linked with myofilament disorientation in atrophied cardiomyocytes.[34] Therefore, it is possible that the basal contractile depression of myocytes in LS with RAS blockade may be due to structural deformation during progressive cardiac atrophy. A high salt intake may prevent these changes, and thereby preserve contractile function.

Our previous studies have shown that, at physiological concentration ranges, Ang II normally elicits a positive inotropic response from rat ventricular cardiomyocytes.[19] Data presented in this study show that under conditions of chronic RAS blockade, regardless of sodium status, the response to exogenous Ang II is converted to one which is predominantly negatively inotropic in character. Considerable cell-to-cell variability in the nature and extent of the Ang II inotropic response was particularly evident in the LS treatment group. A negative inotropic effect of this peptide has been previously reported in cardiomyocytes from hypertrophied and failing hearts.[35-37] Thus, Ang II-induced negative inotropy appears to be a feature of the pathologically deranged heart.

A possible explanation for this inotropic inversion may be a shift in the balance of AT_1- versus AT_2-receptor-mediated signalling. Where there is AT_1 suppression, down-regulation or blockade, a relative increase in AT_2-signalling may result. The role of the AT_2-receptor subtype in inotropic modulation in the heart is virtually unexplored, although there is evidence that increased AT_2 expression occurs in some pathological states. This possibility requires that the AT_2-receptor is in some manner linked to a second messenger or intracrine signalling pathway which induces a reduction in myocyte contractility. Some evidence for these possibilities exists[38,39] and further investigation of the functional role of AT_2-receptor signalling (if any) in the myocardium is required.

In summary, this study provides experimental evidence that combined RAS blockade treatment under sodium restricted dietary conditions can produce impairment of cardiomyocyte contractile function, in parallel with progressive cardiac atrophy. Based on this study, combination treatments with ACE-I and AT_1 antagonist could be

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predicted to have detrimental rather than beneficial effects on cardiac function, particularly in circumstances of restricted dietary sodium intake. The findings of this study also indicate that the effectiveness of *in vivo* RAS blockade can be undermined when dietary sodium intake is high. Therefore, the appropriate sodium intake should be taken into account when combined blockade of RAS is introduced for clinical treatment. More detailed studies in which the therapeutic titration of sodium intake with combined RAS blockade treatment are indicated.

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References

1. Kim S, Iwao H. Molecular and cellular mechanism of angiotensin II-mediated cardiovascular and renal diseases. *Pharmacol Rev* 2000;**52**:11-34.
2. Lui Y-H, Yang X-P, Sharo VG *et al*. Effects of angiotensin-converting enzyme inhibitors and angiotensin type 1 receptor antagonists in rats with heart failure. *J Clin Invest* 1997; **99**:1926-35.
3. Azizi M, Linhart A, Alexander J *et al*. Pilot study of combined blockade of the renin-angiotensin system in essential hypertension patients. *J Hypertens* 2000;**18**:1139-47.
4. Kim S, Zhan Y, Izumi Y, Iwao H. Cardiovascular effects of combination of perindopril, candesartan, and amlodipine in hypertensive rats. *Hypertension* 2000;**35**:769-74.
5. Ohta T, Hasebe N, Tsuji S *et al*. Effects of angiotensin converting enzyme inhibitor and angiotensin II type 1 receptor blocker on cardiac dysfunction induced by isoproterenol in dogs. *J Cardiovasc Pharmacol* 2001;**38**(suppl 1):S63-S67.
6. Easthope SE, Jarvis B. Candesartan cilexetil: An update of its use in essential hypertension. *Drugs* 2002;**62**:1253-87.
7. van den Meiracker AH, Man in't Veld AJ, Admiraal PJJ, Ritsema van Eck HJ, Boomsma F, Derkx FHM. Partial escape of angiotensin converting enzyme (ACE) inhibition during prolonged ACE inhibitor treatment: does it exist and does it affect the antihypertensive response? *J Hypertens* 1992;**10**:803-12.
8. Azizi M, Guyene T-T, Chatellier G, Wargon M, Menard J. Additive effect of losartan and enalapril on blood pressure and plasma active renin. *Hypertension* 1997;**29**:634-40.
9. Menard J, Campbell DJ, Azizi M, Gonzales M-F. Synergistic effects of ACE inhibition and angiotensin II antagonism on blood pressure, cardiac weight, and renin in spontaneously hypertensive rats. *Circulation* 1997;**96**:3072-8.
10. Dostal DE, Baker KM. The cardiac renin-angiotensin system: conceptual, or a regulator of cardiac function? *Circ Res* 1999;**85**:643-50.
11. Kawai H, Stevens SY, Liang CS. Renin-angiotensin system inhibition on noradrenergic nerve terminal function in pacing-induced heart failure. *Am J Physiol* 2000;**279**:H3012-H3019.
12. He FJ, Markandu ND, Sagnella GA, MacGregor GA. Importance of the renin system in determining blood pressure fall with salt restriction in black and white hypertensives. *Hypertension* 1998;**32**:820-4.
13. Cholewa BC, Mattson DL. Role of the renin-angiotensin system during alterations of sodium intake in conscious mice. *Am J Physiol* 2001;**281**:R987-R993.
14. Morgan TO, Aubert J-F, Wang Q. Sodium, angiotensin II, blood pressure, and cardiac hypertrophy. *Kidney Int* 1998; **54**(suppl 67):213-15.
15. Teravainen T-L, Mervaala EMA, Laakso J, Paakkari I, Vapaatalo H, Karppanen H. Influence of age on cardiovascular effects of increased dietary sodium and angiotensin-converting enzyme inhibition in normotensive Wistar rats. *J Pharm Pharmacol* 1997;**49**:912-18.
16. Herlitz H, Dahlof B, Jonsson O, Friberg P. Relationship between salt and blood pressure in hypertensive patients on chronic ACE-inhibition. *Blood Pressure* 1998;**7**:47-52.
17. Griffiths CD, Morgan TO, Belbridge LM. Effects of combined administration of ACE inhibitor and angiotensin II receptor antagonist are prevented by a high NaCl intake. *J Hypertens* 2001;**19**:2087-95.
18. Abro E, Griffiths CD, Morgan TO, Delbridge LM. Regression of cardiac hypertrophy in the SHR by combined renin-angiotensin system blockade and dietary sodium restriction. *JRAAS* 2001;**2**:148-53.
19. Delbridge LM, Morgan TO, Harris PJ. Effects of endothelin-1 on the contractility of cardiomyocytes from the spontaneously hypertensive rats. *Clin Exp Pharmacol Physiol* 1995; **22**:755-62.
20. Di Nicolantonio R, Silvapulle MJ, Spargo S, Morgan TO. High salt diet decreases longevity in the spontaneously hypertensive rats. *Clin Exp Pharmacol Physiol* 1988;**15**:357-9.
21. Delbridge LM, Harris PJ, Morgan TO. Characterization of single heart cells contractility by rapid imaging. *Clin Exp Pharmacol Physiol* 1989;**16**:179-84.
22. Delbridge LM, Harris PJ, Pringle JT, Dally LJ, Morgan TO. A superfusion bath for single-cell recording with high-precision optical depth control, temperature regulation, and rapid solution switching. *Eur J Physiol* 1990;**416**:94-7.
23. Harris PJ, Stewart D, Cullinan MC, Delbridge LM, Dally L, Grinwald P. Rapid measurement of isolated cardiac muscle cell length using a line-scan camera. *IEEE Trans Biomed Eng* 1987;**34**:463-7.
24. Navis G, de Jong PE, Donker AJM, van der Hem K, de Zeeuw D. Diuretic effects of angiotensin-converting enzyme inhibition: comparison of low and liberal sodium diet in hypertensive patients. *J Cardiovasc Pharmacol* 1987;**9**:743-8.
25. Muller DN, Fischli W, Clozel J *et al*. Local angiotensin II generation in the rat heart role of renin uptake. *Circ Res* 1998;**82**:13-20.
26. Chen LY, He Q, Jiang LQ, Cul CJ, Xu L, Liu LS. Transgenic study of the function of chymase in heart remodeling. *J Hypertens* 2002;**20**:2047-55.
27. Xu J, Carretero OA, Lui YH *et al*. Role of AT2 receptors in the cardioprotective effect of AT1 antagonists in mice. *Hypertension* 2002;**40**:244-50.
28. Horiuchi M, Akishita M, Dzau V. Recent progress in angiotensin II type 2 receptor research in the cardiovascular system. *Hypertension* 1999;**33**:613-21.
29. Lee S, Kramer CM, Mankad S, Yoo SE, Sandberg K. Combined angiotensin converting enzyme inhibition and angiotensin AT1 receptor blockade up-regulates myocardial AT2 receptors in remodeled myocardium post-infarction. *Cardiovasc Res* 2001;**51**:131-9.
30. Frohlich ED, Chien Y, Sesoko S, Pegram BL. Relationship between dietary sodium intake, hemodynamics, and cardiac mass in SHR and WKY rats. *Am J Physiol* 1993;**264**:R30-R34.
31. Gu J-W, Anand V, Shek EW *et al*. Sodium induces hypertrophy of cultured myocardial myoblasts and vascular smooth muscle cells. *Hypertension* 1998;**31**:1083-7.
32. Harmsen E Leenen FHH. Dietary sodium induced cardiac hypertrophy. *Can J Physiol Pharmacol* 1992;**70**:580-6.
33. Ito K, Nakayama M, Hasan F, Yan X, Schneider MD, Lorell BH. Contractile reverse and calcium regulation are depressed in myocytes from chronically unloaded hearts. *Circulation* 2003;**107**:1176-82.
34. Thompson EW, Marino TA, Uboh CE, Kent RL, Cooper IV G. Atrophy reversal and cardiocyte redifferentiation in unloaded cat myocardium. *Circ Res* 1984;**54**:367-77.
35. Meissner A, Min J-Y, Simon R. Effects of angiotensin II on inotropy and intracellular Ca^{2+} handling in normal and hypertrophied rat myocardium. *J Mol Cell Cardiol* 1998;**30**:2507-18.
36. Kobayashi T, Hamada M, Okayama H, Shigematsu Y, Sumimoto T, Hiwada K. Contractile properties of left ventricular myocytes isolated from spontaneously hypertensive rats: effect of angiotensin II. *J Hypertens* 1995;**13**:1803-07.
37. Cheng CP, Suzuki M, Ohte N, Wang ZM, Little WC. Altered ventricular myocyte response to angiotensin II in pacing-induced heart failure. *Circ Res* 1996;**78**:880-92.
38. De Mello W. Intracellular angiotensin II regulates the inward calcium current in cardiac myocytes. *Hypertension* 1998;**32**:976-82.
39. Schuijt MP, Danser AH. Cardiac angiotensin II: an intracrine hormone? *Am J Hypertens* 2002;**15**:1109-16.

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