High Dietary Salt and Angiotensin II Chronically Increase Renal Sympathetic Nerve Activity
A Direct Telemetric Study

Sarah-Jane Guild, Fiona D. McBryde, Simon C. Malpas, Carolyn J. Barrett

Abstract—Overactivity of the sympathetic nervous system has long been implicated in the hypertensive response to elevated angiotensin II (Ang II) levels. Although recent studies suggest that high dietary salt may alter cardiovascular responses to Ang II, direct evidence demonstrating chronic activation of sympathetic nerve activity is lacking. The objective of this study was to determine whether a low dose of Ang II, on a background of high salt intake, would result in a chronic increase in renal sympathetic nerve activity (RSNA). Arterial pressure and RSNA were recorded via telemetry. Two groups of rabbits were studied: 1 group drank a 0.9% NaCl solution and received Ang II (20 ng/kg per minute for 21 days, Salt+Ang), and the other drank tap water throughout and was not infused with Ang II (Control).

In the Salt+Ang group, mean arterial pressure increased over the first week and remain elevated by 18.5 ± 4.1 mm Hg at day 21. RSNA was not significantly different between groups on day 7 but was significantly elevated in the Salt+Ang group on day 21 (13.5 ± 3.2% compared with 6.8 ± 0.8% in the Control group; P < 0.05). Baroreflex control of RSNA showed a rightward shift on day 21, but not day 7, and baroreflex responses indicated that RSNA could not be completely suppressed when arterial pressure was increased. No changes were observed in either mean arterial pressure or RSNA variables in the Control group. Our results support the hypothesis that elevated Ang II levels, in conjunction with a high salt diet, have the ability to chronically increase RSNA and, thus, potentially contribute to the maintenance of hypertension. (Hypertension. 2012;59:00-00.) • Online Data Supplement

Key Words: sympathetic nervous system • hypertension • angiotensin II • salt • renal sympathetic nerve activity • telemetry

Elevated sympathetic nerve activity (SNA), in particular to the kidneys and heart, and blunting of arterial baroreflexes are well-established hallmarks of hypertension. Although a novel surgical approach of removing the renal nerves is showing initial promise in treating resistant hypertension, it is clear that we need to better understand the long-term modulators of the level of renal SNA (RSNA). One potential regulator of SNA in hypertension is angiotensin II (Ang II). In addition to the actions of elevated Ang II in the periphery, to increase volume retention and total peripheral resistance, it has been proposed that Ang II acts via central nervous system pathways to increase SNA.

The ability of elevated Ang II to chronically increase SNA is highly controversial. Initially, using indirect methods to assess sympathetic tone, enhanced responses to ganglionic blockade and a delayed development of hypertension in renal denervated animals supported the involvement of an increase in SNA in animal models of Ang II–induced hypertension. However, sympathoexcitation is not a universal finding, with some studies indicating no change in nerve activity, both when nerve activity has been directly recorded or assessed with ganglionic blockade. Furthermore, in studies where Ang II caused an immediate increase in arterial pressure, a renal sympathetic-inhibitory effect has been reported. Specifically, we have shown, using direct recordings of RSNA in the rabbit, that, in response to a 7-day infusion of Ang II, there is a pronounced and sustained decrease in RSNA. The dose of Ang II in that study was immediately pressor (50 ng · kg⁻¹ · min⁻¹ · IV), and the decrease in RSNA was eliminated in baroreceptor denervated animals, suggesting that the decrease in RSNA was baroreflex mediated.
Recent work as outlined by Osborn et al.\textsuperscript{16} now considers that it is the link between Ang II and dietary salt intake that is a central factor in driving the level of SNA. The broad concept is that “moderate” elevations in Ang II levels increase blood pressure through a modest increase in SNA to specific regions but that this effect can be potentiated by a high-salt diet.\textsuperscript{16} Recently, the depressor response to ganglionic blockade was used to assess sympathetic drive in rabbits during different 21-day infusions of Ang II (20 or 50 ng · kg\textsuperscript{-1} · min\textsuperscript{-1}).\textsuperscript{17} Consistent with the earlier studies, the higher dose was associated with a rapid increase in blood pressure, and the response to ganglionic blockade provided evidence of sustained sympathoinhibition. However, the lower dose of Ang II was associated with a slow onset of hypertension, reaching the same level of pressure as the higher dose but over 7 to 10 days. In animals with normal dietary salt, the slow-onset hypertension was accompanied by evidence of sympathoinhibition. However, in a further group with increased dietary salt (0.9% NaCl in drinking water), there was no evidence of decreased SNA. Ganglionic blockade gives an indirect measurement of the direct vasoconstrictor effect of global sympathetic drive. SNA to specifically the kidney has the potential to have a much larger influence on long-term levels of arterial pressure, with RSNA not only altering blood flow but also renin release and sodium reabsorption.\textsuperscript{18} The interaction between salt and Ang II on RSNA remains unclear, because no direct nerve recordings have assessed the effect of a dose of Ang II that results in a slow onset of hypertension in combination with a high-salt diet.

The aim of this study was to determine the effect of an Ang II–induced slow increase in arterial pressure, in the presence of a high-salt diet, on specifically SNA to the kidney. We hypothesized that a slow pressor action of Ang II, in conjunction with high dietary salt, produces a chronic renal sympathoexcitation.

Methods

A full description of the methods used and statistical analysis can be found in the online-only Data Supplement.

Experiments were conducted on 12 male and female New Zealand white rabbits divided into 2 groups, Control (n=6) and Salt+Ang (n=6). Control animals received tap water to drink, whereas the drinking water of the Salt+Ang group was replaced with 0.9% NaCl ≥4 days before surgery to implant telemetry devices to measure arterial blood pressure (model PA-D70, Data Sciences International, St Paul, MN) and RSNA (model TR76S, Telemetry Research Ltd, Auckland, New Zealand).\textsuperscript{15}

Experimental Protocol

After the animals had recovered from surgery (>5 days) and after recording mean arterial pressure (MAP), heart rate (HR), and RSNA for a 5 day baseline period (“baseline”), baroreflex responses were determined in response to infusions of phenylephrine and sodium nitroprusside, as described previously.\textsuperscript{15} In addition, the HR and RSNA responses to nasopharyngeal stimulation were assessed by exposing the rabbit to cigarette smoke dispensed by a 50-ml syringe for 2 seconds.\textsuperscript{19}

At the completion of the baroreflex responses, a mini-osmotic pump (model 2ML4; ALZET) was implanted in the Salt+Ang group to continuously infuse Ang II (Auspep Pty Ltd) at a rate of 20 ng · kg\textsuperscript{-1} · min\textsuperscript{-1} IV into the right jugular vein. The Control group underwent similar surgery where the right jugular vein was exposed and tied off but no pump was implanted. MAP, HR, and RSNA were monitored for an additional 3 weeks with baroreflex responses again investigated (and blood samples taken) on days 7 and 21 of the infusion. Responses to nasopharyngeal stimulation were assessed on day 14 in addition to after each set of baroreflex responses.

Data Analysis

The quality of the RSNA signal was checked regularly using systolic wave-triggered averaged records of arterial pressure and RSNA. Figure 1 shows example day 21 recordings from individual rabbits from the 2 experimental groups. Only animals with evidence of “good” sympathetic recordings characterized by the systolic wave-triggered averages showing a distinct phasic relationship between arterial pressure and the RSNA\textsuperscript{20,21} were included in the study (6 additional rabbits began the study but were removed because of poor RSNA signals). The response of the RSNA to nasopharyngeal stimulation was also used to determine whether a recording was still viable.\textsuperscript{20}

RSNA was measured in microvolts, and the 0 level, calculated by taking the minimum of the systolic triggered averages of RSNA,\textsuperscript{20} was subtracted from the daily averages to account for background noise. RSNA is also presented as a percentage, with 100% being the maximal response to nasopharyngeal activation, calculated as the maximum of a 2-second moving average of the 500-Hz data.\textsuperscript{20}

Two-way ANOVAs with repeated measures were used to compare the daily averages of BP, HR, and RSNA at days 0, 7, and 21 both within and between the groups. To evaluate the effects of Salt+Ang II hypertension on the baroreflex responses, 5 parameter sigmoidal curves were fitted to the collected data.\textsuperscript{22} Two-way ANOVAs were used to determine whether there was any difference on each parameter within or between groups. Bonferroni post hoc comparisons were used where appropriate. Changes were considered significant when P<0.05. Data are shown as mean±SEM.

Results

Effect of High Dietary Salt on Baseline Variables

There were no detectable differences in the baseline levels between the Control group drinking tap water and the Salt+Ang group drinking 0.9% NaCl for any of the recorded variables (Figure 2). Both groups of rabbits drank similar amounts of fluid in the baseline period (Figure 2). The baseline level of RSNA in microvolts (with the 0 level subtracted) was 0.7±0.1 μV in the Control group with a range of 0.4 to 1.2 μV. The 0 levels of the RSNA were 1.7±0.4, 2.0±0.5, and 2.2±0.6 μV on days 0, 7, and 21, respectively. For the Salt+Ang group, the baseline level of RSNA was 0.7±0.3 μV (range: 0.1–2.3 μV). The 0 level for this group was 2.2±0.4 μV on each of days 0, 7, and 21. There were no significant differences in the 0 level throughout the experiment (P>0.05). There was no significant change in plasma osmolality either within or between groups throughout the experiment. Plasma osmolality of the Control group was 283±4 and 279±5 mosmol on days 0 and 21, respectively, compared with 285±4 and 289±2 mosmol in the Salt+Ang group.

Chronic Effects of High Dietary Salt and Ang II Infusion

Infusion of Ang II in rabbits drinking 0.9% NaCl caused MAP and RSNA to increase slowly during the 3 week infusion (Figure 2). Hypertension developed over the first week of the infusion, with MAP being significantly elevated in the Salt+Ang group on both days 7 and 21 (P<0.05). MAP reached a maximum of 109±4 mm Hg on day 21 (compared with 91±5 mm Hg on day 0). In contrast, normal-
Dietary Salt and Ang II Infusion Changes in Arterial Baroreflexes During High Dietary Salt and Ang II Infusion

Changes in Arterial Baroreflexes During High Dietary Salt and Ang II Infusion

On examining the baroreflex relationship, in the Salt + Ang group, between MAP and HR (Figure 3), there was a rightward shift toward a higher MAP on both days 7 and 21 with no change in range or gain parameters (a Table of the baroreflex parameters can be found in the online-only Data Supplement). This is illustrated by the increase in the MAP at half the reflex range from 92±4 mm Hg on day 0 to 109±8 mm Hg on day 7 (P<0.05) and 109±7 mm Hg on day 21 (P<0.01). In addition, the resting points remained near the steepest point of the curve throughout, reflecting that HR did not alter with the Ang II infusion. No changes to the baroreflex control of HR were seen in the Control group.

In contrast to the HR curves, the baroreflex relationship, in the Salt + Ang group, between MAP and RSNA (Figure 3), showed an increase in MAP at half the reflex range, suggesting that the curve was shifted to the right, on day 21 (103±7 mm Hg compared with 86±3 mm Hg on day 0; P<0.001) but not day 7 (98±7 mm Hg; P>0.05). The range appeared to be decreased on days 7 and 21 (20±3% and 19±6% compared with 30±3% on day 0), but there was no significant difference between the 2 groups on any day (P>0.05). However, the lower plateau was significantly higher in the Salt + Ang group on day 21 (11±3% compared with 1±1% on day 0; P<0.01) but not day 7 (3±2%), suggesting that the RSNA could not be completely suppressed by phenylephrine on day 21. In all of the cases, the resting point of the RSNA curves lay toward the bottom half of the curve, suggesting that the resting level of RSNA was not greatly reduced by increasing MAP using phenylephrine. The resting level of RSNA in the Salt + Ang group was, however, significantly higher on day 21 (14±3% compared with 7±1% on day 0; P<0.01), consistent with the daily average results (Figure 2). No changes to the baroreflex...
Discussion

In this study, we used a unique telemetry based approach to directly measure the changes in RSNA during low-dose infusion of Ang II for 21 days in rabbits on a high-salt diet (drinking 0.9% NaCl). The slow increase in arterial pressure in response to Ang II was accompanied by a delayed increase in RSNA. Not only was the resting level of RSNA increased, but there was also an inability to suppress RSNA during increases in blood pressure. Our results support the hypothesis that the combination of high dietary salt and elevated Ang II has the ability to chronically increase RSNA and, thus, possibly contribute to the maintenance of hypertension.

Previously we reported that a higher dose of Ang II (50 ng/kg per minute, without the high-salt diet) resulted in an immediate increase in arterial pressure, consistent with a control of RSNA were seen in the Control group. The gains were unaltered during the experiment in either group.
direct vasoconstrictive action. The increase in pressure was accompanied by an immediate, sustained decrease in RSNA, which depended on the presence of the arterial baroreceptor afferents. In contrast, in the present study, whereas the final increase in arterial pressure was similar to that seen with the higher dose of Ang II, the RSNA response was much slower and was increased rather than decreased. Baroreflex control of HR showed a rightward shift on days 7 and 21, suggesting resetting to the higher pressure. In contrast, whereas baroreflex control of RSNA showed some resetting on day 21, the accompanying increased lower plateau suggests that RSNA could not be reduced in response to an acute increase in pressure. Not only does this provide further evidence that baroreflex controls of HR and SNA are differentially regulated, but the lack of baroreflex suppression of the RSNA may also be part of the mechanism that allows the hypertension to develop.

Ang II has long been described as sympathoexcitatory, acting within the central nervous system to increase SNA. Previous studies have demonstrated that the central administration of Ang II to a number of sites within the brain, including the area postrema, subfornical organ, pontine A5, and paraventricular nucleus, results in elevations in SNA. In addition, the expression of Ang II type I receptors in both the paraventricular nucleus and subfornical organs is higher after a 4-week subpressor infusion of Ang II in the rat. However, the present experiments are the first to demonstrate using direct nerve recordings that elevated Ang II indeed results in a chronic increase in RSNA.

The prevailing action of Ang II on RSNA is also dependent on the plasma level of Ang II. With high plasma levels of Ang II, the resulting rapid vasoconstriction and subsequent increase in blood pressure result in sympathoinhibition via the arterial baroreflex. However, at lower levels, where the increase in arterial pressure is much slower, it is suggested that the chronic action of Ang II in the central nervous system dominates, resulting in a slow increase in SNA above baseline. In the present experiments, both the low dose of Ang II, minimizing the inhibitory actions of the arterial baroreflex, and the length of recordings were critical in demonstrating a chronic sympathetic activation in response to Ang II–induced hypertension.

There is growing evidence to suggest that a high-salt diet potentiates the central actions of Ang II. In both the rat and rabbit, it has been shown that the depressor response to ganglionic blockade is larger in Ang II–treated animals on a high-salt diet than those on a normal salt diet. It should be noted that the interpretation of ganglionic blockade studies to indirectly infer SNA is complicated by the possibility that Ang II may act peripherally to augment the vasoconstrictor actions of SNA. Further evidence of a role for a high-salt diet comes from the studies of King et al who showed that a high-salt diet or Ang II infusion alone over a 7-day period in rats had no effect on whole body norepinephrine spillover, whereas together Ang II and a high-salt diet resulted in an increase in the norepinephrine spillover. We have shown previously that adding 0.9% salt to the drinking water for a 6-day period had no effect on RSNA. Also, in the present experiment, the animals on the high-salt diet had been drinking the salt water for ≥9 days before the baseline data being collected (and, therefore, 14 days before the start of the Ang II infusion). The salt alone had no effect baseline levels of blood pressure, water intake, or RSNA, only in combination with the low-dose Ang II infusion was sympathoexcitation observed. Although we cannot totally discount the possibility that we would have seen an increase in RSNA if the rabbits were on a normal salt diet, the evidence would suggest that both a high-salt diet and Ang II are required for the sympathoexcitation to be observed, at least within the time frame studied (21 days).

The role that any increase in RSNA plays in the maintenance of the arterial pressure is not entirely clear. The Ang II itself likely contributes directly to the increase in arterial pressure because of its antinatriuretic actions. Thus, the increase in RSNA may also be part of the mechanism that allows the hypertension to develop.

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The role that any increase in RSNA plays in the maintenance of the arterial pressure is not entirely clear. The Ang II itself likely contributes directly to the increase in arterial pressure because of its antinatriuretic actions. Thus, the possibility must be considered that the delayed increase in RSNA seen in this study may be because of the development of the hypertension rather than a cause of it. There is the potential that renal damage and/or feedback from the renal afferents may be responsible for the increased RSNA developing toward the end of the study. However, our previous study showing renal denervation delayed the onset of the hypertension suggests the renal nerves contribute, at least to some extent, to the development of the hypertension. Given that the increase in RSNA occurred at a time when salt intake was also increasing, it would appear that the upregulation of RSNA is also being countered by other natriuretic mechanisms, ensuring that arterial pressure does not additionally rise.

One of the hallmarks of the sympathetic nervous system is that it is differentially controlled. In the present study, we have focused exclusively on the role of the renal nerves. The focus on the renal nerves is justified by the importance of the kidneys in the long-term control of arterial pressure, with evidence that sympathetic outflow to the kidneys is elevated in patients with primary (essential) hypertension. The recent successes seen with selectively ablatting the renal nerve in humans as a treatment for resistant hypertension further supports the importance of the renal sympathetic nerves in long-term pressure control. In response to a “chronic, slowly developing model of Ang II hypertension” in rats on a high-salt diet (10 ng/kg per minute of Ang II IV and 4.0% NaCl), renal denervation attenuated the increase in arterial pressure. However, this finding is by no means universal, with renal denervation having little effect on the ultimate increase in blood pressure in response to Ang II infusion in studies in both the rat and rabbit. Both the salt intake and dose of Ang II have varied in the previous studies, but again these studies tend to suggest that increased RSNA is most likely observed in the presence of a both a high-salt diet and a comparatively low dose of Ang II.

**Limitations**

In the current study we presented the data as both microvolts and as a percentage of the response to nasopharyngeal activation (Figure 2). Although the trend in RSNA is similar regardless of whether the absolute or normalized values are presented, normalization reduced the variability of the data between animals to allow the significant increase in RSNA to
be identified. The variability in the RSNA levels between individual animals was \( \approx 5 \)-fold, which is similar to that reported in humans.\(^{3,36}\) As discussed previously,\(^{20}\) we believe that this is more an effect of real variation between animals than an effect of electrode contact or other experimental factors as was once thought. One experimental error that could potentially result in an increase in recorded RSNA and lack of suppression of RSNA would be an increase in background electric noise. Electric noise was carefully monitored in the current experiments, with the 0 level repeatedly measured\(^{29}\) and shown to not change with time. Therefore, the increase in RSNA in the Salt+Ang group cannot be ascribed to a change in the noise level. Furthermore no change in RSNA, or any of the other variables, was observed in the Control group throughout the entire 4-week recording (Figure 2). The inclusion of the Control group, with no interventions, was specifically chosen to ensure that any effect of time on the stability of the recordings could be observed. The lack of change in RSNA over time in the Control group supports that the increase in RSNA in the Salt+Ang group was because of the treatment, although we cannot absolutely discriminate whether the increase depends on both the high salt and Ang II.

### Perspectives

Although there is a strong correlation between overactivity of the sympathetic nervous system and cardiovascular disease, the role of renal nerves in the development of hypertension remains controversial. Recent studies show that ablation of renal nerves is producing exciting results in the treatment of human hypertension.\(^{3,34}\) However, the exact mechanism behind the decrease in blood pressure remains in question, and it is possible that this is not necessarily a result of decreased effenter RSNA but a consequence of reduced feedback from the renal afferents.\(^{37}\) The present study indicates that, with a background of high dietary salt, a modest elevation of Ang II could initiate renal sympathoexcitation, which may contribute to sustaining the increase in arterial pressure through a cascade of renal responses. It would appear that this renal sympathoexcitation is not critical in the initial increase in arterial pressure, with increases in arterial pressure observed before the increase in SNA. The differential nature of SNA\(^{33}\) raises the possibility that sympathetic activation to other vascular beds, possibly the splanchnic circulation,\(^{35}\) may be important in mediating the initial increase in arterial pressure.

### Sources of Funding

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### Disclosures

S.C.M. is a director in the company Telemetry Research. S.-J.G. is a scientific advisor to Telemetry Research.

### References


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High dietary salt and angiotensin II chronically increase renal sympathetic nerve activity; a direct telemetric study.

Short title: Chronic increased RSNA in hypertension

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Expanded methods section
Experiments were conducted on 12 male and female New Zealand White rabbits with initial weights of 3.1±0.1 kg and were approved by the University of Auckland Animal Ethics Committee. 6 additional rabbits began the study but were removed due to poor RSNA signals. The rabbits were housed individually in cages (height 45 cm, width 65 cm, and depth 65 cm). The rabbits were fed daily (100 g standard rabbit pellets with 0.5% NaCl content, supplemented with hay, carrot, and apple) at 0900, and water was available ad libitum. Food and fluid intake were monitored daily. The room was kept at a constant temperature (18°C) and dark-light cycle (lights on from 0600 to 1800). Animals were divided into two groups: Control (n=6) and Salt+Ang (n=6). Control animals received tap water to drink throughout the study whilst the drinking water of the Salt+Ang group was replaced with a 0.9% NaCl at least 4 days prior to surgery.

Telemetry devices to measure arterial blood pressure (model PA-D70, Data Sciences International, St Paul, MN) and RSNA (model TR76S, Telemetry Research Ltd, Auckland, New Zealand) were surgically implanted into all animals as has been described in detail previously. At the beginning of surgery, the rabbits were treated prophylactically with an antibiotic (enrofloxacin, Baytril, Bayer, New Zealand; 5 mg/kg sc) and analgesic (carprofen, Rimadyl, Pfizer, New Zealand; 4mg/kg sc, continued daily for 2 further days). As soon as the rabbits regained consciousness they were returned to their home cages. A heating pad was placed in the cage for 24 hr after the surgery.

Data Collection
Animals were allowed to recover from surgery (~5 days) before data were recorded for a five day baseline period. Recovery from surgery was characterized by the rabbits eating and drinking normally and an observable circadian variation in heart rate. Arterial pressure and RSNA signals were sampled at 500Hz using an analog-digital data acquisition card (PCI 6024E National Instruments, Austin, Texas) and continuously displayed by a data acquisition program (Universal Acquisition 11, University of Auckland, Auckland, New Zealand). Heart rate (HR) was derived from the arterial pressure waveform. The original RSNA signal was amplified, filtered between 50-5000 Hz, full-wave rectified and integrated using a low pass filter with a 20ms time constant.

Data recording was scheduled for 15 minutes every two hours, a protocol which has been shown to accurately represent the underlying average. For the calculation of the overall mean levels of mean arterial pressure (MAP) and RSNA, the 500 Hz sampled signals were averaged every two seconds and saved to disk along with HR. All subsequent data collection and analysis were performed using a data analysis program (Universal Analysis 11, University of Auckland, Auckland, New Zealand).

Experimental Protocol
After recording MAP, HR and RSNA for a five day baseline period (“baseline”), baroreflex responses were determined in response to infusions of phenylephrine and sodium nitroprusside as described previously. In addition, the heart rate and sympathetic responses to nasopharyngeal stimulation were assessed by exposing the rabbit to cigarette smoke dispensed by a 50-mL syringe for 2 seconds. Immediately prior to the baroreflex responses, 0.5mL of blood was collected from the central ear artery for measurement of plasma osmolarity.

At the completion the baroreflex responses, a short surgery was performed to implant a mini-osmotic pump (Model 2ML4; Alzet) in the Salt+Ang group to continuously infuse AngII
(Auspep Pty Ltd, Australia) at a rate of 20 ng.kg$^{-1}$min$^{-1}$ iv with the infusion catheter inserted into the right jugular vein. The Control group underwent a similar surgery where the right jugular vein was exposed and tied off but no pump was implanted. MAP, HR and RSNA were monitored for a further 3 weeks with baroreflex responses again investigated (and blood samples taken) on days 7 and 21 of the infusion. Responses to nasopharyngeal stimulation were assessed on day 14 in addition to after each set of baroreflex responses.

**Data Analysis**

The quality of the RSNA signal was checked regularly using systolic wave-triggered averaged records of arterial pressure and RSNA. Figure 1 shows example Day 21 recordings from individual rabbits from the two experimental groups. Only animals with evidence of “good” sympathetic recordings characterized by the systolic wave-triggered averages showing a distinct phasic relationship between arterial pressure and the RSNA$^4,5$ were included in the study. The response of the RSNA to nasopharyngeal stimulation was also used to determine if a recording was still viable$^4$.

Arterial pressure, heart rate and RSNA were collected as 2 second averages for the duration of the experiment and converted to daily averages to examine the chronic effects of salt and AngII. The RSNA signal was measured in microvolts and the zero level, calculated by taking the minimum of the systolic triggered averages of RSNA$^4$, was subtracted from the daily averages to account for background noise. RSNA is also presented as a percentage with 100% being the maximal response to nasopharyngeal activation, calculated as the maximum of a 2 s moving average of the 500 Hz data$^4$.

Two-way ANOVA with repeated measures were used to compare the daily averages of BP, HR and RSNA at Days 0, 7 and 21 both within and between the groups. To evaluate the effects of Salt+AngII hypertension on the baroreflex responses, 5 parameter sigmoidal curves were fitted to the collected data$^6$. Two-way ANOVA were used to determine if there was any difference on each parameter within or between groups. Bonferroni post-hoc comparisons were used where appropriate. Changes were considered significant when P < 0.05. Data are shown as mean±SEM.

**References**


Table S1: Baroreflex parameter values obtained for the Control and Salt+Ang groups on Days 0, 7 and 21

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<th>Parameter name</th>
<th>Day 0</th>
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<th>Day 21</th>
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<tr>
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<td>Control</td>
<td>Salt+Ang</td>
<td>Control</td>
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<td>Resting MAP, mmHg</td>
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<td>Resting HR, bpm</td>
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</tr>
<tr>
<td>Range, bpm</td>
<td>181±10</td>
<td>193±3</td>
<td>174±10</td>
</tr>
<tr>
<td>Lower Curvature, bpm/mmHg</td>
<td>-0.09±0.01</td>
<td>-0.10±0.02</td>
<td>-0.08±0.01</td>
</tr>
<tr>
<td>BP50, mmHg</td>
<td>87±2</td>
<td>92±4</td>
<td>88±3</td>
</tr>
<tr>
<td>Upper Curvature, %/mmHg</td>
<td>-0.16±0.02</td>
<td>-0.18±0.04</td>
<td>-0.17±0.02</td>
</tr>
<tr>
<td>Gain, %/mmHg</td>
<td>-5.66±0.66</td>
<td>-6.5±0.95</td>
<td>-5.63±0.92</td>
</tr>
<tr>
<td>Fit, %</td>
<td>97.0±0.6</td>
<td>94.5±1.7</td>
<td>97.7±0.3</td>
</tr>
<tr>
<td>RSNA parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower Plateau, %</td>
<td>3±1</td>
<td>1±1</td>
<td>4±1</td>
</tr>
<tr>
<td>Range, %</td>
<td>21±3</td>
<td>30±3</td>
<td>19±2</td>
</tr>
<tr>
<td>Lower Curvature, %/mmHg</td>
<td>-0.18±0.03</td>
<td>-0.23±0.07</td>
<td>-0.23±0.03</td>
</tr>
<tr>
<td>BP50, mmHg</td>
<td>78±5</td>
<td>86±3</td>
<td>84±3</td>
</tr>
<tr>
<td>Upper Curvature, %/mmHg</td>
<td>-0.30±0.07</td>
<td>-0.32±0.06</td>
<td>-0.28±0.06</td>
</tr>
<tr>
<td>Gain, %/mmHg</td>
<td>-1.30±0.32</td>
<td>-1.98±0.31</td>
<td>-1.17±0.15</td>
</tr>
<tr>
<td>Fit, %</td>
<td>92.4±2.3</td>
<td>92.4±1.9</td>
<td>94.5±0.7</td>
</tr>
</tbody>
</table>

Data are mean ±SEM. *P<0.05; †P<0.01; ‡P<0.001, where data are compared to the Control group. Note the resting variables are taken at the time of determining baroreflexes.