Abstract—We examined the contribution of the renal nerves to mean arterial pressure (MAP) during 5-week chronic infusion of angiotensin II (Ang II; 50 ng/kg per minute SC) in conscious rabbits. Basal MAP was 68±1 mm Hg, and the maximum depressor response to ganglion blockade was −20±2 mm Hg. MAP increased by 25±2 mm Hg after 1 week and remained stable over the next 4 weeks. Depressor responses to pentolinium (6 mg/kg IV) were similar to control during the first week of hypertension but thereafter became increasingly greater in Ang II–treated rabbits but not vehicle-treated rabbits. After 5 weeks, the fall in MAP was 54% greater in Ang II- than in vehicle-treated rabbits (−34±2 versus −22±2 mm Hg), but renal sympathetic nerve activity was similar in both groups. Renal denervation produced a small fall in MAP in all of the vehicle-treated rabbits after 4 days (−6±2 mm Hg; P=0.01), but there was no consistent effect in hypertensive rabbits. The depressor response to ganglion blockade was enhanced in vehicle-treated but not Ang II–treated rabbits. The finding that renal sympathetic nerve activity is not altered by Ang II hypertension nor is the hypertension altered by renal denervation suggests that renal sympathetic nerves do not contribute to the hypertension. The greater depressor effect of acute ganglion blockade in hypertensive rabbits suggests that the sympathetic nervous system exerts increased vasoconstriction in the peripheral vasculature in Ang II–induced hypertension. (Hypertension. 2008;51:878-883.)

Key Words: Ang hypertension ■ sympathetic nervous system ■ ganglion blockade ■ renal denervation ■ rabbits ■ blood pressure ■ heart rate

Elevated sympathetic activity has long been associated with the development of essential hypertension, but whether this is also the case in hypertension induced by chronic infusion of angiotensin (Ang) II has yet to be established. Evidence to date suggests that the responses to ganglionic blockade and sympatholytic agents are enhanced in Ang II–induced hypertension in rats and rabbits. Collectively, these observations suggest that there may be greater sympathetic nervous system (SNS)–mediated vasoconstrictor activity, but this does not necessarily mean that activation of the SNS initiates or maintains Ang II–induced hypertension.

However, the precise role of the renal nerves in the pathogenesis of Ang II hypertension is still somewhat controversial. Previous renal denervation has been observed to delay or blunt the development of hypertension during chronic infusion of Ang II in rats, although this is not a universal finding. Furthermore, a 7-day infusion of Ang II reduced renal sympathetic nerve activity (RSNA) measured directly in rabbits and renal spillover of norepinephrine in dogs. Furthermore, the renal nerves appear to promote sodium excretion in Ang II–induced hypertension in dogs via a baroreflex-dependent mechanism. Collectively, these observations suggest that the renal nerves may make little contribution to hypertension induced by chronic infusion of Ang II in the rabbit and dog. To date, however, the effects of renal denervation on arterial pressure in Ang II–induced hypertension have received relatively little attention in these species. Furthermore, measurements of RSNA have only been made for a relatively short period of 1 week, which is inadequate to determine whether in the long-term RSNA and/or its contribution to blood pressure control are enhanced in chronic Ang II–induced hypertension. Therefore, in the present study we have established the Ang II–induced hypertension for 5 weeks, measured RSNA and tested the effects of renal denervation on blood pressure, as well as the acute response to ganglionic blockade. We hypothesized that, if the renal nerves contribute to the maintenance of Ang II–induced hypertension, then renal denervation should result in a greater fall in mean arterial pressure (MAP) in hypertensive rabbits than in normotensive control rabbits. If renal vasomotor drive were enhanced in Ang II–induced hypertension, we would expect to see greater resting levels of RSNA and a diminution of the effect of ganglion blockade after renal denervation. Furthermore, we might also expect that renal sympathetic baroreceptor reflexes would be reset to a higher level of...
arterial pressure with time, thus allowing RSNA and also heart rate (HR) to return to normal levels or even elevated levels if there is additional sympathetic drive associated with the Ang-induced hypertension. Therefore, we also assessed HR and RSNA baroreflex curves after chronic Ang II infusion.

Methods

Experiments were conducted in 25 conscious New Zealand white rabbits of either sex (2.7 to 3.5 kg), bred and housed at the Baker Heart Research Institute, in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Experimental Procedures and Protocol

Experiments to measure ear MAP and HR were performed before and at weekly intervals during 6 weeks of subcutaneous infusion of Ang II (50 ng/kg per minute, Auspep) or saline as vehicle. Ang II was delivered by 2 sequential 28-day osmotic minipumps (Alzet Model 2ML4, Durect Corp) implanted under local anesthesia. On each day the rabbits were studied, MAP and HR were recorded for 1 hour, and then the maximum fall in MAP during ganglion blockade (pentolinium 6 mg/kg, Sigma-Aldrich) was measured over 1 minute, 2 to 3 minutes after administration. At 5 weeks, rabbits underwent surgery under halothane anesthesia (3 mg/kg of carprofen IV for analgesia before and 24 hours after surgery and 10 mg/kg of propofol IV for induction) to surgically denervate or sham denervate the kidneys. MAP and HR before and during ganglion blockade were again determined 4 and 8 days after surgery.

The acute hypotensive response to ganglionic blockade may be affected by renin release from the kidney, which may confound the interpretation when comparing normal and Ang II–treated animals. This is because the infused Ang II is likely to suppress renin release through its feedback mechanism. Thus, in 5 vehicle-treated rabbits, the response to ganglion blockade was also examined after clamping the renin-Ang system. MAP was measured for 1 hour before treatment with the Ang converting enzyme inhibitor enalaprilat (2 mg/kg plus 10 μg/kg per minute IV, Merck Laboratories). After 10 min, Ang II was infused IV at a dose that returned MAP to the control level, and MAP was held at this level for 30 minutes by adjusting the rate of infusion. The MAP and HR responses to ganglion blockade were then measured. The time periods of measurement were identical to those used in the main experiment.

In another 8 rabbits (4 vehicle- and 4 Ang II–treated), a recording electrode was implanted on the left renal nerve under halothane anesthesia.17 One week later, MAP, RSNA, and HR were recorded for 1 hour and then the maximum responses during ganglion blockade were measured as described above. RSNA was normalized in each rabbit relative to the maximum 2 seconds of RSNA evoked by 50 mL of smoke directed at the rabbit’s nose at the start of each experiment.18 Baroreflex curves for HR and RSNA were obtained from phenylephrine and nitroprusside infusions.19 At the end of the experiments, the animals were euthanized, and the left ventricle was removed and weighed. The kidneys were also removed, snap frozen in liquid nitrogen, and stored at −80°C for later measurement of noradrenaline content in the cortex.20

Data Analysis

MAP and HR derived from the pressure pulse were digitized online and averaged over 2 seconds. RSNA was normalized to the maximum RSNA recorded during the nasopharyngeal response evoked by smoke, taken to be 100 normalized units.18 Values were expressed as means±SEM or mean differences±SE of the difference. Data were analyzed by split plot repeated-measures ANOVA, which allowed for within-animal and between-animal (group) contrasts. Bonferroni and Greenhouse-Geisser corrections were applied to control risk of type 1 error.21 Dependent and independent t tests were used to compare basal responses with those after clamping the renin-Ang system and left ventricular weights in vehicle- and Ang II–treated rabbits.
Effects of Renal Denervation
Five weeks after beginning treatment with either Ang II or vehicle, rabbits underwent either denervation or sham denervation of both kidneys. In all of the vehicle-treated rabbits, MAP was lower 4 days after denervation (−6±2 mm Hg; P=0.01), but this response was more variable in the Ang II–treated rabbits with a fall in only 4 of the 6 animals (−6±5 mm Hg). The depressor response to ganglion blockade was 33% greater 4 days after renal denervation in the vehicle-treated animals (−21±2 mm Hg at week 5 compared with −27±3 mm Hg; P<0.05) and 43% greater 8 days after renal denervation (−29±4 mm Hg; P<0.01; Figure 2). By contrast, in Ang II–treated animals, renal denervation had no significant effect on MAP or the responses to ganglion blockade (n=6; Figure 2). Sham denervation in 5 normotensive rabbits had no significant effect on MAP or the responses to ganglion blockade (data not shown).

Effects of Clamping the Renin-Angiotensin System
The renin-angiotensin system was inactivated in 5 vehicle-treated rabbits by intravenous treatment with the Ang-converting enzyme inhibitor enalaprilat, and MAP was normalized by intravenous infusion of Ang II. The final average dose of Ang II required to return MAP to pretreatment levels was 4 ng/kg per minute. MAP after clamping (75±2 mm Hg) was not significantly different from MAP determined in the basal experiment (74±3 mm Hg), and HR was also similar (185±7 bpm and 194±12 bpm, respectively). The maximum fall in MAP (−25±3 mm Hg from control), measured 2 to 3 minutes after ganglion blockade, was not significantly altered by clamping the renin/Ang system (Figure 3). However, the rebound increase in MAP, which occurred 7 to 8 minutes after pentolinium administration of +18±2 mm Hg (−8±5 mm Hg from control in the basal experiment; Figure 3), was attenuated by clamping the renin-Ang system (+3±2 mm Hg; −18±3 mm Hg from control; Figure 3).

Effects of Chronic Ang II on RSNA and Baroreflex Curves
RSNA (normalized by nasopharyngeal stimulation) was closely similar in vehicle-treated and Ang II–treated rabbits, being 6.9±1.1 and 6.9±1.6 normalized units, respectively (Figure 4). Although the raw μV signals during the control period showed a tendency to be greater in the Ang II animals than vehicle-treated rabbits (43±5 versus 28±7 μV; P = 0.113), the same trend was also seen in response to nasopharyngeal stimulation (482±70 versus 309±96 μV; P = 0.2). By contrast, MAP and the fall in MAP to ganglion blockade were greater in Ang II compared with vehicle-treated rabbits (P<0.01; Figure 4).

Full sigmoidal HR and RSNA baroreflex curves were assessed at the end of the Ang II treatment. Both baroreflex curves were shifted parallel to the right in line with the increase in MAP (Figure 5). Although HR range and gain were similar in vehicle- and Ang–treated rabbits, RSNA range was significantly reduced by 25% in the hypertensive rabbits (P<0.05). However RSNA gain was not different in the 2 groups (Figure 5; −15±26%; P>0.05).
Thus, in rats and rabbits, the evidence suggests that the development of hypertension during chronic Ang II infusion did not result in enhanced depression of baseline blood pressure and/or maintenance of Ang II–induced hypertension. Interestingly, a recent study in the rat also demonstrated that renal denervation did not blunt the hypertension reached during the maintenance phase of Ang II infusion. In normotensive animals (2.7 ± 0.1 versus 2.5 ± 0.1 g/kg, respectively; P<0.07). Total kidney weight was 10% greater in hypertensive compared with normotensive animals (2.7 ± 0.1 versus 2.5 ± 0.1 g/kg, respectively; P<0.05). Noradrenaline concentration in denervated kidneys was 8% of that in intact kidneys (369 ± 117 versus 30 ± 8 ng/g; P<0.01).

**Discussion**

In the present study we were unable to detect a significant chronic effect of renal denervation on arterial blood pressure in established Ang II–induced hypertension. In normotensive rabbits, renal denervation was followed by a small fall in renal nerves making little contribution to the maintenance of Ang II–induced hypertension. These data accord with the recent finding of McBryde et al that previous renal denervation did not reduce the level of hypertension associated with activation of the renin-angiotensin system. Indeed, the baroreflex was completely reset. Thus, the suppression of RSNA observed with a 1-week infusion of Ang II, which has been attributed to incomplete resetting of the sympathetic baroreflex, does not appear to persist in the longer term. We also found that the RSNA baroreflex gain was similar in Ang II hypertensive rabbits compared to control rabbits. Because we have performed full baroreflex curves, we can be certain that both the HR and RSNA baroreflexes have completely reset. Our findings also accord with the results of other studies in 2-kidney 1-clip and renal wrap hypertensive rabbits, and together these data suggest that the renal sympathetic baroreflex is relatively normal (albeit reset) during the established phase of rabbit models of hypertension associated with activation of the renin-angiotensin system. We did observe that the upper plateau of the RSNA curve was suppressed, which also has been observed after 2 days and 1 week of Ang II infusion.

In our present study we found that the depressor response to ganglion blockade progressively increased over the first 4 weeks of Ang II infusion, suggesting that the vasoconstrictor effects of the SNS are enhanced in Ang II–induced hypertension. These data are consistent with our previous observations using a centrally acting sympathoinhibitory agent, rilmenidine, where we observed a markedly enhanced depressor response in 2-kidney 1-clip hypertensive rabbits and studies using a centrally acting sympathoinhibitory agent, rilmenidine, where we observed a markedly enhanced depressor response in 2-kidney 1-clip hypertensive rabbits.
by others in Ang II-induced hypertension using ganglionic blockade in both rats and rabbits. Nevertheless, the depressor response to ganglion blockade should be interpreted with caution at 2 levels. First, although a greater depressor response indicates a greater degree of sympathetic vasoconstrictor tone, this is not necessarily attributable to increased SNA. Rather, development of vascular hypertrophy or vascular remodelling, increased neurotransmitter release, increased responsiveness of the vasculature to sympathetic neurotransmitters, or even alterations in the balance between the relative contributions of cardiac output and total peripheral resistance to MAP could all contribute. Second, the greater depressor response to ganglion blockade in rabbits with Ang II-induced hypertension does not necessarily indicate that sympathetic vasomotor drive contributes to development or even maintenance of hypertension in this model. Rather, it simply indicates that the SNS exerts increased vasoconstrictor effects on the peripheral vasculature in Ang II-induced hypertension.

Our present observations are novel in that they show that the enhanced response to ganglion blockade in Ang II-induced hypertension can take much longer to develop than previously thought, gradually increasing over a 2-month period. This effect is unlikely to be mediated via the renal nerves, both because of our findings with renal denervation and because the level of RSNA after several weeks of hypertension was similar to that measured in normotensive animals. The enhanced depressor response to ganglion blockade must, therefore, arise because of enhanced vasoconstrictor actions of the SNS in nonrenal beds. This is supported by recent evidence that greater sympathetic tone in the splanchnic circulation contributes to Ang II-induced hypertension in rats on a high-salt diet.

Our novel observation of a greater depressor response to ganglion blockade after renal denervation in normotensive rabbits suggests that the absence of the renal nerves enhances the role of sympathetic vasomotor tone in maintenance of MAP. This observation might be expected as a consequence of unloading of arterial baroreceptors because of some degree of hypovolemia caused by the acute natriuretic effect of renal denervation.

This notion is consistent with the small fall in MAP observed after renal denervation in this and previous studies in rabbits. The enhanced depressor response to ganglion blockade after renal denervation is unlikely to be because of removal of renal afferents, because this would be expected to reduce SNA. Considering the complete resetting of baroreflex mechanisms that we in the present study and others have observed in long-term hypertensive rabbits, it is not unreasonable to expect that the enhanced response to ganglion blockade observed after renal denervation in normotensive rabbits would also be observed in the hypertensive group. The absence of a consistent effect of renal denervation on MAP in hypertensive rabbits and no change in the depressor response to ganglion blockade may both be related to Ang II actions within the kidney. This could include its antinatriuretic effects, which might act to reduce the impact of removing renal nerves.

The depressor response to ganglion blockade could potentially be confounded by renin released in response to hypotension. To avoid this, we assessed maximum responses to ganglion blockade 2 to 3 minutes after pentolinium administration. When assessed in this way, depressor responses to pentolinium under control conditions were indistinguishable from those during combined treatment with an Ang-converting enzyme inhibitor and exogenous Ang II ("Ang clamp"). Thus, we can be confident that our present observations were not confounded by renin release. A limitation of our study is that we have measured blood pressure, RSNA, and other variables during a relatively limited period within the laboratory rather than 24-hour measures using telemetry.

Our previous comparisons of telemetered blood pressure in the home cage with measurements made in a similar setting in the laboratory to the present study showed that the laboratory measurements were $4 \pm 1$ mm Hg higher than in the home cage ($P < 0.001$; $n = 21$; unpublished data), but there was a very strong correlation between the home cage telemetry blood pressure and the values measured during a 1-hour quiet period in the laboratory ($r = 0.89$). This was similar to the correlation between values measured from 12 hours (during daytime) using telemetry measured on separate occasions ($r = 0.85$). Thus, we would not expect that measurements in the laboratory have undermined our ability to detect changes in variables produced by long-term Ang II infusion or renal denervation.

**Perspectives**

Using the same experimental model used in the present study, we did not observe any effect of chronic Ang II infusion on the acute natriuretic response to renal denervation, and the antinatriuretic responses to reflexive and electric stimulation of the renal nerves, in pentobarbitone-anesthetized rabbits. Moreover, neurally mediated renin release was blunted in Ang II-induced hypertension. In our present study we observed normal levels of RSNA and no consistent effect of renal denervation in the hypertensive animals. Taken together, these findings provide strong evidence against a role of the renal nerves in the maintenance of chronic Ang II-induced hypertension. Nevertheless, the consistent finding that depressor responses are enhanced in Ang II hypertension indicate enhanced sympathetic-mediated vasoconstriction. The present experiments do not elucidate the underlying mechanism, but a recent study using Ang II-induced hypertension in rats on a high-salt diet might suggest a role of the splanchnic rather than the renal circulation.

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**Disclosures**

None.
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