Sustained influence of the renal nerves to attenuate sodium retention in angiotensin hypertension

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Lohmeier, Thomas E., Justin R. Lohmeier, Jane F. Reckelhoff, and Drew A. Hildebrandt. Sustained influence of the renal nerves to attenuate sodium retention in angiotensin hypertension. Am J Physiol Regulatory Integrative Comp Physiol 281: R434–R443, 2001.—Recent studies indicate that baroreflex suppression of renal sympathetic nerve activity is sustained for up to 5 days of ANG II infusion; however, steady-state conditions are not associated with ANG II hypertension of this short duration. Thus the major goal of this study was to determine whether neurally induced increments in renal excretory function during chronic intravenous infusion of ANG II are sustained under more chronic conditions when hypertension is stable and sodium balance is achieved. Experiments were conducted in five conscious dogs subjected to unilateral renal denervation and surgical division of the urinary bladder into hemibladders to allow separate 24-h urine collection from denervated (Den) and innervated (Inn) kidneys. ANG II was infused after control measurements for 10 days at a rate of 5 ng·kg⁻¹·min⁻¹. Twenty-four-hour control values for mean arterial pressure (MAP) and the ratio for urinary sodium excretion from Den and Inn kidneys (Den/Inn) were 92 ± 4 mmHg and 0.99 ± 0.05, respectively. On days 8–10 of ANG II infusion, MAP was stable (+30 ± 3 mmHg) and sodium balance was achieved. Whereas equal amounts of sodium were excreted from the kidneys during the control period, throughout ANG II infusion there was a greater rate of sodium excretion from Inn vs. Den kidneys (day 10 Den/Inn sodium = 0.56 ± 0.05), indicating chronic suppression of renal sympathetic nerve activity. The greater rate of sodium excretion in Inn vs. Den kidneys during renal sympathoinhibition also revealed a latent impairment in sodium excretion from Den kidneys. Although the Den/Inn for sodium and the major metabolites of nitric oxide (NO) decreased in parallel during ANG II hypertension, the Den/Inn for cGMP, a second messenger of NO, remained at control levels throughout this study. This disparity fails to support the notion that a deficiency in NO production and action in Den kidneys accounts for the impaired sodium excretion. Most importantly, these results support the contention that baroreflex suppression of renal sympathetic nerve activity is sustained during chronic ANG II hypertension, a response that may play an important role in attenuating the rise in arterial pressure.

baroreflexes; sympathetic nervous system; kidneys; nitric oxide

AN IMPORTANT UNRESOLVED ISSUE relating to blood pressure control is whether the sympathetic nervous system contributes to long-term regulation of body fluid volumes and arterial pressure. This uncertainty is a result of technical limitations that prevent determination of both long-term changes in sympathetic activity and the sustained influence of the sympathetic nervous system on sodium excretion. At present, it is not clear what mechanisms, if any, respond to disturbances in body fluid volumes and arterial pressure to evoke sustained compensatory changes in sympathetic activity. One hypothesis is that arterial baroreflexes play a role in the chronic, as well as the acute, regulation of arterial pressure. However, skepticism for this mechanism has mounted since the seminal studies of McCubbin et al. (22) demonstrating arterial baroreceptor resetting in chronically hypertensive dogs. Indeed, numerous acute studies have subsequently shown that arterial baroreceptors reset quite rapidly, within minutes, after a step change in arterial pressure (3). Furthermore, additional baroreceptor resetting occurs under more chronic conditions. Nonetheless, it is still unclear whether the arterial baroreflex completely resets in chronic hypertension. Additionally, even less is known about the time course and extent of resetting of cardiac mechanoreceptors, which produce compensatory changes in renal sympathetic nerve activity and sodium excretion when body fluid volumes are altered (7, 37). Importantly, if baroreflexes do completely reset in chronic hypertension, then they cannot possibly have long-term effects on sympathetic activity, body fluid volumes, and arterial pressure.

It is well established that the kidneys play a critical role in the long-term regulation of arterial pressure (11). A key feature of this control system is pressure natriuresis or the ability of the kidneys to respond to changes in arterial pressure by altering the renal excretion of salt and water. Clearly, chronic alterations in renal adrenergic activity—achieved either by renal denervation or long-term infusions of norepinephrine directly into the renal artery—alter pressure natriuresis and produce sustained changes in arterial pressure (14, 15, 31). However, it is not clear whether compensatory changes in renal sympathetic nerve activity...
occur in response to long-term perturbations in body fluid volumes and arterial pressure and, if so, whether they are of sufficient magnitude to alter renal excretory function.

To ascertain whether the renal sympathetic nerves might mediate chronic changes in renal excretory function in states of altered body fluid volumes and arterial pressure, we have used a split-bladder preparation in dogs combined with unilateral renal denervation. This is a powerful experimental model for exposing a functional role of the renal nerves because both kidneys are exposed to the same perfusion pressure and hormonal influences. Consequently, any differences in sodium excretion between the kidneys can be attributed to either the direct or indirect effects of the renal nerves on renal excretory function. Our studies using the split-bladder preparation in combination with unilateral renal denervation indicate that the changes in renal sympathetic nerve activity do play a role in the chronic regulation of sodium excretion. During high salt intake or hypertension induced by chronic intravenous infusion of either norepinephrine or ANG II, there is a relative increase in sodium excretion from innervated (Inn) vs. denervated (Den) kidneys (16–18, 21). This indicates that renal sympathetic nerve activity is suppressed in these states of chronic volume excess and/or hypertension. Furthermore, we have determined that the chronic renal sympathoinhibition during ANG II hypertension is abolished by denervation of sinoaortic and cardiopulmonary receptors, suggesting that baroreflexes play a critical role in this long-term response (18). However, in all of these previous studies, the duration of the experimental period has been no longer than 5 days. In regards to the above ANG II hypertension experiments, it is relevant that sodium balance and a stable level of arterial pressure were not achieved after 5 days of ANG II infusion (16, 18). Therefore, because the baroreflex lags behind changes in body fluid volumes and arterial pressure, it is not clear whether baroreflex suppression of renal sympathetic nerve activity persists under more chronic conditions when sodium balance is ultimately achieved and arterial pressure is stable. The major goal of the present study was to determine whether baroreflex-mediated renal sympathoinhibition during ANG II hypertension is sustained chronically under steady-state conditions.

A final goal of the present study was to provide further insight into the interactions among the renal nerves, ANG II, and nitric oxide (NO) in long-term control of sodium excretion. A number of studies in dogs with a split bladder and unilateral renal denervation have demonstrated that under conditions of renal sympathoinhibition, the Inn kidney actually excretes more sodium than the contralateral kidney devoid of innervation (16–18, 21, 39). This paradox suggests that there is a deficiency of a natriuretic substance (or, alternately, a surplus of an antinatriuretic compound) in chronically Den kidneys. Nitrodergic nerves are present in dog kidneys, and full expression of the natriuretic effects of NO is observed only in the presence of the renal nerves (1, 27, 39). Therefore, one possibility to account for the impaired excretion of sodium in chronically Den kidneys is that the nephrogenous production and/or the renal actions of NO are deficient in kidneys without innervation. This hypothesis is also consistent with our preliminary findings demonstrating that the greater rate of sodium excretion in Inn vs. Den kidneys during ANG II hypertension is associated with a similar pattern in the excretion of the major metabolites of NO, nitrate and nitrite (NOX) (20). Knowledge of the simultaneous excretion rates of NOX and cGMP, a second messenger of NO, would provide further insight into this hypothesis. That is, if deficient renal actions of NO do, in fact, account for impaired sodium excretion in Den kidneys during ANG II hypertension, then one would expect this to be reflected in the excretion of cGMP as well as NOX. Elucidating the relationship between NOX and cGMP excretion in Inn and Den kidneys during ANG II hypertension was a final objective of this study.

**METHODS**

**Surgical procedures.** The experiments were performed in 5 female dogs weighing 19–24 kg. All procedures were in accordance with National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committee. Before their initial surgery, the dogs were administered atropine sulfate (0.05 mg/kg sc), sedated with acepromazine maleate (0.15 mg/kg sc), and then anesthetized with pentobarbital sodium (25 mg/kg iv). Catheters made of Tygon microbore tubing were implanted in the lower abdominal aorta and in the inferior vena cava near the right atrium; the catheters were exteriorized between the scapulas. Then, the left kidney was denervated, and the urinary bladder was surgically divided. Each half was then sutured to form hemi-bladders, and Silastic catheters were implanted to allow continuous 24-h urine collection from each kidney. The catheters were exteriorized in the flank region and connected to sterile plastic bags that were changed daily. Postoperatively, the dogs were treated with antibiotics (Cefazolin sodium, 0.5 g im 2 times/day) for 5 days. Additionally, an analgesic (buprenorphine hydrochloride, 0.015 mg/kg im 2 times/day) was administered as needed for the first 24–48 h after surgery. The above surgical procedures have been described in more detail in previous communications (16–19, 21).

**General procedures.** The dogs were housed in a room maintained at 22 ± 2°C and 70% humidity with a 12:12-h light-dark cycle. They were fitted with a specially designed harness containing a pressure transducer (model P23 ID, Statham Laboratories, Hato Rey, PR) positioned at heart level for measurement of arterial pressure. Isotonic saline (350 ml/day) was infused continuously in one of the femoral vein catheters by means of a Wiz peristaltic pump (Isco, Lincoln, NE). A disposable filter (Cathivex, Millipore, Bedford, MA) was connected in series with the infusion to prevent passage of bacteria and other contaminants.

During a 2- to 3-wk training and equilibration period and throughout the entire experiment, the dogs were given free access to water and maintained on a fixed diet of two 15.5-oz cans of prescription heart diet (HD; Hill’s Pet Products, Topeka, KS) supplemented with 5 ml of vitamin syrup (V.A.L. Syrup, Fort Dodge Laboratories, Fort Dodge, IA). Two cans of HD provide ~5 meq of sodium and ~60 meq of potassium. Thus, with the intravenous sodium infusion, so-
Results

Figure 1 shows that ANG II infusion produced chronic hypertension in the absence of bradycardia. The immediate on- and off-arterial pressure transients to ANG II infusion were not quantitated in this study because they occurred during the hours of feeding and cage cleaning. However, at the infusion rate of ANG II used in the present study, most of the day 1 increase in MAP occurs during the first 1–2 h of ANG II infusion (4; T. E. Lohmeier, personal observations). MAP increased 20–25 mmHg during the first day of ANG II infusion before reaching a steady state in all dogs by day 8. On days 5–7 of ANG II infusion, there were still either small increases or decreases in MAP in all dogs. During the last 3 days of ANG II infusion, however, MAP varied no more than 1 mmHg in all dogs, and on days 9–10, MAP was elevated 30 ± 3 mmHg above control (control = 92 ± 4 mmHg). Heart rate (control = 63 ± 4 beats/min) tended to decrease during ANG II infusion, but the changes were not statistically significant.

The changes in urinary electrolyte and creatinine excretion during ANG II infusion are illustrated in Figs. 2-4. During the control period, sodium and potassium balance was achieved and approximately equal excretion rates of sodium, potassium, creatinine, and volume were excreted from Inn and Den kidneys. This is reflected by the control values for the Den/Inn for sodium (0.99 ± 0.05), potassium (0.94 ± 0.02), creatinine (0.98 ± 0.05), and volume (1.01 ± 0.05) excretion.

As expected, chronic ANG II infusion caused sodium retention for 2 days; however, much of the sodium that...
was retained initially was subsequently excreted on days 4–7 before sodium balance was once again achieved on days 8–10 of ANG II infusion (Fig. 2). For the 10-day period of ANG II infusion, there was no significant net retention of sodium. Most importantly, in contrast to the control and recovery periods when approximately equal amounts of sodium were excreted by both kidneys, substantially more sodium (≈2-fold) was excreted from Inn vs. Den kidneys throughout the entire 10 days of ANG II infusion. During ANG II hypertension, the Den/Inn for sodium excretion decreased from a control value of 0.99 ± 0.05 to a nadir of 0.45 ± 0.05 on day 4 and a steady-state level of 0.56 ± 0.05 on days 9–10 of ANG II infusion. This response indicates that suppression of renal sympathetic nerve activity is sustained under steady-state conditions and well beyond 5 days of ANG II infusion, the duration of our previous studies (16, 18). Finally, in association with the pronounced fall in MAP after cessation of ANG II infusion, the Den/Inn for sodium excretion increased to above control levels on days 1 and 2 before subsequently returning to pre-ANG II infusion levels. Presumably, this transient increase in the Den/Inn for sodium excretion was mediated by baroreflex activation of the renal sympathetic nerves. In general, there were parallel changes in the Den/Inn for sodium and volume excretion throughout this study (data not shown).

Although there were no significant changes in potassium balance, the Den/Inn for potassium excretion decreased in parallel with the Den/Inn for sodium excretion during ANG II infusion (Fig. 3). By day 10 of ANG II infusion, the Den/Inn for potassium excretion decreased from a control value of 0.94 ± 0.02 to 0.74 ± 0.04. Thus more potassium, as well as sodium, was excreted from Inn vs. Den kidneys during ANG II hypertension. After cessation of ANG II infusion, the Den/Inn for potassium excretion returned to control levels after a transient increase on days 1 and 2 of the recovery period.

As illustrated in Fig. 4, there were no significant changes in total creatinine excretion during ANG II infusion. Furthermore, in marked contrast to the fall in the Den/Inn for sodium and potassium excretion during ANG II infusion, there were no significant changes in the Den/Inn for creatinine excretion throughout the duration of ANG II hypertension. This indicates that the higher rates of sodium and potassium excretion from Inn vs. Den kidneys during ANG II infusion were independent of the filtered load of these electrolytes.
Like the Den/Inn for sodium and potassium secretion, the Den/Inn for NOX excretion decreased during chronic ANG II infusion (Fig. 5). The total daily excretion of NOX tended to fall throughout the 10-day period of ANG II hypertension, but the reduced rate of NOX excretion achieved statistical significance only on day 1 of ANG II infusion (Fig. 5). In contrast, there was a sustained decrease in the Den/Inn for NOX excretion throughout the 10 days of ANG II infusion, and on day 10, the Den/Inn for NOX excretion was reduced from a control value of 0.97 ± 0.04 to 0.64 ± 0.03. Thus, as with the excretion of sodium and potassium, the excretion of NOX was considerably higher from Inn vs. Den kidneys during ANG II infusion. In addition, the sharp increase in the Den/Inn for NOX excretion on day 1 of the recovery period also mimicked the response for sodium and potassium excretion.

In marked contrast to the fall in Den/Inn for urinary NOX excretion during ANG II hypertension, the Den/Inn for cGMP excretion (control = 0.98 ± 0.05) was unchanged throughout the 10-day period of ANG II hypertension (Fig. 6). Thus, in comparing Den to Inn kidneys, a clear dichotomy existed between the relative excretion rates of NO metabolites and the well-established intracellular mediator of the physiological actions of NO, cGMP. Finally, it should be noted that there were statistically significant increases in the total excretion of cGMP on days 3–5 of ANG II infusion.

During ANG II infusion, PRA (control = 0.46 ± 0.14 ng ANG I·ml⁻¹·h⁻¹) decreased to undetectable levels and plasma potassium concentration (control = 4.5 ± 0.2 meq/l) fell 0.7 ± 0.2 meq/l (day 10). Hematocrit and plasma protein concentration reflected changes in salt and water balance. During the initial days of ANG II infusion when there was appreciable volume retention, hematocrit (control = 39 ± 1%) and plasma protein concentration (control = 6.5 ± 0.2 mg/dl) decreased 10–15%. However, in parallel with the subsequent loss of body fluid volume during the more chronic phase of ANG II infusion, these reductions in hematocrit and plasma protein concentration were not sustained. Finally, there were no significant changes in plasma sodium concentration (control = 144 ± 1 meq/l) throughout this study. Recovery values for all of the above were similar to control.

**DISCUSSION**

The present study provides further insight into the potential role of the sympathetic nervous system and baroreflexes in the chronic regulation of sodium excre-
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Fig. 6. Daily excretion of cGMP from both Den and Inn kidneys and changes in the Den/Inn for cGMP excretion during chronic ANG II infusion. Values are means ± SE. *P < 0.05 vs. control.

A number of studies in chronically instrumented animals subjected to infusions of ANG II lasting up to 10 days support the hypothesis that baroreflex-mediated suppression of renal sympathetic nerve activity contributes to long-term volume and arterial pressure homeostasis in ANG II hypertension. First, measurements of renal norepinephrine overflow (an indirect index of renal sympathetic nerve activity) and direct recordings of renal nerve activity have clearly shown that renal sympathetic nerve activity is suppressed in chronic ANG II hypertension (2, 5). However, suppression of renal sympathetic nerve activity in ANG II hypertension does not necessarily indicate that similar changes in sympathetic activity occur in other tissues (8). Second, in the present study and in earlier investigations from our laboratory (16, 18), the relatively greater rate of sodium excretion in Inn vs. Den kidneys during ANG II hypertension indicates that chronic renal sympathoinhibition is of sufficient magnitude to produce functionally significant increments in renal excretory function. Third, as the fall in the Den/Inn for sodium excretion in response to ANG II infusion is totally abolished after denervation of cardiopulmonary and sinoaortic baroreceptors (18), it would appear that chronic renal sympathoinhibition is mediated by baroreflexes. In fact, the potential importance of baroreflex-mediated suppression of renal sympathetic nerve activity as a compensatory mechanism in ANG II hypertension is highlighted by the response to ANG II infusion after baroreceptor deafferentation. After deafferentation of cardiopulmonary and sinoaortic baroreceptors, the Den/Inn for sodium excretion actually increases during chronic ANG II infusion, indicating that in the absence of baroreflexes, ANG II has sustained renal sympathoexcitatory effects (18). Fourth, the present results are especially important in that they suggest that baroreflex-mediated suppression of renal sympathetic nerve activity is truly a long-term compensatory response to ANG II hypertension. As in our earlier studies (16, 18), arterial pressure was not stable and sodium balance did not occur after 5 days of ANG II infusion. Indeed, negative sodium balance on days 4–7 of ANG II infusion suggests diminution of the initial volume expansion induced by ANG II, a response that could reduce renal sympathetic nerve activity by deactivating cardiopulmonary baroreceptors. Nonetheless, suppression of renal sympathetic nerve activity was sustained throughout the entire 10 days of ANG II hypertension, including days 8–10 of ANG II infusion when sodium balance was achieved and arterial pressure was stable. Thus, in contrast to studies of shorter duration not associated with steady-state conditions, it is unlikely that sustained renal sympathoinhibition during chronic ANG II hypertension in the present study was a result of baroreceptors responding to subtle transient changes in either body fluid volumes or arterial pressure.

Because of technical limitations that prevent faithful quantitative determinations of time-dependent changes in nerve activity, there is relatively little information on the time course and extent of arterial baroreflex resetting in chronic hypertension. Krieger (13) subjected rats to coarctation of the aorta to produce an abrupt and constant increase in arterial pressure. From recordings of aortic baroreceptor activity during anesthesia, Krieger concluded that complete baroreceptor resetting occurs within 48 h of hypertension. Cowley and DeChue (4) took a different approach.
to assess baroreflex function by determining the influence of the baroreflex on the time course and severity of ANG II hypertension. These investigators induced a fairly rapid and sustained increase in arterial pressure by chronic infusion of ANG II at the same rate as employed in the present study while monitoring arterial pressure continuously, 24-h/day, in dogs with and without sinoaortic denervation. In this study, acute increases in arterial pressure in response to ANG II were more pronounced after deafferentation of arterial baroreceptors than before when the arterial baroreflex was intact. However, by the 28th hour of ANG II infusion, the increase in blood pressure was the same in both groups of dogs, and, furthermore, the final steady-state level of arterial pressure on day 7 of ANG II infusion was comparable under both conditions. Thus the baroreceptors appeared to have no sustained influence on the severity of the hypertension. In summary, both of the above studies suggest that complete baroreceptor resetting occurs within several days of achieving a stable elevation in arterial pressure. In contrast, because deafferentation of cardiopulmonary and arterial baroreceptors abolishes chronic renal sympathoinhibition during ANG II hypertension (18), our current findings indicate that baroreflex control of renal sympathetic nerve activity does not reset according to the rapid time course expected from the above studies.

How then does one reconcile the observations by Cowley and DeClue (4) suggesting fairly rapid arterial baroreceptor resetting in ANG II hypertension to our findings that sustained renal sympathoinhibition during chronic ANG II infusion is abolished by deafferentation of cardiopulmonary and arterial baroreceptors? One possibility is that arterial baroreflexes do have a sustained influence on sympathetic activity and arterial pressure in chronic ANG II hypertension, but after arterial baroreceptor denervation, their compensatory role in blood pressure regulation is assumed by other neurohormonal mechanisms. Alternately, other neural mechanisms may simply be more important than arterial baroreflexes in mediating sustained reductions in renal sympathetic nerve activity in ANG II hypertension. In this regard, it is possible that cardiopulmonary (cardiac) reflexes may play an especially important role in chronically inhibiting renal sympathetic nerve activity during ANG II hypertension. Indeed, although both arterial and cardiopulmonary baroreflexes influence renal sympathetic nerve activity, most studies have shown that cardiopulmonary receptors with vagal afferents play the more predominant and significant role in the regulation of sodium excretion, at least in response to acute alterations in intravascular volume (7, 37). Furthermore, whereas little is known about the resetting characteristics of cardiopulmonary baroreflexes, studies lasting up to 2 h in chronically instrumented dogs suggest that cardiopulmonary baroreflex control of renal sympathetic nerve activity is refractory to resetting (23, 24). Although it is not known whether cardiac reflexes have a sustained influence on sympathetic activity, it is possible that ANG II could chronically stimulate atrial and/or ventricular mechanoreceptors by increasing cardiac mechanoreceptors. This could occur as a result of the volume-retaining effects of ANG II and/or the effects of ANG II to increase afterload. Clearly, more selective denervation procedures that eliminate either cardiac or sinoaortic afferents, but not both in combination, are needed to determine the specific reflexes that account for chronic renal sympathoinhibition in ANG II hypertension. Finally, it is possible that the antinatriuretic effects of ANG II are far more potent than are the compensatory effects on renal excretory function mediated via renal sympathoinhibition. As a result, chronic suppression of renal sympathetic nerve activity may have little influence on the severity of ANG II hypertension. In this regard, an important future study will be to determine whether the severity of ANG II hypertension is exacerbated after deafferentation of cardiopulmonary receptors as well as arterial baroreceptors.

A number of studies in conscious dogs with surgical division of the bladder and unilateral renal denervation, including the present, has shown that under conditions in which renal sympathetic nerve activity is suppressed, the Inn kidney actually excretes more sodium than the contralateral Den kidney (16–18, 21, 33). These findings are consistent with the observation that the natriuretic response to acute volume expansion, which normally is associated with reflex suppression of renal sympathetic nerve activity, is blunted after chronic bilateral renal denervation (7, 37). These studies clearly demonstrate that intact kidneys subjected to either acute or chronic inhibition of renal sympathetic nerve activity may actually excrete more sodium than kidneys completely devoid of innervation, a response that does not reflect renal denervation supersensitivity (21). Thus these observations suggest that chronic renal denervation not only abolishes the antinatriuretic effects of the renal nerves, but it eliminates natriuretic (or induces sodium retaining) mechanisms as well. As renal denervation is a time-honored technique for assessing the physiological and pathophysiological effects of the renal nerves, this consideration indicates an important caveat in data interpretation that has not been widely appreciated.

For several reasons, we considered the possibility that a deficiency in the renal production of NO might account for the impaired excretion of sodium in Den vs. Inn kidneys during the inhibition of renal sympathetic nerve activity associated with ANG II hypertension. Nitrooxidergic nerves are present in dog kidneys, and NO promotes sodium excretion (10, 27). Furthermore, renal NO production is increased by both norepinephrine and ANG II, and NO generation by the kidneys plays an important role in counteracting the acute and chronic vasoconstrictor and antinatriuretic effects of these agents (12, 26, 35, 36, 40, 41). Additionally, the renal vasoconstrictor and antinatriuretic effects associated with pharmacological inhibition of NO generation are attenuated after renal denervation (9, 39); alternately, the natriuretic response to L-arginine administration, thought to be mediated by renal NO
production, is reduced by renal denervation (1). All of the above support the premise that NO influences are greater in the presence than in the absence of the renal nerves. Finally, our preliminary experiments demonstrated a greater rate of NOX excretion in Inn vs. Den kidneys during the renal sympathoinhibition associated with ANG II hypertension (20). Thus we hypothesized that a deficiency in NO production in the Den kidney, although of little significance under control conditions, may manifest in exaggerated sodium retention during renal NO stimulation by ANG II.

Although the renal excretion of NOX has been used as an index of endogenous NO production, it is a rather poor indicator of renal NO production (34). This is because large quantities of NO are produced in the systemic circulation and because NOX is freely filtered. Indeed, although ANG II increases the renal production of NO (12, 35, 41), NOX excretion actually decreased in the present study on day 1 of ANG II infusion, presumably reflecting the transient fall in cardiac output (and endothelial shear stress) produced by this rate of ANG II infusion (4). Subsequently, coincident with the expected recovery of cardiac output (4), NOX excretion returned to control levels during chronic ANG II hypertension, an observation consistent with an earlier report that NOX excretion was unchanged during chronic ANG II infusion in rats (6). Nonetheless, despite the limited insight into renal NO production from measurements of total urinary NOX excretion from both kidneys, the greater rate of NOX excretion in Inn vs. Den kidneys during ANG II hypertension suggests either enhanced renal actions of NO or altered tubular handling of NOX. This is because the constant Den/Inn for creatinine excretion throughout this study indicates that the filtered load of NO was comparable in Inn and Den kidneys, both before and during ANG II infusion.

To differentiate between these two possibilities, we determined the excretion rates of the second messenger of NO, cGMP, as well as the excretion rates of the major NO metabolites, NOX. Recent studies do indicate that some of the renal actions of NO may be cGMP independent (32). Nonetheless, given this caveat, we hypothesized that if the relative increase in NOX excretion in Inn vs. Den kidneys reflected a greater rate of NO production and action in the former, then there should be a parallel decrease in the Den/Inn for NOX and cGMP excretion. Contrary to this hypothesis, the Den/Inn for cGMP excretion remained at control levels during the marked decrease in the Den/Inn for NOX excretion associated with chronic ANG II infusion. This divergence in the excretion patterns of NOX and cGMP in Inn and Den kidneys suggests differences in the tubular handling of NOX rather than differences in NO production and action (34). NOX is extensively reabsorbed in the proximal tubule, a predominant site of the renal nerves on sodium transport, and inhibition of sodium reabsorption with proximally acting diuretics increases NOX excretion (34). Thus it is likely that the relative increase in NOX excretion in Inn vs. Den kidneys and the parallel decrease in the Den/Inn for sodium and NOX excretion during ANG II hypertension was secondary to inhibition of renal sympathetic nerve activity and concomitant suppression of sodium reabsorption. Additionally, as we have discussed previously (16, 18), such a mechanism could also account for the parallel fall in the Den/Inn for potassium excretion, because potassium transport is closely coupled to sodium reabsorption in the proximal tubule. The handling of cGMP is quite different to that of NOX. cGMP is neither reabsorbed nor secreted and has a very high fractional excretion rate, exceeding 100% when the renal production of this second messenger is stimulated (38). Because the Den/Inn for cGMP did not change during ANG II hypertension, this would suggest that the nephrogenous production of cGMP was similar in both kidneys, providing no support for the hypothesis that the impaired sodium excretion in chronically Den kidneys was due to deficient renal NO production. As the excretion pattern of sodium suggests that appreciable volume expansion occurred only during the initial days of ANG II infusion, it is likely that the transient increase in urinary cGMP excretion from both kidneys during this time was due to the renal actions of atrial natriuretic peptide. This natriuretic hormone is stimulated by atrial stretch, and its physiological effects, such as those of NO, are dependent on the second messenger cGMP (38).

In summary, these results indicate that the renal nerves have a sustained, long-term influence to attenuate the sodium retention induced by chronically elevated plasma levels of ANG II. Importantly, taken in the context of our earlier studies (16–18), these findings provide direct support for the concept that baroreflex suppression of renal sympathetic nerve activity is a long-term compensatory response to ANG II hypertension. Additionally, this study exposes a latent impairment in sodium excretion that is present in chronically Den kidneys. The nature of this impairment is unresolved, but it does not appear to be a deficiency in NO production or action.

Perspectives

As discussed above, a pattern emerging from our recent studies is that suppression of renal sympathetic nerve activity and attendant increments in renal excretory function are long-term responses to excess body fluid volumes and hypertension (16–18, 21). These results support the contention that the sympathetic nervous system responds chronically, as well as acutely, to regulate body fluid volumes and arterial pressure. Furthermore, a follow-up investigation demonstrated that long-term suppression of renal sympathetic nerve activity in ANG II hypertension is dependent on baroreflexes (18). This latter finding is particularly important given the technical limitations in determining whether baroreflexes completely reset in chronic hypertension. In light of numerous acute studies demonstrating that ANG II actually increases sympathetic activity (8, 28), it is a particularly intriguing finding that chronic suppression of renal sympa-
thetic nerve activity is the integrated response to ANG II hypertension. Whereas acute observations are often (inappropriately) extrapolated to infer that similar mechanisms are operative under more long-term conditions, it should be emphasized that there is little chronic data to support the contention that the sympathetic nervous system contributes to ANG II hypertension (8, 28). The above considerations raise several important issues. First, it is important to recognize that the mechanisms by which the sympathetic nervous system regulates arterial pressure acutely and chronically are quite different (11). Therefore, acute blood pressure responses to either sympathetic stimulation or inhibition may provide little insight into the contribution of the sympathetic nervous system to the chronic maintenance of arterial pressure. Second, the renal nerves appear to be the critical link between the sympathetic nervous system and long-term regulation of arterial pressure, because they impact renal excretory function (11). Accordingly, as the sympathetic nervous system is differentially regulated, chronic changes in sympathetic activity to the kidneys, and not to other tissues, are of paramount importance in producing long-term alterations in arterial pressure. Consequently, measurements of sympathetic activity in nonrenal tissues cannot be used as a surrogate for changes in renal sympathetic nerve activity. Finally, novel techniques are needed to determine the extent of baroreflex resetting and the sustained influence of baroreflexes on body fluid volumes and arterial pressure in physiological and pathophysiological states. Our findings indicating that baroreflexes play a critical role in opposing the chronic renal sympathoexcitatory effects of ANG II highlight the potential importance of baroreflexes and baroreflex dysfunction as chronic determinants of sympathetic activity in disease states such as hypertension and heart failure (18).

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