Baroreflexes prevent neurally induced sodium retention in angiotensin hypertension

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Baroreflexes prevent neurally induced sodium retention in angiotensin hypertension. Am J Physiol Regulatory Integrative Comp Physiol 279: R1437–R1448, 2000.—Recent studies indicate that renal sympathetic nerve activity is chronically suppressed during ANG II hypertension. To determine whether cardiopulmonary reflexes and/or arterial baroreflexes mediate this chronic renal sympathoinhibition, experiments were conducted in 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. Dogs were studied 1) intact, 2) after thoracic vagal stripping to eliminate afferents from cardiopulmonary and aortic receptors [cardiopulmonary denervation (CPD)], and 3) after subsequent denervation of the carotid sinuses to achieve CPD plus complete sinoaortic denervation (CPD + SAD). After control measurements, ANG II was infused for 5 days at a rate of 5 ng·kg\(^{-1}\)·min\(^{-1}\). In the intact state, 24-h control values for mean arterial pressure (MAP) and the ratio for urinary sodium excretion from Den and Inn kidneys (Den/Inn) were 98 ± 4 mmHg and 1.04 ± 0.04, respectively. ANG II caused sodium retention and a sustained increase in MAP of 30–35 mmHg. Throughout ANG II infusion, there was a greater rate of sodium excretion from Inn vs. Den kidneys (day 5 Den/Inn sodium = 0.51 ± 0.05), indicating chronic suppression of renal sympathetic nerve activity. CPD and CPD + SAD had little or no influence on baseline values for either MAP or the Den/Inn sodium, nor did they alter the severity of ANG II hypertension. However, CPD totally abolished the fall in the Den/Inn sodium in response to ANG II. Furthermore, after CPD + SAD, there was a lower, rather than a higher, rate of sodium excretion from Inn vs. Den kidneys during ANG II infusion (day 5 Den/Inn sodium = 2.02 ± 0.14). These data suggest that cardiac and/or arterial baroreflexes chronically inhibit renal sympathetic nerve activity during ANG II hypertension and that in the absence of these reflexes, ANG II has sustained renal sympathoexcitatory effects.

THE RENIN-ANGIOTENSIN SYSTEM plays an important role in both short- and long-term regulation of sodium excretion and arterial pressure. This role of the renin-angiotensin system is achieved by the actions of ANG II on vascular smooth muscle, adrenal cortex, and renal tubules. Additionally, numerous acute studies have shown that ANG II has several effects that increase sympathetic activity, including a central action to increase sympathetic outflow (33). However, the physiological and pathophysiological significance of the interactions between the renin-angiotensin and sympathetic nervous systems in the control of sodium excretion and arterial pressure is not fully understood.

Although a number of studies indicates that endogenous ANG II stimulates the sympathetic nervous system in hypovolemic and/or hypotensive states, it is controversial whether the sympathetic nervous system contributes to the hypertension induced by ANG II (8, 11, 18, 33). On one hand, arterial baroreflexes and/or cardiac reflexes might be expected to chronically oppose the sympathoexcitatory actions of ANG II. On the other hand, this hypothesis is inconsistent with the notion that baroreceptors undergo rapid adaptation and resetting and, therefore, would be unable to participate in long-term regulation of sodium excretion and arterial pressure (2, 4, 5, 15, 38). It should be emphasized, however, that due to the inherent difficulty in achieving chronic nerve recordings that provide quantitative time-dependent changes in sympathetic activity, it is unknown to what extent baroreflex control of sympathetic activity may adapt in chronic hypertension. Thus it is not clear whether baroreflexes have the capacity to chronically influence sodium excretion and arterial pressure.

The kidneys play a critical role in the long-term regulation of arterial pressure (5, 15, 38). Long-term control of arterial pressure is achieved by the renal body fluid feedback mechanism whereby the kidneys slowly regulate arterial pressure by altering body fluid volumes by the effect of pressure on sodium excretion, referred to as pressure natriuresis. Because chronic alterations in renal adrenergic activity alter pressure natriuresis and produce sustained changes in arterial pressure (17, 35), it is conceivable that the renal nerves may play a critical role in mediating long-term effects...
of the sympathetic nervous system on body fluid volumes and arterial pressure. Therefore, in regards to understanding the long-term interactions between the renin-angiotensin and sympathetic nervous systems in the control of sodium excretion and arterial pressure, it would be important to determine whether ANG II has sustained effects on renal sympathetic nerve activity that impact renal excretory function. Furthermore, a related issue is whether baroreflexes chronically influence any direct sympathoexcitatory actions of ANG II that promote sodium retention.

The split-bladder preparation combined with unilateral renal denervation has provided considerable insight into the functional effects of the renal nerves in a number of chronic hypertensive and hypertensive conditions (18, 19, 21, 22, 27, 28). 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. In light of the large number of acute studies demonstrating that ANG II increases sympathetic activity, we recently used the above model to investigate whether the renal nerves promote sodium retention in ANG II hypertension (18). Our results were just the opposite. During chronic infusion of ANG II, pathophysiological levels of ANG II in the circulation were associated with a relative increase in sodium excretion from innervated (Inn) vs. denervated (Den) kidneys, suggesting that renal sympathetic nerve activity is suppressed, not elevated, during ANG II hypertension. However, the afferent mechanisms leading to chronic suppression of renal sympathetic nerve activity in ANG II hypertension were not investigated. Accordingly, the goal of the present study was to determine whether sympathoinhibitory reflexes emanating from cardiac and arterial baroreceptors account for the enhanced sodium excretory response induced by the renal nerves during ANG II hypertension. To this end, we used the split-bladder preparation combined with unilateral renal denervation and studied dogs first in the intact state and then after progressive stages of deafferentation of cardiopulmonary and arterial baroreceptors. We hypothesized that if cardiopulmonary and/or arterial baroreflexes account for sustained inhibition of renal sympathetic nerve activity during chronic ANG II hypertension, then the relative increase in sodium excretion in Inn vs. Den kidneys during ANG II hypertension should be abolished after cardiopulmonary and arterial baroreceptor denervation.

METHODS

Surgical Procedures

Catheters and split bladder. The experiments were performed in five female dogs weighing 20–24 kg. All procedures were in accordance with National Institutes of Health Guidelines and approved by the Institutional Animal Care and Use Committee. Before the 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 through a flank approach. All visible nerves along the renal artery and vein were removed, the adventitia was stripped, and the vessels were painted for 20 min with a solution of 10% phenol in ethanol. As we have reported in previous studies (22, 36), this procedure produces more than a 30-fold difference in norepinephrine content between Inn and Den kidneys, indicating pronounced norepinephrine depletion in Den kidneys. Finally, the urinary bladder was surgically divided. Each half was sutured to form hemibladders, 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. The above surgical procedures have been described in more detail in previous communications (18–22).

Cardiopulmonary denervation. Deafferentation of cardiopulmonary and aortic baroreceptors was achieved with the use of the technique described by Persson et al. (31, 32). The dogs were sedated with acepromazine and then anesthetized with isoflurane (1.5–2.0%) after induction with thiopental (10 mg/kg iv). Through a left thoracotomy at the third intercostal space, cardiopulmonary and aortic baroreceptors were denervated by sectioning all branches of both vageosympathetic trunks between the thoracic aperture and ~2 cm below the origin of the aortic arch. A successful denervation was confirmed during surgery by stimulating the stripped vagal nerves (10 V, 20 Hz). The absence of short-latency bradycardia during vagal stimulation was taken to indicate denervation. Further tests for cardiopulmonary denervation (CPD) were made ~2 wk later after complete recovery from surgery.

Carotid sinus denervation. To achieve denervation of carotid as well as aortic baroreceptors, a final surgery was performed with the use of the same preanesthetic and anesthetics described above for CPD. Through a ventral midline neck incision, carotid baroreceptors were denervated by stripping the adventitia in the area of the carotid bifurcation, including the initial 1–2 cm of the external and internal carotid arteries, and painting all vessels in this region with 10% phenol in ethanol. Thus, after this surgery, there was deafferentation of both cardiopulmonary and sinoaortic baroreceptors [CPD + sinoaortic denervation (SAD)].

Postoperative medication. 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.

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 saline infusion, sodium intake was ~60 meq/day. Water consumption was monitored daily, and 24-h urine samples were collected at 10:00 AM, ~1 h before feeding. Body temperature was measured each morning to ensure the dogs were free of infection. Amoxicillin (250 mg), dicloxacillin (250 mg), and a trimethoprim (400 mg)-sulfamethoxazole (80 mg) combination were given orally twice a day as a prophylactic measure.

**Measurement of Hemodynamics**

Throughout the study, arterial pressure was monitored continuously from an arterial catheter connected to the pressure transducer in the harness and recorded on a Grass polygraph (model 7D, Grass Instruments, Quincy, MA). A microcomputer and customized software were used to sample the signal from the Grass recorder at 200 Hz for a duration of 12 s, once a minute, 24 h/day (20, 29). The digitized data for each 12-s burst were immediately processed to extract systolic and diastolic events and to compute mean arterial pressure (MAP) and heart rate. The daily hemodynamic values presented were averaged from the 20-h period extending from noon to 8:00 AM. The variability for MAP and heart rate was expressed from the standard deviation of the 20-h data set (1,200 data points/day). The hours excluded from the 24-h analysis included the time required for flushing catheters, calibrating pressure transducers, feeding, and cleaning cages.

**Experimental Protocol**

During the initial 3-wk training and equilibration period, the intact dogs were trained to lie quietly in their cages for collection of blood samples. After a 3-day control period, the dogs were continuously infused with ANG II ([Asp^1 Val^3]ANG II, Ciba-Geigy, Summit, NJ) for 5 days by adding the peptide to the 24-h saline infusion. ANG II was infused at a rate of 5 ng·kg⁻¹·min⁻¹ that increases plasma levels to approximately five times control (34). The chronic infusion of ANG II was followed by a 5-day recovery period. On intermittent days, 5 ml arterial blood samples were taken for determination of hematocrit, plasma renin activity (PRA), and the plasma concentrations of sodium, potassium, and protein. Throughout the study, urine samples were collected daily for determination of 24-h urinary excretion rates of sodium, potassium, and creatinine from Den and Inn kidneys.

After completion of the above experimental protocol in intact dogs, an identical study was repeated in the same dogs after CPD and subsequently after CPD + SAD. Control periods for CPD and CPD + SAD experiments were initiated ~1 and 2 wk postoperatively, respectively.

**Verification of CPD**

In addition to intraoperative stimulation of the vagus, elimination of cardiac vagal innervation was quantified postoperatively by two additional methods. First, reflex bradycardia in response to bolus intravenous administration of phenylephrine (10 µg/kg) was tested before and after CPD. CPD was considered effective if heart rate decreased <5 beats/min in response to this pressor dose of phenylephrine (31, 32). Second, CPD was evaluated by determining the reflex hypertensive and bradycardia induced by veratridine. Responses mediated by excitation of cardiopulmonary receptors with vagal afferents. For this test, a bolus injection of veratridine (50 µg) was administered into one of the vena cava catheters positioned in the vicinity of the right atrium. Responses to veratridine were evaluated in the intact state and after CPD.

**Verification of SAD**

Marked attenuation of the reflex reduction in heart rate in response to acute administration of an α-adrenergic receptor agonist (e.g., phenylephrine) is commonly used as an index for effective sinoaortic baroreceptor denervation. This test could not be used in the present study because of the absence of cardiac vagal innervation. However, sinoaortic baroreceptor denervation was verified by an increase in blood pressure variability after CPD + SAD (see RESULTS).

**Analytic Methods**

PRA was measured by radioimmunoassay (20). Plasma and urine concentrations of sodium and potassium were determined by flame photometry (IL 943, Instrumentation Laboratories, Lexington, MA), plasma protein concentration by refractometry (American Optical, Buffalo, NY), and hematocrit by a micromethod (Autocrit II, Clay Adams, Franklin, NJ). Urinary creatinine concentration was determined with a creatinine analyzer (model 2, Beckman, Brea, CA).

**Statistics**

Results are expressed as means ± SE. A one-way ANOVA was used to compare experimental and recovery responses to control within each condition (intact, CPD, CPD + SAD). Significant differences were established with the use of Dunnett’s t-test for multiple comparisons. Additionally, differences in control values among the three conditions were compared by the Bonferroni t-test. Statistical significance was considered to be P < 0.05. The relative excretion rates of sodium, potassium, and creatinine from Den and Inn kidneys are expressed by the ratio Den/Inn.

**RESULTS**

**Responses in the Intact State**

Figure 1 shows that chronic infusion of ANG II produced hypertension in the absence of a significant reduction in heart rate. MAP increased 20–25 mmHg during the first day of ANG II infusion, and on day 5, MAP was elevated 32 ± 2 mmHg above control (control = 98 ± 4 mmHg). Although heart rate tended to decrease during ANG II hypertension, the only significant change in heart rate (control = 72 ± 9 beats/min) occurred on the day after cessation of ANG II infusion when MAP decreased 25–30 mmHg. During this 24-h period, heart rate increased 22 ± 6 beats/min before returning to control levels on subsequent recovery days.

The changes in urinary electrolyte and creatinine excretion during ANG II infusion were similar to those we have reported previously (18). As expected, sodium retention occurred on the first day of ANG II infusion before sodium balance was achieved subsequently (Fig.
2). Most importantly, in contrast to the control and recovery periods when approximately equal amounts of sodium were excreted by both kidneys, substantially more sodium (approximately twofold) was excreted from Inn vs. Den kidneys throughout the entire 5 days of ANG II infusion. This is reflected by the fall in the Den/Inn for sodium excretion from a control value of 1.04 ± 0.04 to 0.51 ± 0.05 on day 5 of ANG II infusion, a response that indicates chronic suppression of renal sympathetic nerve activity during ANG II hypertension. 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 recovery days 1 and 2. Presumably, this transient increase in the Den/Inn for sodium excretion was mediated by baroreflex activation of the renal nerves. Although there were no significant changes in potassium balance, the Den/Inn for potassium excretion decreased parallel with the Den/Inn for sodium excretion during ANG II infusion (Fig. 3). By day 5 of ANG II infusion, the Den/Inn for potassium excretion decreased from a control value of 0.96 ± 0.02 to 0.63 ± 0.07. 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 (and sodium) excretion returned to control levels after a transient increase on days 1 and 2 of the recovery period. Though not illustrated, there were no significant changes in the Den/Inn for creatinine excretion (control = 1.01 ± 0.02) during ANG II hypertension. This indicates that the neurally induced natriuresis and kaliuresis during ANG II infusion were independent of a higher glomerular filtration rate in Inn vs. Den kidneys.

During ANG II infusion, PRA (control = 0.34 ± 0.12 ng ANG I·ml⁻¹·h⁻¹) decreased to undetectable levels, and plasma potassium concentration (control = 4.2 ± 0.1 meq/l) fell 0.2 ± 0.1 meq/l. In contrast, there were no significant sustained changes in hematocrit (control = 39 ± 3%) or in the plasma concentrations of sodium (control = 144 ± 1 meq/l) or protein (control = 6.9 ± 0.3 mg/dl) during ANG II infusion. Recovery values for all of the above were similar to control.

**Responses after CPD**

**Acute postoperative responses.** The hemodynamic and the sodium excretory responses in Den and Inn kidneys during the initial 18 h after CPD are illustrated in Fig. 4. Compared with the previous daily values in the intact state, heart rate increased markedly from 74 ± 6 to 125 ± 6 beats/min after elimination of cardiac vagal innervation. In contrast, CPD had no
significant effect on MAP or the Den/Inn for sodium, potassium, or creatinine excretion.

It should be noted that for several days after surgery for CPD, some dogs had gastrointestinal disturbances and therefore did not consume all of their food. However, this response was transient, and within 5 postoperative days, daily food consumption was normal.

Responses to ANG II. Figure 1 and Table 1 illustrate the influence of CPD on MAP and heart rate before, during, and after ANG II infusion. Approximately 2 wk after CPD, values for both MAP and variability of MAP were similar to those observed in the presence of cardiopulmonary and aortic baroreceptor innervation. In contrast, in the absence of cardiac vagal innervation, control values for heart rate were elevated ~30 beats/min, and variability of heart rate was depressed ~50% compared with the intact state. Moreover, the chronic MAP and heart rate responses to ANG II were not influenced by CPD. After CPD, MAP increased 29 ± 2 mmHg by day 5 of ANG II vs. 32 ± 2 mmHg before denervation. Although tachycardia tended to occur in response to ANG II after CPD, as in the intact state, there were no significant changes in heart rate during ANG II hypertension.

CPD had a marked influence on the relative excretion rates of sodium and potassium from Den and Inn kidneys during ANG II infusion. After CPD, control values for the excretion of electrolytes (and creatinine) from Den and Inn kidneys were similar to the intact state (Figs. 2 and 3). CPD did not alter the net excretion of sodium and potassium from both kidneys during ANG II infusion. However, CPD had a pronounced effect on the neurally induced natriuresis and kaliuresis associated with ANG II hypertension. In contrast to

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Table 1. Effect of CPD on daily values for MAP and HR

<table>
<thead>
<tr>
<th></th>
<th>MAP, mmHg</th>
<th>SD, mmHg</th>
<th>HR, beats/min</th>
<th>SD, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>98 ± 4</td>
<td>13 ± 1</td>
<td>72 ± 9</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>CPD</td>
<td>91 ± 4</td>
<td>10 ± 1</td>
<td>101 ± 11*</td>
<td>11 ± 2*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 dogs. MAP, mean arterial pressure; HR, heart rate; SD, standard deviation; CPD, cardiopulmonary denervation. *P < 0.05 vs. intact.
the marked reductions in the Den/Inn for sodium and potassium excretion during ANG II infusion in the intact state, after CPD there were no significant changes in the Den/Inn for either sodium or potassium (or creatinine) excretion in response to ANG II. That is, although CPD had no effect on control values, it totally abolished the differential changes in sodium and potassium excretion of Den and Inn kidneys that were observed during ANG II infusion with reflexes intact. Finally, as in the intact state, a transient increase in the Den/Inn for urinary sodium and potassium excretion occurred on day 1 of the recovery period, concomitant with the marked hypotensive response associated with cessation of ANG II infusion.

CPD had no influence on basal values for PRA (control = 0.28 ± 0.07 ng ANG I·ml⁻¹·h⁻¹), hematocrit (control = 38 ± 3%), or the plasma concentrations of sodium (control = 144 ± 1 meq/l), potassium (control = 4.2 ± 0.1 meq/l), and protein (control = 7.0 ± 0.3 mg/dl). As in the intact state, PRA decreased to undetectable levels, and plasma potassium concentration decreased 0.2 ± 0.1 meq/l during ANG II infusion. There were no other significant changes in any of the above during ANG II hypertension.

Postoperative verification of CPD. In the intact state, phenylephrine increased MAP 36 ± 5 mmHg resulting in a compensatory reduction in heart rate of 36 ± 4 beats/min. Additionally, veratridine induced reductions in MAP and heart rate of 37 ± 6 mmHg and 50 ± 9 beats/min, respectively. After CPD, the reflex bradycardia in response to both drugs was virtually abolished, as was the reflex hypotension induced by veratridine. These results indicate effective elimination of cardiac vagal innervation.

Responses After CPD + SAD

Acute postoperative responses. MAP, heart rate, and the Den/Inn for sodium (and potassium) excretion all increased during the initial 18 h after CPD + SAD (Fig. 4). Immediately after denervation of both carotid sinuses, MAP increased markedly (60–80 mmHg), but over the next 9–12 h, it gradually returned to preoperative values. Tachycardia also occurred after CPD + SAD and for the 18-h postoperative period. MAP and heart rate were elevated 13 ± 3 mmHg and 22 ± 4 beats/min, respectively (Fig. 4). In addition, the Den/Inn for urinary sodium (and potassium) excretion increased substantially during the 18-h period after CPD + SAD, indicating activation of the renal sympathetic nerves. Once again, these transient elevations in MAP and in the Den/Inn for sodium excretion did not occur after surgery for CPD alone (Fig. 4).

Responses to ANG II. Table 2 and Figs. 5–7 illustrate the influence of CPD + SAD on control values and the responses to ANG II infusion. In one of five dogs, there were paroxysmal elevations in MAP (60–80 mmHg) and heart rate (80–100 beats/min) after CPD + SAD. These daily paroxysms lasted 5–10 min and occurred approximately six times/day. They persisted until one of the paroxysms ended in sudden death (presumably due to ventricular fibrillation) 3 days after CPD + SAD. Consequently, the data illustrated in Figs. 5–7 and in Table 2 represent the findings from only four of the five dogs.

The control values for MAP and heart rate for the three conditions in these four dogs are presented in Table 2. Although causing only a transient elevation in MAP lasting several hours (Fig. 4), CPD + SAD did produce a sustained increase in MAP variability (approximately twofold). In comparison, CPD + SAD had no prolonged effects on either heart rate or heart rate variability. Heart rate was elevated ~35 beats/min, and heart rate variability was reduced ~50% compared with the intact state; however, these control values after CPD + SAD were similar to those observed after CPD alone (Table 2).

CPD + SAD had little or no sustained influence on the Den/Inn for sodium and potassium excretion in the

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**Table 2. Effect of sequential baroreceptor denervation on daily values for MAP and HR**

<table>
<thead>
<tr>
<th>Condition</th>
<th>MAP (mmHg)</th>
<th>SD (mmHg)</th>
<th>HR (beats/min)</th>
<th>SD (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>95 ± 3</td>
<td>13 ± 1</td>
<td>66 ± 7</td>
<td>19 ± 1</td>
</tr>
<tr>
<td>CPD</td>
<td>88 ± 2</td>
<td>10 ± 1</td>
<td>96 ± 11</td>
<td>10 ± 2*</td>
</tr>
<tr>
<td>CPD + SAD</td>
<td>88 ± 1</td>
<td>20 ± 1*</td>
<td>101 ± 11</td>
<td>8 ± 1*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 4. SAD, sinoaortic denervation. *P < 0.05 vs. intact.
absence of ANG II infusion (Figs. 6 and 7). The marked increase in the Den/Inn for sodium and potassium excretion observed during the 18-h period after complete baroreceptor denervation (Fig. 4) returned almost to preoperative values over the next 7 days. After CPD + SAD, the Den/Inn control values for urinary sodium and potassium excretion were 1.30 ± 0.09 and 1.12 ± 0.04, respectively, vs. 1.04 ± 0.05 and 0.95 ± 0.02 in the intact state (the Den/Inn control values for urinary creatinine excretion were comparable under the 2 conditions). However, this small but statistically significant difference in the Den/Inn for sodium and potassium excretion did not persist during the recovery period after ANG II infusion.

CPD + SAD altered the heart rate but not the arterial pressure responses to ANG II (Fig. 5). Although the on-and-off transient changes in MAP in response to ANG II were more pronounced after CPD + SAD than in the intact state, the final rise in MAP during ANG II infusion was virtually the same in the two conditions. By day 5 of ANG II, MAP was elevated 33 ± 2 mmHg after CPD + SAD vs. 32 ± 2 mmHg when all reflexes were intact. On the other hand, CPD + SAD had a significant influence on heart rate during ANG II hypertension. In contrast to the intact state in which heart rate tended to fall during ANG II infusion, in the absence of cardiopulmonary and sinoaortic baroreflexes, there was a sustained tachycardia of ~15 beats/min during ANG II hypertension. With cessation of ANG II infusion, heart rate returned to control levels during the first recovery day.

CPD + SAD also had substantial effects on the Den/Inn for sodium and potassium excretion during ANG II infusion (Figs. 6 and 7). In contrast to the marked reductions in the Den/Inn for sodium and potassium excretion during ANG II infusion in the intact state, the Den/Inn for urinary sodium and potassium excretion actually increased during ANG II infusion after CPD + SAD. On day 5 of ANG II, the Den/Inn for urinary sodium and potassium excretion reached values of 2.02 ± 0.14 and 1.30 ± 0.66, respectively. These elevated values fell abruptly to control levels on termination of ANG II infusion. Most importantly, the rise in the Den/Inn for urinary sodium and potassium excretion during ANG II infusion suggests that ANG II chronically increased renal sympathetic nerve activity, a response entirely opposite to that achieved when all baroreflexes were intact. Finally, although having a major impact on the relative excretion rates of sodium and potassium from Den and Inn kidneys during ANG II hypertension, CPD + SAD did not alter the total excretion of these electrolytes from both kidneys.

Although PRA tended to be higher after CPD + SAD (control = 0.59 ± 0.28 ng ANG I·ml⁻¹·h⁻¹) compared with the intact state (control = 0.33 ± 0.15 ng ANG I·ml⁻¹·h⁻¹), there were no significant differences in PRA, hematocrit, or the plasma concentrations of

Fig. 6. Daily sodium excretion from both Den and Inn kidneys and changes in the Den/Inn for sodium excretion during chronic ANG II infusion in the intact state and after CPD + SAD. Values are means ± SE; n = 4. *P < 0.05 vs. control.

Fig. 7. Daily potassium excretion from both Den and Inn kidneys and changes in the Den/Inn for potassium excretion during chronic ANG II infusion in the intact state and after CPD + SAD. Values are means ± SE; n = 4. *P < 0.05 vs. control.
protein, sodium, or potassium between the two conditions. Furthermore, as in the intact state, PRA decreased to undetectable levels and plasma potassium concentration decreased modestly (0.3 ± 0.1 meq/l) during ANG II infusion. There were no other significant changes in any of the above during ANG II hypertension.

**DISCUSSION**

The present study elucidates the long-term interactions between ANG II and the renal nerves that result in sustained alterations in sodium excretion. Once again, our results in dogs indicate that the renal nerves promote sodium excretion, not retention, during ANG II hypertension (18). Therefore, our findings do not support the notion that the sympathetic nervous system contributes to the hypertension induced by pathophysiological levels of ANG II. On the contrary, the present results indicate that suppression of renal sympathetic nerve activity is a chronic compensatory response that may actually attenuate the antinatriuretic and hypertensive effects of ANG II. Furthermore, an important new finding in the present study is that chronic renal sympathoinhibition during ANG II hypertension is dependent on afferent input from cardiopulmonary (presumably cardiac) receptors and arterial baroreceptors. This suggests that baroreflexes do indeed have the capacity to exert long-term changes in renal sympathetic nerve activity that result in sustained alterations in sodium excretion. Another important observation in the present study is that in the absence of cardiac vagal and arterial baroreceptor input into the central nervous system, chronic renal sympathoexcitatory effects of ANG II are manifested, leading to neurally induced sodium retention.

An important unresolved issue relating to blood pressure control is whether the sympathetic nervous system contributes to the long-term regulation of arterial pressure (2, 5, 15, 38). More specifically, it is not clear whether compensatory changes in renal sympathetic nerve activity chronically alter pressure natriuresis during perturbations in body fluid volumes and arterial pressure. This uncertainty is a result of technical limitations that prevent determination of both long-term changes in renal sympathetic nerve activity and the sodium excretory responses to chronic alterations in renal sympathetic activity. Nonetheless, a number of studies in animals made hypertensive by chronic infusion of ANG II suggests that the renal nerves contribute to long-term volume and arterial pressure homeostasis. First, in the present study, and in an earlier investigation from our laboratory (18), the finding of a relative increase in sodium excretion in Inn vs. Den kidneys throughout a 5-day period of ANG II infusion indicates that renal sympathetic nerve activity is chronically suppressed during ANG II hypertension. Furthermore, we recently extended the duration of ANG II infusion from 5 to 10 days and found little change in the depressed Den/Inn for sodium excretion during this more prolonged infusion period (T. E. Lohmeier, J. R. Lohmeier, D. A. Hildebrandt, unpublished observations). Thus, at least within the time frame of our experiments, there appears to be little or no time-dependent adaptation in the differential rates of sodium excretion between Den and Inn kidneys during ANG II hypertension. These functional responses are consistent with the measurements of renal norepinephrine overflow reported by Carroll et al. (3) in chronically instrumented dogs. In the study by Carroll and associates (3), renal norepinephrine overflow, an indirect index of renal sympathetic nerve activity, was markedly suppressed after 6 days of ANG II hypertension. More recently, Cox and Bishop (8) directly recorded renal sympathetic nerve activity in conscious rabbits subjected to ANG II hypertension. They found that renal sympathetic nerve activity was depressed after 10 days of ANG II infusion. Taken together, these studies suggest that suppression of renal sympathetic nerve activity is a compensatory response that shifts pressure natriuresis to lower arterial pressure levels and counteracts the antinatriuretic and hypertensive actions of ANG II.

Other studies using the split-bladder preparation indicate that chronic suppression of renal sympathetic nerve activity is not specific for ANG II hypertension, but it may be a general compensatory response to volume excess and/or hypertension. The substantially greater rate of sodium excretion in Inn vs. Den kidneys during ANG II hypertension is also a distinctive feature of the hypertension induced by chronic infusion of norepinephrine (21). In addition, although not associated with a statistically significant elevation in arterial pressure, increased sodium intake in dogs also leads to a relatively higher rate of sodium excretion in Inn vs. Den kidneys (19). Thus the above studies are all consistent with the hypothesis that suppression of renal sympathetic nerve activity and the attendant natriuresis play a compensatory role in the chronic maintenance of arterial pressure in states of volume excess and/or hypertension.

Having established that suppression of renal sympathetic nerve activity and concomitant increases in renal excretory function are sustained responses associated with ANG II hypertension, a major goal of this study was to investigate the afferent mechanisms that lead to renal sympathoinhibition. It is widely accepted that arterial baroreflexes adapt to changes in arterial pressure and shift their set point and operating range for controlling arterial pressure in the direction of the prevailing level of arterial pressure (4, 5, 15, 38). Nonetheless, we hypothesized that baroreflexes mediate chronic suppression of renal sympathetic nerve activity in ANG II hypertension for several reasons. First, due to technical limitations, in particular the inability to record time-dependent changes in nerve activity in conscious animals, it is unclear whether baroreceptors or baroreceptor reflexes completely reset in chronic hypertension (4, 5, 15, 38). Secondly, although both arterial and cardiopulmonary (cardiac) 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 (1, 9, 30, 38). Thus it is conceivable that in states of chronic volume excess and/or hypertension, cardiopulmonary reflexes may have a particularly important long-term influence on pressure natriuresis. In this regard, much less is known about the resetting characteristics of cardiopulmonary vs. arterial baroreflexes, and recent elegant studies in conscious dogs suggest that cardiopulmonary baroreflex control of renal sympathetic nerve activity may be relatively refractory to chronic resetting (23, 24). Finally, studies by Persson et al. (31, 32) in dogs provide further support for the hypothesis that cardiopulmonary reflexes may be important in long-term control of arterial pressure. From 1-h daily recordings of arterial pressure, these investigators reported that elimination of the sympathoinhibitory effects of either sinoaortic baroreceptors or cardiopulmonary receptors failed to produce sustained increases in arterial pressure; however, combined deafferentation of both receptor regions led to chronic hypertension. Their results suggest that one set of baroreceptors can compensate for the loss of the other and that sinoaortic and cardiopulmonary afferents have nonadditive effects on long-term blood pressure control. More recent findings from this same group, however, indicate that MAP is not chronically elevated after CPD + SAD (16). In this latter study, MAP was recorded 24 h/day.

To determine whether cardiac reflexes and/or arterial baroreflexes mediate chronic suppression of renal sympathetic nerve activity during ANG II hypertension, deafferentation of cardiac receptors and arterial baroreceptors was achieved by a procedure developed by Persson et al. (31, 32). We used the method of Persson and associates for several reasons. First, this procedure eliminates cardiopulmonary vagal as well as sinoaortic baroreceptor afferents, and an objective of this study was to achieve complete, or at least nearly complete, deafferentation of both groups of baroreceptors. Second, one would expect denervation of aortic and cardiopulmonary baroreceptors by either complete or partial ligation of the vagosympathetic trunks to produce adverse effects in the chronic state (especially gastrointestinal problems). In the present study, baroreceptor denervation by section of the thoracic vagal branches, which innervate cardiac and aortic baroreceptors, did not lead to decreased food consumption or impaired health due to achalasia. However, stripping of the vagosympathetic trunk did abolish cardiopulmonary reflexes mediated by vagal afferents, as reflected by the absence of a response to intravenous injection of veratridine. Finally, by determining the functional responses after CPD alone, as well as after denervation of the carotid sinuses (CPD + SAD), we were able to gain some insight into the origin of receptors that chronically influence renal sympathetic nerve activity and sodium excretion during ANG II hypertension. As expected, elimination of afferents from both aortic and carotid baroreceptors (CPD + SAD) was associated with approximately a twofold increase in arterial pressure variability. This indicates that SAD was complete or, at the very least, nearly complete (5, 7, 31, 32).

A particularly pertinent observation relating to the potential role of baroreflexes in long-term control of arterial pressure is that CPD and CPD + SAD had little or no sustained influence on basal values for arterial pressure, heart rate, or the relative excretion rates of sodium from Inn vs. Den kidneys. Because arterial baroreflexes tonically inhibit sympathetic tone, the marked increases in arterial pressure and heart rate immediately after carotid sinus denervation (CPD + SAD) in dogs with CPD were expected. However, over the subsequent 9–12 h, MAP and heart rate returned toward control levels, as reflected by the modest hypertension and tachycardia for the daily values on postoperative day 1 (Fig. 4). Additionally, there was a large increase in the Den/Inn for sodium excretion during the 24-h period immediately after CPD + SAD (Fig. 4), suggesting substantial activation of the renal nerves. However, this response gradually waned, and within 7 postoperative days, the Den/Inn for sodium excretion returned almost to preoperative levels. Furthermore, the small increases in the Den/Inn for sodium excretion present 7 days after CPD + SAD did not persist during the subsequent recovery period ~1 wk later. The failure to chronically increase renal sympathetic nerve activity and achieve a sustained increase in the Den/Inn for sodium excretion after CPD + SAD is consistent with 24-h recordings of arterial pressure in a number of studies. These studies demonstrate that SAD and even SAD + CPD results in little or no sustained hypertension (5–7, 16, 37). It should be pointed out, however, that even if appreciable renal sympathetic activation were sustained in the present study, one would expect little hypertension because of pressure natriuresis in the Den kidney. Taken together, it appears that elimination of sinoaortic afferents, even in combination with cardiopulmonary deafferentation, does not chronically shift pressure natriuresis to higher arterial pressure levels by increasing renal sympathetic nerve activity.

Although deafferentation of cardiac receptors and arterial baroreceptors had little or no chronic influence on basal levels of sodium excretion from Den and Inn kidneys, an important finding in the present study is that CPD alone completely abolished the marked fall in the Den/Inn for sodium excretion during ANG II hypertension. One interpretation for this altered sodium excretory response to ANG II infusion is that CPD eliminated cardiac reflexes that play a critical role in chronically inhibiting renal sympathetic nerve activity during ANG II hypertension. A number of acute studies have shown that stimulation of atrial receptors by volume expansion, head-out water immersion, or a localized increase in atrial pressure inhibits renal sympathetic nerve activity (1, 9, 10, 23, 24, 30, 38). This represents an important feedback mechanism for controlling intravascular volume and related variables such as arterial pressure. Furthermore, activation of
vagal receptors (by volume expansion and ventricular hypertrophy) and chemoreceptors also inhibit sympathetic activity (25, 38). Although it is not clear whether these sympathoinhibitory reflexes are sustained chronically, ANG II could chronically stimulate atrial and/or ventricular mechanoreceptors by increasing cardiac pressures. This may occur as a result of the volume-retaining effects of ANG II and/or the effects of ANG II to increase afterload. Alternatively, because CPD eliminates aortic baroreceptor inputs as well as vagal afferents from the heart, it is conceivable that after CPD, abolition of the fall in Den/Inn for sodium excretion during ANG II hypertension is solely due to loss of the sympathoinhibitory effects of aortic baroreceptors. Finally, it is possible that cardiac reflexes and aortic baroreflexes interact to chronically suppress renal sympathetic nerve activity and that after CPD the absence of a fall in the Den/Inn for sodium excretion during ANG II hypertension is due to the elimination of inputs from both receptor populations. Clearly, more selective denervation procedures that eliminate either cardiac or aortic afferents, but not both in combination, are needed to determine the specific receptors that contribute to this response. Nevertheless, our findings suggest that cardiac reflexes and/or aortic baroreceptor reflexes play an important role in chronically suppressing renal sympathetic nerve activity in ANG II hypertension.

Another important observation in the present study is that after deafferentation of carotid baroreceptors in dogs with CPD (to achieve CPD + SAD), the Den/Inn for sodium excretion not only failed to decrease during chronic ANG II infusion, it actually increased. This finding provides further support for the contention that arterial baroreceptors, either alone or in combination with cardiac reflexes, chronically suppress renal sympathetic nerve activity in ANG II hypertension. Moreover, this finding indicates that when central input from cardiac receptors with vagal afferents and arterial baroreceptors is absent or appreciably diminished, ANG II chronically stimulates renal sympathetic nerve activity. Furthermore, the sustained tachycardia during ANG II hypertension, parallel with the increase in the Den/Inn for sodium excretion, suggests that the sustained sympathoexcitatory effects of ANG II include the heart as well as the kidneys. These observations may be particularly relevant to pathophysiological conditions such as hypertension and heart failure. In these conditions, baroreflex function has been reported to be impaired during the progression of these disease states (9, 38, 39). Consequently, the sympathoexcitatory effects of ANG II may play an increasingly important role in the pathogenesis of hypertension and heart failure as the ability of baroreflexes to inhibit sympathetic tone deteriorates.

In the absence of baroreceptor input into the central nervous system, it would be reasonable to assume that activation of renal sympathetic nerves is an immediate response to increased plasma levels of ANG II. If this is indeed the case, the explanation for the delayed increase in the Den/Inn for sodium excretion during the 5-day period of ANG II infusion (Fig. 6) is not clear. Because there was marked sodium retention in both Den and Inn kidneys during the first 24 h of ANG II infusion, this would indicate that the initial antinatriuresis induced by ANG II was largely independent of the renal nerves. In contrast, as arterial pressure increased further and total sodium excretion returned to control levels on days 3–5 of ANG II infusion, the renal nerves had a progressively greater effect to promote sodium retention. The mechanisms that amplify the antinatriuretic effects of the renal nerves during escape from the sodium-retaining effects of ANG II may have important pathophysiological significance and merit further investigation.

Because our results indicate that baroreflexes chronically suppress renal sympathetic nerve activity in ANG II hypertension, it would be reasonable to expect that the sympathetic nervous system chronically attenuates the severity of ANG II hypertension. In light of this hypothesis, a study by Cowley and DeClue (6) with a seemingly different conclusion merits discussion. These investigators chronically infused ANG II, at the same rate as employed in the present study, into intact dogs and dogs with sinoaortic baroreceptor deinnervation. They measured arterial pressure 24 h/day and reported that although the initial rise in arterial pressure was more pronounced in SAD vs. intact dogs, that on the second day of ANG II infusion and thereafter, the severity of hypertension was the same in both groups of dogs. Their results imply that in this model of hypertension, the arterial baroreceptors adapt within 2 days and therefore have no long-term influence on the final level of arterial pressure, a contention that would appear to be inconsistent with our results. However, three important issues should be raised. First, Cowley and DeClue (6) denervated only arterial baroreceptors in their study. Consequently, as discussed above, the presence of cardiac afferents in their study may have been sufficient to sustain a degree of renal sympathoinhibition comparable to that achieved in intact dogs. As a result, this may account for the fact that the final degree of hypertension was the same in arterial baroreceptor Den and intact dogs. Second, their procedure for arterial baroreceptor deinnervation included complete section of the left vagosympathetic trunk and section of the medial bundle of the right vagus. Although not discussed in their study, if gastrointestinal problems occurred as a result of extensive sectioning of the vagi, this could have influenced the degree of hypertension. This issue is particularly relevant because ANG II hypertension is sodium sensitive, and a significant amount of the daily sodium intake in their study was contained in the diet (6, 15). Thus, if dietary sodium absorption were impaired after SAD in this earlier study, this would be expected to attenuate the rise in arterial pressure in response to ANG II infusion and therefore underestimate the true influence of arterial baroreceptors on the final level of arterial pressure. Finally, one must acknowledge the possibility that the renal sympathoinhibition associ-
ated with ANG II infusion is insufficient to have a quantitatively important long-term influence on pressure natriuresis. As a result, chronic suppression of renal sympathetic nerve activity may have little or no influence on the severity of ANG II hypertension. In this regard, it will be important in future studies to determine whether CPD + SAD exacerbates the severity of ANG II hypertension in dogs with renal innervation to both kidneys.

As previously observed in dogs with split bladders and unilateral renal denervation subjected to a number of physiological and pathophysiological conditions (18, 19, 21), alterations in sodium excretion between Den and Inn kidneys during ANG II hypertension were associated with parallel changes in potassium excretion and were independent of alterations in the Den/Inn for creatinine excretion. The constant Den/Inn for creatinine excretion during ANG II infusion suggests that chronic neurally induced alterations in sodium excretion are primarily mediated by tubular mechanisms. Furthermore, if the proximal tubule is the predominant site of the renal nerves on sodium reabsorption under long-term (as well as short term) conditions, altered sodium transport in this nephron segment could readily account for parallel changes in the Den/Inn for sodium and potassium excretion. This is because potassium reabsorption is closely coupled to sodium reabsorption in the proximal tubule, and potassium secretion is strongly dependent on distal sodium delivery (12).

In summary, it is well established that the kidneys play a preeminent role in the long-term regulation of arterial pressure (5, 15, 38). Therefore, to determine whether the sympathetic nervous system might contribute to the long-term regulation of arterial pressure in ANG II hypertension, the present study focused on chronic changes in sodium excretion induced by the renal nerves. For reasons discussed above, the split-bladder preparation in combination with unilateral renal denervation provides a unique model to assess the long-term influence of changes in renal sympathetic nerve activity on sodium excretion. The results of the present study are novel because they elucidate the chronic interactions between ANG II and the renal nerves in the control of sodium excretion. These results indicate that chronic suppression of renal sympathetic nerve activity during ANG II hypertension is mediated by baroreflexes. Furthermore, they suggest that in the absence of the sympathoinhibitory effects of cardiac and arterial baroreflexes, increased plasma levels of ANG II chronically increase renal sympathetic nerve activity and promote sodium retention. Additional studies are needed, however, to determine the relative importance of cardiac reflexes vs. arterial baroreflexes in mediating chronic renal sympathoinhibition during ANG II hypertension and the quantitative significance of suppression of renal sympathetic nerve activity in ameliorating the hypertension.

Perspectives

A number of studies using the split-bladder preparation in combination with unilateral renal denervation indicates that suppression of renal sympathetic nerve activity plays a role in the chronic regulation of body fluid volumes and arterial pressure in states of volume excess and hypertension (18, 19, 21). But are these experiments lasting 5–10 days relevant to the more chronic states of hypertension in humans? Direct recordings of muscle sympathetic nerve activity by the technique of microneurography suggest that they are indeed relevant. In contrast to patients with essential hypertension and elevated sympathetic activity, patients with pheochromocytoma and primary aldosteronism have suppressed muscle sympathetic nerve activity compared with normal subjects (13, 14, 26). These clinical observations of chronic sympathoinhibition in patients with secondary hypertension are consistent with our findings suggesting that renal sympathetic nerve activity decreases during either hypertension induced by chronic infusion of norepinephrine or increased salt intake (19, 21). On the other hand, our results indicating suppression of renal sympathetic nerve activity during ANG II hypertension appear to differ with reports that muscle sympathetic nerve activity is either normal or increased in patients with renovascular hypertension and elevated plasma levels of ANG II (13, 26). One possibility to account for the apparent discrepant results is that there may be differential sympathetic responses in kidneys and muscles during ANG II hypertension. Alternately, in long-standing renovascular hypertension, the direct sympathoexcitatory actions of ANG II may become more dominant relative to the opposing influence of baroreflexes to inhibit sympathetic activity. Whether this reflects progressive abnormalities in baroreflex control of sympathetic activity or merely baroreflex adaptation is not clear at present. Clearly, additional time-dependent studies are needed to further elucidate the long-term interactions between baroreflexes and the renin-angiotensin system on sympathetic activity in hypertension and in other pathophysiological states such as heart failure.

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