

# Acute and Chronic Dose-Response Relationships for Angiotensin, Aldosterone, and Arterial Pressure at Varying Levels of Sodium Intake

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**SUMMARY** We examined the acute and chronic dose-response relationships between intravenously infused angiotensin II (A II) and the resulting changes in arterial pressure and plasma aldosterone concentration at varying levels of sodium intake. Sequential analysis of plasma aldosterone at each A II infusion rate resulted in an acute dose-related increase in plasma aldosterone which was markedly attenuated after the first 24 hours of infusion, the final level being directly related to the dose of A II and inversely related to sodium intake. A II infused at 5, 15, and 23 ng/kg per min was associated with an initial increase (2nd to 8th hour) in plasma aldosterone to 2, 6, and 9 times control values, respectively, in dogs receiving 40 mEq Na<sup>+</sup>/day. But, after the 1st day, aldosterone averaged only 1, 1.7, and 3 times control values for

the next 2 weeks at the same rates of A II infusion. Dogs receiving 120 mEq Na<sup>+</sup>/day during A II infusion exhibited only a transient increase in plasma aldosterone during the 1st day. Sustained hypertension developed over a period of a week at all doses of A II at normal and high sodium intake, but did not occur at any dose of A II in sodium-depleted dogs. Increasing sodium intake from 40 to 120 mEq/day resulted in higher levels of hypertension, 125% compared to 140% of control values for dogs infused with A II, 5.0 ng/kg per min. We conclude that primary angiotensin-induced hypertension need not be associated with increased levels of plasma aldosterone, which appears to remain elevated only with amounts of A II greater than those required to sustain a significant degree of hypertension.

THE MECHANISMS responsible for the gradual development of hypertension during chronic administration of small doses of angiotensin have not been clearly elucidated. Hypertension induced in this manner is characterized by only a slight elevation in arterial pressure during the first several hours of angiotensin infusion, followed by a gradual increase over the following week to a fixed hypertensive state.

It is commonly thought that one of the important factors responsible for the gradual elevation of pressure is stimulation of aldosterone secretion by angiotensin, leading to sodium retention and the gradual expansion of body fluid volumes. This concept evolved from the observations that the renin-angiotensin system can cause an increased secretion of aldosterone and that hypertension occurs with excess mineralocorticoid secretion, as seen in patients with primary aldosteronism. In addition, frequent reports of hyperaldosteronism secondary to renovascular hypertension have served to emphasize the role of aldosterone in hypertension.<sup>1</sup> On the other hand, there is reason to believe that the role of aldosterone secretion in primary angiotensin-induced hypertension may have been exaggerated. For example, the reported overall incidence of hyperaldosteronism associated with renovascular hypertension in man varies widely in the literature and ranges from 25% to 90%.<sup>2</sup> Laragh et al.<sup>3</sup> reported that small amounts

of infused angiotensin could cause hypertension without stimulating increased aldosterone secretion. Dickinson and Yu<sup>4</sup> were able to sustain hypertension in adrenalectomized rabbits with chronic administration of angiotensin. It was therefore one of the primary goals of the present study to evaluate the role of angiotensin and aldosterone in hypertension by determining the sequential pattern of aldosterone secretion during the development of hypertension caused by chronic infusion of small amounts of angiotensin. Refinement of techniques for chronic experimental animal monitoring and data analysis,<sup>5</sup> together with the application of a sensitive radioimmunoassay procedure<sup>6</sup> to measure plasma aldosterone concentration, permitted quantification of the transient changes so that we could assess the role of aldosterone secretion in this form of hypertension.

At the same time that the hypertension was studied, we also determined the acute and long-term dose-response relationships between rate of angiotensin infusion and rate of aldosterone secretion. Also, the influence of sodium intake on these relationships was studied because it is widely recognized that the renin-angiotensin-aldosterone system and arterial blood pressure are significantly influenced by daily levels of sodium intake. Daily sodium intake has been shown to be one of the major determinants of the rate of secretion of renin and aldosterone. It also influences both the vascular sensitivity to angiotensin in a variety of animals and man and the sensitivity of the adrenal zona glomerulosa to angiotensin.<sup>1</sup> However, interpretation of the role of sodium in angiotensin-induced hypertension in earlier studies has been complicated by the fact that sodium intake frequently was ignored or variously controlled. In the present study the influence of sodium intake on aldosterone secretion and on the degree

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of hypertension was evaluated in dogs receiving a low, normal, or high sodium intake.

The effects of three rates of chronic infusion of angiotensin (5.0, 15.0, and 23.0 ng/kg per min) were studied for periods of 2–3 weeks. The lower dose represented a rate that we found capable of producing a sustained moderate elevation of arterial pressure at a normal sodium intake of 40 mEq/day. The higher dose represented that used by previous investigators in earlier chronic infusion studies to determine the long-term effects of angiotensin on aldosterone secretion.<sup>3,7</sup>

The results indicate that both the degree of chronic hypertension and plasma aldosterone concentrations are dependent on the dose of angiotensin administered. Low doses of angiotensin (5.0 ng/kg per min) resulted in a moderate degree of hypertension but no chronic elevation of plasma aldosterone levels. Higher doses of angiotensin (15.0–23.0 ng/kg per min) resulted in increasingly severe levels of hypertension and dose-dependent sustained elevations of plasma aldosterone concentrations.

### Methods

#### PREPARATION OF DOGS

Thirty chronic angiotensin infusion experiments were performed on 30 mongrel dogs weighing  $18.6 \pm 1.2$  (SE) kg. Indwelling polyvinyl arterial and venous catheters were surgically implanted under sodium pentobarbital anesthesia (30 mg/kg, iv) several weeks prior to the angiotensin infusion. Arterial pressure was measured through a femoral artery catheter placed in the abdominal aorta at a site distal to the left renal artery. Intravenous infusions were made through another catheter placed in the inferior vena cava through the ipsilateral femoral vein. The catheters were tunneled subcutaneously to the cephalad portion of the dog's back and when not in use were kept filled with solution containing 1,000 USP units of heparin.

#### GENERAL EXPERIMENTAL PROTOCOL

Several weeks after implantation of the catheters, the dogs were placed in a large recording pen, given free access to water, and maintained on the various fixed sodium and potassium diets described below (prescription diets h/d and K/d, Riviana Foods) for a period of 5 to 7 days.

Angiotensin II (A II) (Hypertensin, CIBA) was prepared daily by diluting to a concentration of 12  $\mu$ g/ml isotonic saline, and infused at rates between 10 and 50 ml/24 hours in order to deliver the appropriate amount of angiotensin per kilogram of body weight, either 5.0, 15.0, or 23.0 ng/kg per min. In dogs maintained on a dietary Na intake of 5.0 or 40 mEq/day this resulted in an additional 1.5–7.5 mEq Na<sup>+</sup>/day, depending on the administered dose. In dogs studied at 120 mEq Na<sup>+</sup>/day, the sodium in excess of the dietary intake of 40 mEq/day was administered by continuous intravenous infusion of isotonic saline (80 mEq Na<sup>+</sup>/day) into which the angiotensin was diluted.

During the 2 days immediately preceding the start of A II infusion, control arterial blood samples were withdrawn for analysis of plasma aldosterone, renin activity, cortisol, and Na<sup>+</sup> and K<sup>+</sup> concentrations. All samples throughout

the experiment were obtained between 8:00 and 10:00 a.m., prior to daily feeding. After the start of angiotensin infusion, serial plasma samples were withdrawn at 0.5, 2, 6, 24, and 48 hours. After the first 2 days of infusion, samples were generally drawn only every other day until the end of the experiment to minimize the loss of blood volume over the 2- to 3-week experimental period.

Arterial blood pressure was generally recorded continuously, 24 hours per day, for at least 1 day preceding A II infusion and then continuously throughout the experiment. The automated techniques used in our laboratory for the continuous measurement of pressure using computerized data analysis have been previously described in detail.<sup>5</sup> The group of dogs receiving angiotensin at 15.0 ng/kg per min was used primarily to determine the aldosterone responses, and arterial pressure was monitored daily for 1 hour throughout the experiment.

#### ANALYSIS OF BLOOD SAMPLES

A total of 18 ml of blood was withdrawn for each plasma analysis. After removal of the plasma, the packed red cells were resuspended in normal saline to give a hematocrit of 50%, and reinjected.

Blood for analysis of plasma aldosterone and cortisol was collected into plastic syringes, rapidly transferred to tubes pretreated with ethylenediaminetetraacetate (EDTA), and centrifuged for 20 minutes at 4°C; the plasma was frozen until the experiment was complete. Plasma aldosterone concentration was determined in duplicate using the radioimmunoassay procedure described by Mayes et al.<sup>8</sup> and modified by McCaa et al.<sup>6</sup>

Plasma cortisol concentration was determined using the competitive protein-binding procedure described by Murphy.<sup>9</sup> Samples for plasma renin activity were collected in iced Na-EDTA Vacutainer tubes and centrifuged for 20 minutes at 4°C, and the plasma was frozen until the experiment was complete. At this time samples from each experiment were analyzed together by radioimmunoassay of angiotensin I generated in vitro according to the method of Haber et al.<sup>10</sup> Plasma sodium and potassium concentrations were measured by flame photometry (Instrumentation Laboratories, IL 343) using a 3-ml blood sample collected in a separate heparinized, disposable syringe.

#### SPECIFIC EXPERIMENTAL PROTOCOLS

##### *Angiotensin Infusion (5.0, 15.0, and 23.0 ng/kg per min); Normal Sodium Intake (40 mEq/day)*

Eight dogs received angiotensin for 2–3 weeks at a rate of 5.0 ng/kg per min, six dogs were infused at a rate of 15.0 ng/kg per min, and three dogs received angiotensin at a rate of 23.0 ng/kg per min. Fourteen of these dogs received 40 mEq Na<sup>+</sup>/day for 7 days before infusion, throughout the period of angiotensin infusion, and during the 2-day postinfusion period. The dogs receiving 23.0 ng/kg per min were anesthetized during the 15th day of infusion to determine various other unreported hemodynamic variables; and postinfusion data therefore are not meaningful.

Angiotensin was prepared daily by diluting to a concentration of 12  $\mu$ g/ml isotonic saline and then infused with a

Harvard infusion pump (model 944) at rates that yielded 4.0–6.0 ng of A II/kg per min. At these infusion rates, an additional 1.5–7.5 mEq of sodium per day was added with less than 50 ml of water.

*Angiotensin Infusion (5.0 and 23.0 ng/kg per min); High Sodium Intake (120 mEq/day)*

Eleven additional dogs received angiotensin at a rate of 5.0 ng/kg per min, administered with an infusion of isotonic saline such that the total sodium intake was 120 mEq/day. Two additional dogs received a high dose of angiotensin, 23.0 ng/kg per min, for 2–3 weeks while receiving 120 mEq Na<sup>+</sup>/day. The dogs were maintained on a sodium intake of 40 mEq/day for 7 days prior to angiotensin infusion. Angiotensin was diluted in isotonic saline and administered with a Sigmamotor pump (model TM 20) at rates selected to deliver the desired sodium intake and dose of angiotensin.

*Angiotensin Infusion (5.0 and 23.0 ng/kg per min); Sodium-Depleted Dogs (5.0 mEq Na<sup>+</sup>/day)*

Six dogs were maintained on a sodium intake of 5.0 mEq/day prior to angiotensin infusion (prescription diet h/d, Riviana Foods). Four of these dogs were further depleted of sodium with furosemide (15 mg/day), which was administered every other day throughout the angiotensin infusion period; in two dogs administration of furosemide was stopped 24 hours before the start of infusion. Angiotensin was infused at 5.0 ng/kg per min for 11 days and then increased to 23.0 ng/kg per min for the next 11 days.

Unless otherwise stated, all values in this paper are expressed as the mean  $\pm$  SEM. An analysis of paired variance was made between the average control period and each succeeding day of angiotensin infusion. Statistical significance was accepted for a *P* value less than 0.05.

## Results

### COMPARISON OF EFFECTS OF ANGIOTENSIN (5.0 ng/kg per min) AT NORMAL AND HIGH SODIUM INTAKE

The results of chronic angiotensin infusion at 5.0 ng/kg per min in eight dogs receiving 40 mEq Na<sup>+</sup>/day and 11 dogs receiving 120 mEq Na<sup>+</sup>/day are summarized in Figures 1 and 2, respectively.

#### Arterial Pressure

Comparison of the upper bar graphs in Figures 1 and 2 shows that both groups of dogs gradually developed hypertension during the 1st week of infusion. Although the rate of onset of hypertension was similar in the two groups, the degree of hypertension exhibited by the dogs receiving a high sodium intake (Fig. 2) was significantly greater (*P* < 0.05) than that developed by dogs maintained on a normal sodium intake. Dogs receiving 40 mEq Na<sup>+</sup>/day stabilized at arterial pressures nearly 125% of control values by the 7th day of infusion. In contrast, nearly twice this rise in pressure was exhibited by dogs receiving 120 mEq Na<sup>+</sup>/day, in which pressure reached nearly 140% of control values by the 7th day of infusion. Control mean arterial pressure with continuous collections (24 hours/day) averaged  $107 \pm 3$  mm Hg for the group

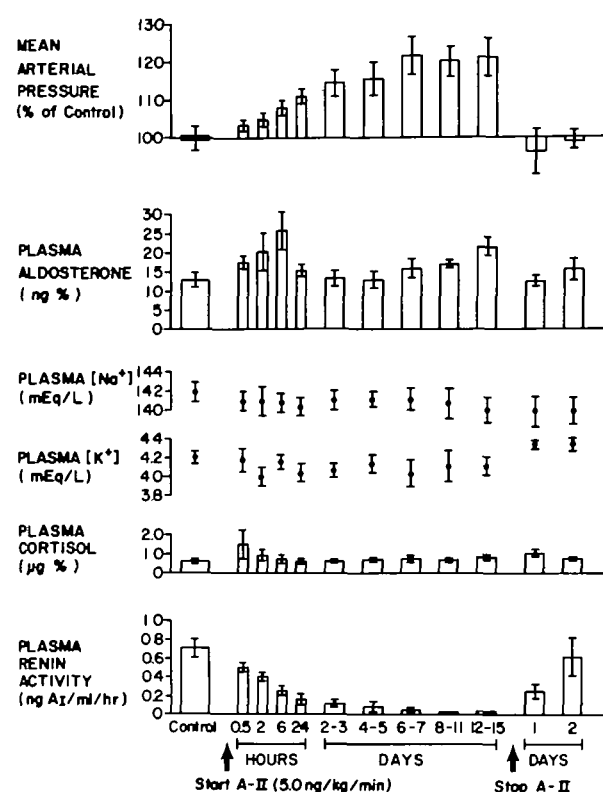


FIGURE 1 The average sequential changes resulting from continuous intravenous infusion of angiotensin II (5.0 ng/kg per min) to eight normal dogs receiving 40 mEq Na<sup>+</sup>/day. Mean and SEM are plotted. See text for statistical data.

receiving 40 mEq Na<sup>+</sup>/day and  $101 \pm 4$  mm Hg for the groups receiving 120 mEq Na<sup>+</sup>/day. The average arterial pressure values shown in these bar graphs represent the averages of the mean 24-hour pressure values for each dog, attained by digitizing the mean pressure to about 1,500 points/hour.

After the end of infusion, the average 24-hour mean arterial pressure fell below control levels in dogs receiving 40 mEq Na<sup>+</sup>/day (Fig. 1), but remained mildly elevated during the same period in dogs receiving the high sodium intake (Fig. 2).

#### Aldosterone

Plasma aldosterone concentration increased within 10–30 minutes after the start of angiotensin infusion in dogs receiving either the normal (Fig. 1) or high (Fig. 2) sodium intake. Control aldosterone values averaged  $12.8 \pm 2.0$  ng/100 ml and  $8.1 \pm 2.1$  ng/100 ml, respectively, in each group. Between the 2nd and 8th hours after the start of infusion, plasma aldosterone concentrations reached maximum levels of nearly 2 times the control values in both groups of dogs ( $25.9 \pm 5.0$  and  $15.5 \pm 2.4$  ng/100 ml, respectively), *P* < 0.05. These elevations of plasma aldosterone were not sustained, however, and by the 24th hour after the start of infusion, plasma concentrations generally had returned to normal in dogs with both normal and high sodium intake. Mean arterial pressure was continuing to rise in both groups at this time.

Between the 2nd and 7th days of infusion, plasma aldosterone concentration was not statistically different from control values in either normal or high sodium groups. However, in seven of the eight dogs receiving a sodium intake of 40 mEq/day, aldosterone concentration rose gradually during the 2nd week of infusion. It was 30% above control values between the 8th and 11th days (Fig. 1) (not statistically significant) and 60% above control values ( $P < 0.05$ ) between the 12th and 15th days of infusion. A similar trend was observed in six of 10 dogs receiving the high sodium intake (Fig. 2), but aldosterone concentrations averaged only 25% above control values between the 8th and 15th days of infusion, not statistically different from control values using analysis of paired variances.

Twenty-four hours after the end of infusion, plasma aldosterone was not significantly different from control in either group of dogs.

#### Plasma Sodium and Potassium

No significant change in plasma sodium concentration was measured in dogs with either normal (Fig. 1) or high (Fig. 2) sodium intake. In the group maintained on 40 mEq Na<sup>+</sup>/day during the infusion plasma sodium averaged  $141.9 \pm 1.2$  mEq/liter before infusion compared to  $140.1 \pm 1.3$  mEq/liter on the final day of infusion. In the group receiving 120 mEq Na<sup>+</sup>/day during the angiotensin infu-

sion plasma sodium averaged  $141.7 \pm 12.0$  mEq/liter during the control period and  $141.7 \pm 1.6$  mEq/liter on the final day of infusion.

Plasma potassium concentration was unchanged in dogs receiving a normal sodium intake (Fig. 1), averaging  $4.20 \pm 0.07$  mEq/liter before infusion and  $4.14 \pm 0.1$  mEq/liter on the final day of infusion. In this group of dogs, however, on the 2 days following the end of infusion, plasma potassium concentration rose to  $4.35 \pm 0.05$  mEq/liter ( $P < 0.05$ ).

In contrast, dogs receiving a high sodium intake, 120 mEq Na<sup>+</sup>/day (Fig. 2), exhibited a marked fall in plasma potassium concentration during the 8th to 15th days of angiotensin infusion ( $P < 0.05$ ). Plasma concentration fell from an average control value of  $4.00 \pm 0.09$  mEq/liter to  $3.56 \pm 0.11$  mEq/liter by the final day of infusion. Twenty-four hours after the end of infusion, concentrations had returned to control levels and averaged  $4.05 \pm 0.14$  mEq/liter.

#### Plasma Cortisol

Plasma cortisol concentrations were not statistically different from control values except for a transient rise consistently seen during the 1st hour of angiotensin infusion. During this period plasma concentrations rose from  $0.6 \pm 0.1$   $\mu$ g/100 ml to  $1.5 \pm 0.9$   $\mu$ g/100 ml in dogs with normal sodium intake (Fig. 1), and from  $1.0 \pm 0.2$   $\mu$ g/100 ml to  $1.5 \pm 0.3$   $\mu$ g/100 ml in dogs with high sodium intake (Fig. 2).

#### Plasma Renin Activity

Infusion of angiotensin was associated with a progressive fall of plasma renin activity which was evident as early as 30 minutes after the start of infusion. By the 6th to 8th hours of infusion, arterial plasma levels had fallen to nearly 30% of control values, which initially averaged  $0.7 \pm 0.1$  ng A I/ml per hour and  $0.9 \pm 0.2$  ng A I/ml per hour, respectively, in normal and high sodium groups. After 24 hours of infusion, plasma renin activity was undetectable by radioimmunoassay and remained at these levels throughout the period of infusion.

Plasma renin activity was still significantly depressed ( $P < 0.05$ ) 24 hours after ending the infusion in both groups of dogs, averaging only 30% of control values. In dogs maintained on a normal sodium intake (Fig. 1) plasma renin returned to normal by the end of the 2nd day following infusion. In the group receiving 120 mEq Na<sup>+</sup>/day (Fig. 2) plasma renin averaged only 45% of control values at this time, although two dogs exhibited an overshoot above control levels at this period.

#### EFFECTS OF ANGIOTENSIN (15.0 ng/kg per min) AT NORMAL SODIUM INTAKE

Chronic infusions of A II (15.0 ng/kg per min) into dogs receiving 40 mEq Na<sup>+</sup>/day throughout the experiment resulted in an acute rise in plasma aldosterone levels from a control value (average of 3 days) of  $7.9 \pm 0.2$  ng/100 ml to  $47.5 \pm 1.8$  ng/100 ml during the first several hours of infusion ( $P < 0.05$ ). By the 6th hour of infusion, plasma aldosterone averaged  $11.6 \pm 1.1$  (both values significantly elevated above control ( $P < 0.05$ )). Thereafter, the aver-

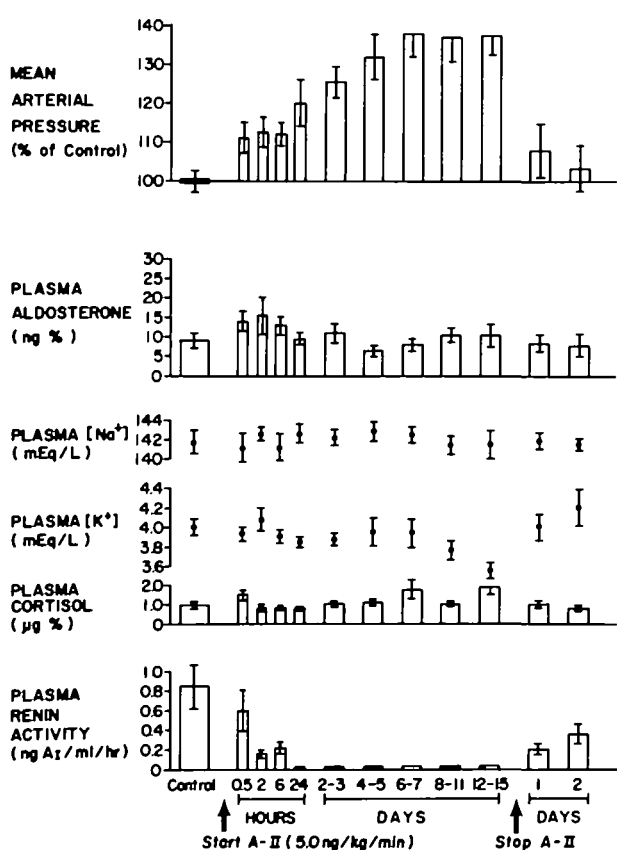


FIGURE 2 The average sequential changes resulting from continuous intravenous infusion of angiotensin II (5.0 ng/kg per min) to 11 normal dogs receiving 120 mEq Na<sup>+</sup>/day during the period of infusion. Mean and SEM are plotted. See text for statistical data.

age plasma aldosterone concentrations ranged between  $11.6 \pm 1.1$  to  $14.8 \pm 0.7$  ng/100 ml over the next 2 weeks of infusion, with all values significantly elevated ( $P < 0.05$ ) except on the 9th day, as determined by an analysis of paired variance. The average aldosterone concentration from the 2nd through the 14th day of infusion was  $12.6 \pm 0.9$  ng/100 ml, a 60% increase above the average control value.

Arterial pressure was measured daily for 30 minutes in this group of dogs. By the end of the 1st week of infusion a sustained elevation of pressure was observed which averaged 135–140% of average control values of  $102 \pm 5$  mm Hg.

Plasma sodium and potassium averaged  $138.3 \pm 0.3$  mEq/liter and  $3.7 \pm 0.1$  mEq/liter during the control period and were not consistently altered through the experimental period.

#### COMPARISON OF EFFECTS OF ANGIOTENSIN (23.0 ng/kg per min) AT NORMAL AND HIGH SODIUM INTAKE

Figures 3 and 4 summarize the results of chronic infusions of the highest rate of angiotensin used (23 ng/kg per min), with three dogs receiving 40 mEq  $\text{Na}^+$ /day and two dogs receiving 120 mEq  $\text{Na}^+$ /day throughout the infusion period.

##### Arterial Pressure

Mean arterial pressure immediately increased after starting the infusion and averaged nearly 130% of the control pressure within 30 minutes after the start of infusion in both normal (Fig. 3) and high sodium (Fig. 4) dogs. By the 24th hour of infusion, arterial pressure had plateaued in both groups at a level nearly 145% of control and remained at these pressures throughout the 2-week infusion period. The average 24-hour mean control arterial pressures averaged  $109 \pm 5$  mm Hg in the former and  $108 \pm 5$  mm Hg in the latter group, respectively.

##### Aldosterone

Plasma aldosterone concentration exhibited nearly a 9-fold increase by the 2nd hour of infusion in dogs receiving a normal sodium intake (Fig. 3) and a 5-fold increase in dogs receiving the high sodium intake (Fig. 4). By the end of the 1st day of infusion, plasma aldosterone tended to return toward control levels in both groups of dogs. However, the dogs maintained on a normal sodium intake exhibited sustained elevations of plasma aldosterone, nearly 3 times control values, throughout the entire 2-week infusion period (Fig. 3). In contrast, plasma aldosterone returned to control levels after the 1st day of infusion in those dogs receiving 120 mEq  $\text{Na}^+$ /day (Fig. 4) and remained within normal limits throughout the 2-week infusion.

##### Plasma Sodium and Potassium

Plasma sodium concentrations were not consistently altered in either group of dogs throughout the infusion period. Plasma potassium concentrations, however, were consistently decreased by 0.2–0.3 mEq/liter in both groups by the 2nd to 3rd day of infusion, and by the 12th to 15th day of infusion had dropped by 0.5–0.7 mEq/liter.

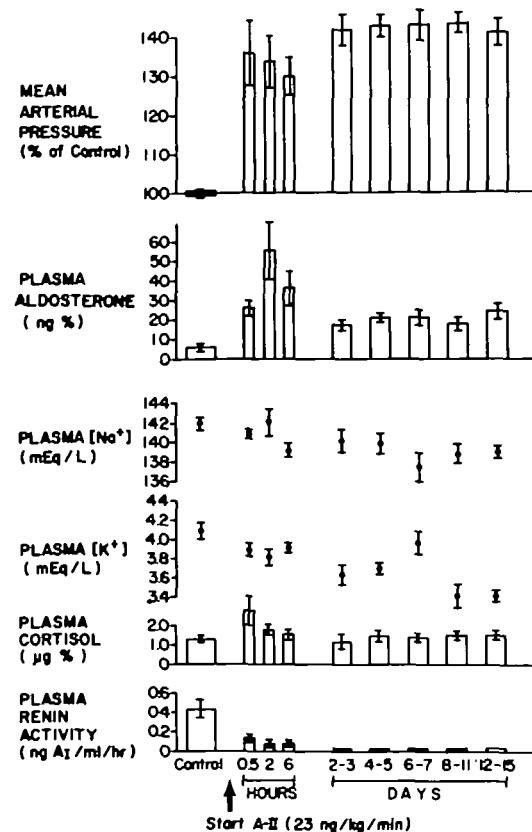


FIGURE 3 The average sequential changes resulting from continuous intravenous infusion of angiotensin II (23.0 ng/kg per min) to three dogs receiving 40 mEq  $\text{Na}^+$ /day.

##### Plasma Cortisol

Concentrations of plasma cortisol showed no consistent changes throughout the experiment, with the exception of transient elevations during the 1st hour of infusion in the dogs on normal sodium intake (Fig. 3).

##### Plasma Renin Activity

As was seen with the lower rate of angiotensin infusion, plasma renin activity was consistently suppressed and fell to less than 30% of control values by the 6th hour of infusion in both groups of dogs. After the 1st day it was not detectable.

#### COMPARISON OF EFFECTS OF ANGIOTENSIN (5.0 and 23.0 ng/kg per min) DURING SODIUM DEPLETION

Angiotensin was administered to six sodium-depleted dogs for a period of 11 days at a dose of 5.0 ng/kg per min. On the 12th day the dose was increased to 23.0 ng/kg per min and maintained at this rate for another 9 days. All dogs were maintained on 5.0 mEq  $\text{Na}^+$ /day for 1 week before infusion. Four of the dogs received furosemide (7.5 mg) twice a day on the last 4 days prior to infusion and every other day throughout the infusion period (Fig. 5, open bars), while two dogs were given furosemide only during the 2 days prior to angiotensin infusion (Fig. 5, solid bars). Hormone and electrolyte values reported below were determined on plasma samples obtained 24 hours following administration of furosemide.

### Arterial Pressure

Mean arterial pressure, which averaged  $104 \pm 4$  mm Hg during the control period, was not changed by either rate of angiotensin infusion in any of the dogs.

### Aldosterone

In the severely sodium-depleted dogs (Fig. 5, open bars), plasma aldosterone concentration averaged  $109.4 \pm 17.8$  ng/100 ml during the control period compared to 40.4 ng/100 ml in the less severely depleted dogs (solid bars). Plasma aldosterone levels were not changed by infusion of angiotensin at either 5 or 23 ng/kg per min in either group of dogs.

### Plasma Sodium and Potassium

Plasma sodium concentration averaged  $136.2 \pm 1.3$  mEq/liter in the four severely depleted dogs and decreased another by 2–3 mEq/liter during the 20-day infusion period (Fig. 5, closed circles). In the mildly depleted dogs sodium averaged 141.2 mEq/liter in the control period and remained nearly unchanged throughout the period of infusion. Plasma potassium concentrations were not consistently changed from control levels in any of the dogs.

### Plasma Cortisol

The only change observed in plasma cortisol concentration throughout the experiment was a transient rise during

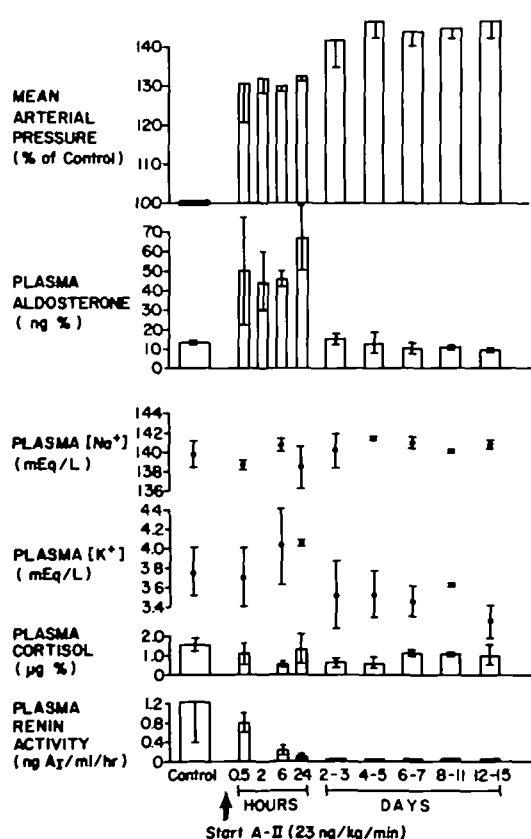


FIGURE 4 The average sequential changes resulting from continuous intravenous infusion of angiotensin II (23.0 ng/kg per min) to two dogs receiving 120 mEq Na<sup>+</sup>/day during the period of infusion.

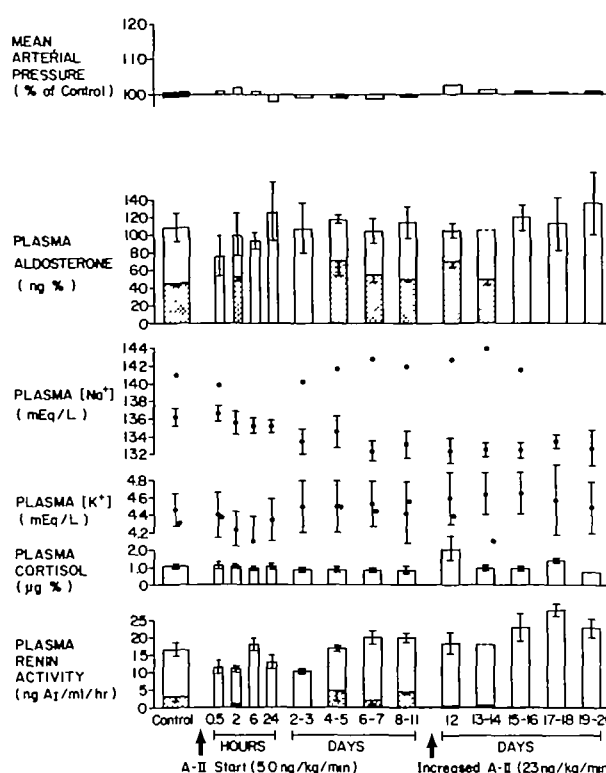


FIGURE 5 The average sequential changes resulting from continuous intravenous angiotensin infusion of 5.0 ng/kg per min for 11 days, followed by 23.0 ng/kg per min for 9 days, to six sodium-depleted dogs. All dogs were maintained on 5 mEq Na<sup>+</sup>/day for 1 week prior to the infusion, and throughout the infusion. Four dogs received furosemide (15 mg) every other day throughout the infusion (open circles and bars); two dogs received furosemide only during the 2 days prior to angiotensin infusion (solid circles and bars).

the 12th day of infusion after the dose was increased to 23 ng/kg per min.

### Plasma Renin Activity

In contrast to dogs receiving normal or high sodium intakes, plasma renin activity remained essentially unchanged throughout the angiotensin infusion in severely sodium-depleted dogs (Fig. 5, open bars). The mildly sodium-depleted dogs (solid bars) did exhibit a transient decrease in plasma renin activity at the low dose of angiotensin and a sustained decrease from a control value of 5.0 to a value of 0.7 ng A<sub>I</sub>/ml per hour during the high dose period of angiotensin infusion.

### ACUTE AND CHRONIC ANGIOTENSIN-ALDOSTERONE DOSE-RESPONSE RELATIONSHIPS

The graph seen in Figure 6 contrasts the changes in plasma aldosterone concentrations observed during the first 8 hours of angiotensin infusion to the average values obtained over the following 2-week period of constant infusion. These data were obtained for the dogs described in Figures 1–5 and receiving 40 mEq Na<sup>+</sup>/day. A dose-response relationship between the three rates of angiotensin infusion and the changes in plasma aldosterone concentration is apparent during the acute and chronic phase.

However, at each infusion rate the short-term rise in plasma aldosterone concentration (upper line) is nearly three times the response obtained during the chronic phase (lower line). Thus, angiotensin exerts its most potent effects on aldosterone secretion during the first 8 hours of infusion. Thereafter the response is attenuated to about  $1/3$  the initial short-term response.

#### INFLUENCE OF SODIUM INTAKE ON THE DEGREE OF HYPERTENSION

Figure 7 illustrates more precisely the strong influence of sodium intake on the degree of hypertension attained during the administration of two doses of angiotensin. Each point represents an average 2-hour mean arterial pressure and was obtained from the computerized analysis of the continuously recorded arterial pressure. The averages of these changes were summarized in Figures 1-5. The upper graph shows the average change of mean arterial pressure (percent of control) obtained over a period of 10 days during infusion of angiotensin, 5.0 ng/kg per min, to dogs receiving either 5.0, 40.0, or 120 mEq of sodium per day. A clear separation of the hypertensive states caused by prolonged angiotensin infusions at the three sodium states is illustrated. Sodium-depleted dogs showed no significant change in arterial pressure throughout the infusion period. Dogs receiving 40 mEq  $\text{Na}^+$ /day attained a pressure level of  $120 \pm 9\%$  of control values. Those receiving 120 mEq  $\text{Na}^+$ /day reached a steady state pressure level of  $137 \pm 10\%$  of control values. Although sodium intake significantly influenced the degree of hyper-

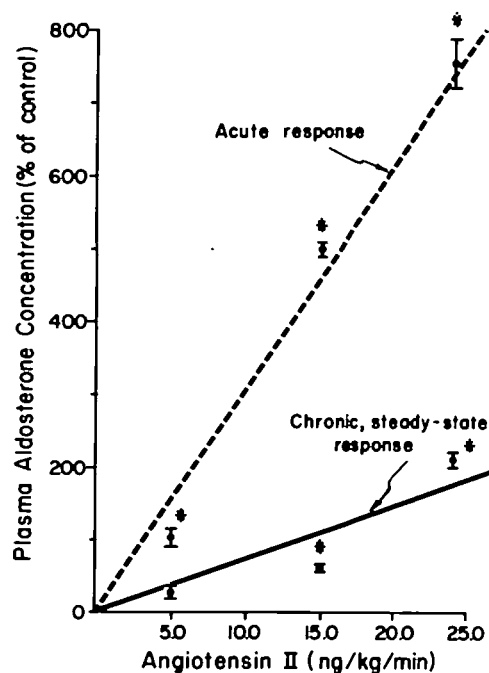


FIGURE 6 Graph of the short-term (lower points) and chronic steady state (upper points) dose-response relationship between angiotensin infusion rates and changes (percent) in plasma aldosterone concentration. Data represent dogs described in Figures 1-5 receiving 40 mEq  $\text{Na}^+$ /day. Asterisks indicate the change was statistically different from the average control values ( $P < 0.05$ ) using an analysis of paired variance.

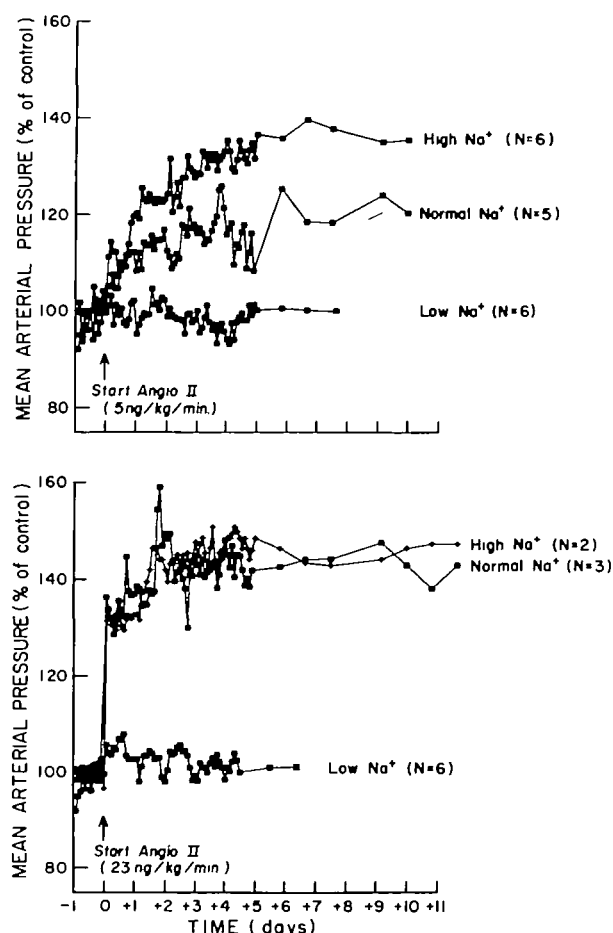


FIGURE 7 The influence of sodium intake on the degree of hypertension at two rates of angiotensin infusion. The continuously recorded mean pressures (upper graph) were obtained from dogs receiving angiotensin (5.0 ng/kg per min) while the dogs received either 5.0, 40, or 120 mEq  $\text{Na}^+$ /day. Average pressures plotted on the lower graph were obtained from dogs receiving 23.0 ng/kg per min at the same three levels of sodium intake.

tension, it appeared to have little influence on the rate of development of hypertension.

The lower graph summarizes the changes in mean arterial pressure obtained in dogs infused with angiotensin, 23 ng/kg per min, at the same three sodium states. At the higher dose of angiotensin the distinction between normal and high sodium states was nearly lost. The degree of hypertension obtained averaged  $148 \pm 9\%$  and  $146 \pm 10\%$  of control values in dogs receiving 40 and 120 mEq  $\text{Na}^+$ /day, respectively, although sodium depletion was capable of preventing a rise in pressure even at this dose of angiotensin.

#### Discussion

The chronic administration of relatively small doses of angiotensin has been shown to cause a sustained elevation of arterial pressure in a variety of animals and in man.<sup>3, 11-13</sup> It is also generally believed that angiotensin can bring about a sustained elevation of aldosterone secretion. And it is well known that the hypertension associated with unilateral renovascular stenosis or parenchymal renal

disease is frequently associated with high levels of renin secretion and secondary increases in aldosterone secretion.<sup>1</sup> Since primary aldosteronism is generally associated with a mild to moderate state of hypertension, it has been suspected that aldosterone secretion contributes significantly to the hypertension associated with the prolonged infusion of angiotensin. The present study helps to clarify the acute and long-term relationships between angiotensin and aldosterone in hypertension.

The results of the present study indicate that moderate hypertension can be sustained at infusion rates of angiotensin (5.0 ng/kg per min) which do not result in sustained elevations of plasma aldosterone. The chronic stimulatory actions of angiotensin on aldosterone secretion occur only at doses of the peptide (15.0 ng/kg per min or higher) which are greater than those required to induce a moderate sustained hypertension. These results extend the findings of McCaa et al.<sup>14</sup> in an independently conducted study and help to explain the apparent discrepancies between those results and findings previously reported in the literature. Examination of earlier studies<sup>3,7</sup> in which chronic infusion of angiotensin caused sustained elevations of aldosterone secretion show that angiotensin was infused at rates ranging from 20 to 60 ng/kg per min, 4–12 times greater than the low dose used in the present study. Sustained chronic elevations of plasma aldosterone at such doses would be predicted from the present study, as seen in Figure 6, in animals maintained on a normal sodium intake.

The present data suggest that aldosterone secretion may influence the degree of hypertension only in the more severe hypertensive states associated with very high levels of circulating angiotensin. At moderate levels of hypertension plasma aldosterone concentrations were dissociated from the arterial pressure responses in several ways. First, they generally reached a maximum level between the 6th and 12th hour of angiotensin infusion and then started to decline. However, arterial pressure by this time was elevated only about 10 mm Hg above control values and did not reach maximum steady state levels for at least 5 days. Second, there was an inverse relationship between plasma aldosterone concentrations and the degree of hypertension obtained. Dogs in which the highest levels of arterial pressure were obtained (high sodium intake dogs) exhibited the least change in plasma aldosterone levels. In these circumstances the aldosterone secretion was changed in a direction which would contribute to normalization of arterial pressure rather than enhancement of the hypertension. Although normal levels of plasma aldosterone observed during conditions of high sodium intake could be considered inappropriately high, it should be noted that, except for a transient increase during the first 24 hours, plasma aldosterone levels were nearly unchanged in those dogs maintained on a normal Na<sup>+</sup> intake throughout the 1st week of angiotensin infusion at the rate of 5 ng/kg per min. This supports the view that the rise in pressure was influenced very little by the aldosterone mechanism.

Work by other investigators also suggests that aldosterone participation in the hypertensive process is minimal. For example, experimental hypertension cannot be produced readily in dogs by chronic administration of miner-

alocorticoids unless accompanied by large increases in sodium intake. Young and Guyton<sup>15</sup> have recently reported that a 2.5-fold increase in long-term aldosterone infusion rates (from 20 to 100  $\mu$ g/day) in adrenalectomized dogs receiving 30 mEq Na<sup>+</sup>/day increased the steady state level of arterial pressure about 10 mm Hg. It appears then that in both man and dog hypertension cannot be readily produced at the levels of plasma aldosterone obtained during the chronic state of the present study.

#### FACTORS INFLUENCING ALDOSTERONE SECRETION

Although the present study was not designed to quantify the relative importance of the various factors known to control the release of aldosterone, nevertheless it is useful to discuss the data in terms of factors which may have influenced the secretion of aldosterone under the various conditions of the study. The renin-angiotensin system generally is considered to be one of the primary mechanisms controlling aldosterone secretion,<sup>16–19</sup> the other major factors being the plasma concentrations of sodium<sup>20–22</sup> and potassium,<sup>14, 23–25</sup> and pituitary secretion of adrenocorticotrophic hormone.<sup>26</sup> Our present results are of particular interest because amounts of angiotensin (5.0 ng/kg per min) which were sufficient to produce a significant chronic elevation of arterial pressure did not result in chronically elevated levels of circulating aldosterone.

Aldosterone secretion thus appears to be less responsive to angiotensin than are some of the other mechanisms involved in the long-term control of arterial pressure. However, one cannot conclude from the present results that angiotensin fails to chronically stimulate the secretion of aldosterone. First, the higher levels of angiotensin infused, which were in excess of the minimal doses required to produce moderate hypertension, resulted in mild sustained elevations of plasma aldosterone levels. Second, other potent factors involved in the long-term control of aldosterone secretion could override the stimulatory actions of angiotensin and mask the response. For example, if sodium retention occurred at this low rate of angiotensin infusion, as has been suggested in recent studies by DeClue et al.,<sup>27</sup> then a decreased sensitivity of the adrenal cortex to the steroidogenic effects of angiotensin could account for the observed changes in aldosterone. Such alterations in adrenal cortex sensitivity after sodium repletion recently have been reported by several investigators.<sup>28–29</sup> Gradual changes in potassium balance also could have influenced aldosterone secretion or prevented angiotensin stimulation of aldosterone synthesis.<sup>23–25</sup>

Although plasma sodium concentration was unchanged throughout the infusions, plasma potassium levels generally exhibited a progressive fall in those dogs receiving the high sodium intake, especially during the 2nd week of angiotensin infusion. This hypokalemic tendency associated with the chronic infusion of angiotensin has generally been thought to be the result of increased circulating levels of aldosterone leading to sodium retention and potassium loss in the distal tubules of the kidney. However, since the plasma aldosterone levels did not change in the present study, it is possible that a high sodium load to the distal tubule was responsible for increased distal tubular exchange of potassium resulting in the observed changes in



plasma potassium levels seen at the end of the 2-week A II infusion period.

The commonly observed rise in aldosterone secretory rate associated with elevation in plasma renin activity during chronic states of negative sodium balance, and the ability to block this response with a competitive inhibitor of A II (saralasin acetate, P-113)<sup>30</sup> has lent strong support to the belief that aldosterone secretion is mediated by the renin-angiotensin system in these circumstances. It has also become clear, however, that there are other mechanisms capable of controlling the secretion of aldosterone. Nephrectomized man and animals<sup>31-33</sup> have been observed to have a normal basal level of secretion of aldosterone which increases in response to sodium depletion. Aldosterone secretion has also been reported to be separated from the behavior of the renin-angiotensin system.<sup>34</sup> The relative importance of these various control systems remains to be quantified.

The data from the present study cannot resolve the question of whether angiotensin can chronically stimulate the release of aldosterone, because the changes observed in plasma aldosterone are compatible with other interpretations, especially if the chronic actions of plasma electrolytes are carefully considered.

#### EFFECTS OF SODIUM INTAKE ON DEGREE OF HYPERTENSION

A number of experiments have indicated that the pressor sensitivity to brief infusions or injections of A II is influenced by the state of sodium balance.<sup>1</sup> The present studies extend this observation and demonstrate that sodium intake (and presumably sodium balance) greatly influence the degree of hypertension associated with a given plasma level of angiotensin over prolonged periods. In going from a sodium-depleted state to sodium intakes nearly 3 times normal, the arterial pressure response to angiotensin infusion varied from no change to nearly 150% of control values.

It is interesting to speculate on the reasons for the differences in pressor sensitivity during acute and chronic infusions of angiotensin. The decreased pressor sensitivity to acute infusions of angiotensin in the sodium-depleted state is perhaps most plausibly explained by a recent study by Thurston and Laragh,<sup>35</sup> which indicates that the phenomenon is a result of prior occupancy of receptor sites by endogenous hormone at various salt states. In contrast, the gradual rise in pressure over a period of 7 days which is increasingly exaggerated at higher levels of sodium intake suggests a different mechanism. These chronic results suggest that a high sodium intake, together with elevated levels of A II, may lead to a greater overall retention of salt and water which in turn enhances the hypertension through volume expansion. Regardless of the mechanism, it appears that sodium and water depletion can effectively prevent both the acute and chronic pressor effects of angiotensin and that excess sodium and water exaggerate the acute and chronic hypertensive effects of angiotensin.

#### References

1. Laragh JH, Sealey JE: The renin-angiotensin-aldosterone hormonal system and regulation of sodium, potassium, and blood pressure ho-

- meostasis. In *Handbook of Physiology*, Section 8, Renal Physiology, edited by J Orloff, RW Berliner. Washington, D.C., American Physiological Society, 1973, pp 864-872
2. Muller J: Regulation of aldosterone biosynthesis. New York, Springer-Verlag, 1971, pp 55-67
3. Laragh JH, Sealey JE, Sommers SC: Patterns of adrenal secretion and urinary excretion of aldosterone and plasma renin activity in normal and hypertensive subjects. *Circ Res* 18/19 (suppl 1): 158-174, 1966
4. Dickinson CJ, Yu R: Mechanism involved in the progressive pressor response to very small quantities of angiotensin. *Circ Res* 21 (suppl II): 157-165, 1967
5. Cowley AW Jr, Liard JF, Guyton AC: Role of the baroreceptor reflex in daily control of arterial blood pressure and other variables in dogs. *Circ Res* 32: 564-576, 1973
6. McCaa RE, McCaa CS, Read DG, Bower JD, Guyton AC: Increased plasma aldosterone concentration in response to hemodialysis in nephrectomized man. *Circ Res* 31: 473-480, 1972
7. Urquhart J, Davis JO, Higgins JT Jr: Effects of prolonged infusion of angiotensin II in normal dogs. *Am J Physiol* 205: 1241-1246, 1963
8. Mayes D, Furuyama S, Kem DC, Nugent CA: A radioimmunoassay for plasma aldosterone. *J Clin Endocrinol Metab* 30: 682-685, 1970
9. Murphy BEP: Some studies of protein binding of steroids and their application to the routine micro and ultra-micro measurement of various steroids in body fluids by competitive protein-binding radioassay. *J Clin Endocrinol Metab* 27: 973-990, 1967
10. Haber E, Hoerner T, Page LB, Kliman B, Purnode A: Application of a radioimmunoassay for angiotensin I to the physiologic measurement of plasma renin activity in normal human subjects. *J Clin Endocrinol Metab* 29: 1349-1355, 1969
11. McCubbin JW, DeMoura RJ, Page IH, Olmsted F: Arterial hypertension elicited by suppressor amounts of angiotensin. *Science* 149: 1394-1395, 1965
12. Dickinson CJ, Lawrence JR: A slowly developing pressor response to small concentrations of angiotensin; its bearing on pathogenesis of chronic renal hypertension. *Lancet* 1: 1354-1356, 1963
13. Koletsky S, Riviera-Velez JM, Pritchard NH: Production of hypertension and vascular disease by angiotensin. *Arch Pathol* 82: 99-106, 1966
14. McCaa RE, McCaa CS, Guyton AC: Role of angiotensin II and potassium in the long-term regulation of aldosterone secretion in intact conscious dogs. *Circ Res* 36 (suppl 1): 57-67, 1975
15. Young DB, Guyton AC: Steady-state responses to changes in aldosterone levels. *Physiologist* 18: 459, 1975
16. Laragh JH, Angers M, Kelly WG, Lieberman S: Hypotensive agents and pressor substances; the effect of epinephrine, norepinephrine, angiotensin II, and others on the secretory rate of aldosterone in man. *JAMA* 174: 234-240, 1960
17. Biron P, Koiw E, Nowaczynski W, Brouillet J, Genest J: Effects of intravenous infusion of valine-5 angiotensin II and other pressor agents on urinary electrolytes and corticosteroids, including aldosterone. *J Clin Invest* 40: 338-347, 1961
18. Genest J, Nowaczynski W, Koiw E, Sandor T, Biron P: Adrenocortical function in essential hypertension. In *Essential Hypertension*, edited by KD Bock, PT Cottier. New York, Springer-Verlag, 1960, pp 126-146
19. Fraser R, James VH, Brown JJ, Isaac P, Lever AF, Robertson JIS: Effect of angiotensin and of furosemide on plasma aldosterone, corticosterone, cortisol, and renin in man. *Lancet* 2: 989-991, 1965
20. Palmore WP, Marieb NJ, Mulrow PJ: Stimulation of aldosterone secretion by sodium depletion in nephrectomized rats. *Endocrinology* 84: 1342-1351, 1969
21. McCaa RE, Bower JD, McCaa CS: Relative influence of acute sodium and volume depletion on aldosterone secretion in nephrectomized man. *Circ Res* 33: 555-562, 1973
22. McCaa RE, Young DB, Guyton AC, McCaa CS: Evidence for a role of an unidentified pituitary factor in regulating aldosterone secretion during altered sodium balance. *Circ Res* 34 (suppl 1): 15-25, 1974
23. Cannon PJ, Ames RP, Laragh JH: Relation between potassium balance and aldosterone secretion in normal subjects and in patients with hypertensive or renal tubular disease. *J Clin Invest* 45: 865-879, 1966
24. Davis JO, Urquhart J, Higgins JT Jr: The effects of alterations of plasma sodium and potassium concentration on aldosterone secretion. *J Clin Invest* 42: 597-609, 1963
25. Dluhy RG, Axelrod L, Underwood RH, Williams GH: Studies of the control of plasma aldosterone concentration in normal man. *J Clin Invest* 51: 1950-1957, 1972
26. Mulrow PJ, Ganong WF: The effect of hemorrhage upon aldosterone secretion in normal and hypophysectomized dogs. *J Clin Invest* 40: 579-585, 1961
27. DeClue JW, Cowley AW Jr, Coleman TG, McCaa RF, Guyton AC: Influence of long-term low dosage infusion of angiotensin II on arterial pressure. *Physiologist* 19: 165, 1976
28. Hollenberg NK, Chenitz WR, Adams DF, Williams GH: Reciprocal

- influence of salt intake on adrenal glomerular and renal vascular responses to angiotensin II in normal man. *J Clin Invest* **54**: 34-42, 1974
29. Oelkers W, Brown JJ, Frasier R, Lever AF, Morton JJ, Robertson JIS: Sensitization of the adrenal cortex to angiotensin II in sodium-deplete man. *Circ Res* **34**: 69-77, 1974
  30. Spielman WS, Davis JO: The renin-angiotensin system and aldosterone secretion during sodium depletion in the rat. *Circ Res* **35**: 615-624, 1974
  31. McCaa RE, Bower JD, McCaa CS: Relative influence of acute sodium and volume depletion on aldosterone secretion in nephrectomized man. *Circ Res* **33**: 555-562, 1973
  32. McCaa RE, McCaa CS, Cowley AW Jr, Ott CE, Guyton AC: Stimulation of aldosterone secretion by hemorrhage in dogs after nephrectomy and decapitation. *Circ Res* **32**: 356-362, 1973
  33. Palmore WP, Marieb NJ, Mulrow PJ: Stimulation of aldosterone secretion by sodium depletion in nephrectomized rats. *Endocrinology* **84**: 1342-1351, 1969
  34. Blair-West JR, Coghlan JP, Denton DA, Goding JR, Wintour M, Wright RD: The control of aldosterone secretion. *Recent Prog Horm Res* **19**: 311-383, 1963
  35. Thurston H, Laragh JH: Prior receptor occupancy as a determinant of the pressor activity of infused angiotensin II in the rat. *Circ Res* **36**: 113-117, 1975

## The Effect of Cardiac Contraction on Collateral Resistance in the Canine Heart

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**SUMMARY** We determined whether the coronary collateral vessels develop an increased resistance to blood flow during systole as does the cognate vascular bed. Collateral resistance was estimated by measuring retrograde flow rate from a distal branch of the left anterior descending coronary artery while the main left coronary artery was perfused at a constant pressure. Retrograde flow rate was measured before and during vagal arrest. We found

that in 10 dogs the prolonged diastole experienced when the heart was stopped caused no significant change in the retrograde flow rate, which indicated that systole has little effect on the collateral resistance. However, when left ventricular end-diastolic pressure was altered by changing afterload or contractility, a direct relationship between end-diastolic pressure and collateral resistance was noted.

CONTRACTION of the heart compresses the coronary arteries and impedes blood flow through them.<sup>1</sup> Systole does not completely occlude the coronary vasculature, but rather creates a gradient for flow across the heart wall ranging from little changes in flow at the subepicardium to near zero flow at the subendocardium.<sup>2, 3</sup> Thus a considerable fraction of coronary inflow occurs during systole. Several workers have examined the collateral vasculature to see whether it is compressed during systole as is the cognate bed. Cibulski et al.<sup>4</sup> analyzed phasic flow records in acute canine preparations and concluded that only 5% of the collateral flow occurred during systole. More recently Brown et al.<sup>5</sup> independently varied either the systolic or the diastolic component of the aortic pressure and measured retrograde flow from an occluded branch. Their data indicated that the collateral vessels, unlike the cognate bed, are completely pinched off during systole. This is a surprising finding, since blood flowing retrograde from an open artery is thought to derive primarily from collateral vessels near the epicardium,<sup>6</sup> where tissue pressure during systole is low.

The present experiment further tested the hypothesis that systole completely occludes collateral channels. This was done by measuring retrograde flow as the coronary

arteries were perfused at constant pressure and the heart was arrested by vagal stimulation. If, indeed, the collateral vessels were totally occluded during the systolic period, then the prolonged diastole associated with arrest should cause retrograde flow to increase in proportion to the time the heart previously was in systole.

### Methods

Ten mongrel dogs of either sex, weighing 11-17 kg were anesthetized with sodium pentobarbital (30 mg/kg, iv). The chest was opened in the 5th interspace and the dog was ventilated with 100% O<sub>2</sub>. The left common coronary artery was exposed by blunt dissection at its origin. A coronary cannula with perfusion tubing attached was inserted through the subclavian artery and advanced into the left common coronary artery, where it was tied securely in place. Heparin (10,000 U, iv) prevented clotting. The perfusion apparatus is shown in Figure 1. The cannula was a double-lumen type which withdrew blood from the aorta via the outer lumen. After passing through the exterior circuit, blood entered the coronary artery through the inner lumen. The tubing (Tygon) with inner diameter (i.d.) =  $\frac{3}{16}$  in., passed through the fingers of a Harvard model 1215 pump. It then led to a 20-ml air-filled buffer bottle which damped the pulsations from the pump. An extracorporeal electromagnetic flow probe (Carolina Medical Electronics) was used to measure flow in the circuit near the coronary cannula. To provide a constant perfusion pressure the perfusion pressure signal was compared to a set point voltage by an integrating circuit. The output of the integrating circuit controlled the pump speed

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