

Quantification of Baroreceptor Influence on Arterial Pressure Changes Seen in Primary Angiotensin-Induced Hypertension in Dogs

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SUMMARY We studied the role of the sino-aortic baroreceptors in the gradual development of hypertension induced by prolonged administration of small amounts of angiotensin II (A II) in intact dogs and dogs with denervated sino-aortic baroreceptors. Short-term 1-hour infusions of A II (1.0–100 ng/kg per min) showed that conscious denervated dogs had twice the pressor sensitivity of intact dogs. Long-term infusions of A II at 5.0 ng/kg per min (2–3 weeks) with continuous 24-hour recordings of arterial pressure showed that intact dogs required 28 hours to reach the same level of pressure attained by denervated dogs during the 1st hour of infusion. At the 28th hour the pressure in both groups was 70% of the maximum value attained by the 7th day of infusion. Both intact and denervated dogs reached nearly the same plateau level of pressure, the magnitude being directly

related both to the A II infusion rate and the daily sodium intake. Cardiac output in intact dogs initially decreased after the onset of A II infusion, but by the 5th day of infusion it was 38% above control, whereas blood volume was unchanged. Heart rate returned to normal after a reduction during the 1st day of infusion in intact dogs. Plasma renin activity could not be detected after 24 hours of A II infusion in either intact or denervated dogs. The data indicate that about 35% of the hypertensive effect of A II results from its acute pressor action, and an additional 35% of the gradual increase in arterial pressure is in large measure a result of baroreceptor resetting. We conclude that the final 30% increase in pressure seems to result from increased cardiac output, the cause of which may be decreased vascular compliance, since the blood volume remains unaltered.

ANGIOTENSIN II (A II) is the most potent endogenous vasopressor agent known, and there has been an unending effort to link the endogenous release of this peptide to the cause of various types of hypertension, especially those of renal origin. The precise role of the renin-angiotensin system in the etiology of various types of renal hypertension awaits clarification, but it has been clearly demonstrated that hypertension gradually develops over a period of 1 week when angiotensin is administered continuously in a low dose to dogs, rabbits, rats, and man.^{1–4} The hypertension observed under these circumstances has been characterized by only 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.

The mechanism or mechanisms responsible for the gradual increase in pressor sensitivity to angiotensin are poorly understood. Moreover, there is little information regarding the many hemodynamic changes that occur in this model of hypertension. Various explanations have been offered to explain the gradual development of hypertension. Some investigators feel that it results from an enhanced output of aldosterone that leads to increased body salt and water content.⁴ Others suggest that it results from vascular wall damage induced by angiotensin.⁵ Perhaps the most commonly evoked explanation for the gradual increase in pressure is that it results from an increase in sympathetic tone to the vasculature, as has been demon-

strated through the use of ganglionic and sympathetic blocking agents.⁶

Two of these general concepts have been examined in our laboratory: the role of aldosterone secretion and the role of changes in the sympathetic tone to the vasculature. The role of aldosterone and the influence of sodium intake were reported separately.⁷ The present study was designed to quantify the contribution of arterial baroreceptor reflex adaptation to the apparent changes in sympathetic tone and thus to the gradual increase in pressure and the final steady state levels of angiotensin-induced hypertension.

Potent baroreceptor reflexes can readily buffer acute changes in arterial pressure in the conscious dog and effectively mask the initial changes brought about by infusion of small amounts of pressor agents.⁸ However, continuous stress of the vascular walls lasting days and weeks leads to adaptation of the baroreceptors.⁸ This adaptation process, referred to as "resetting," theoretically could account for all or part of the observed increase in arterial pressure during continuous low-dose infusion of angiotensin.

To evaluate the role of the baroreceptor system, the hemodynamic events in conscious dogs with denervated sino-aortic baroreceptor were compared with those in normal dogs during the transient and steady state responses resulting from low-dose infusions of angiotensin. The short-term hemodynamic responses during 1-hour infusions of varying doses of A II first were studied in conscious normal and baroreceptor-denervated dogs. The results of these short-term infusions suggested that baroreceptor resetting could be responsible for much of the observed long-term pressure changes occurring during prolonged infusion of angiotensin. Experiments were therefore conducted in which angiotensin was infused continuously for 2–3 weeks. Quantitative comparison of these

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changes in normal and denervated dogs was facilitated by computerized techniques that accurately determined small changes in mean arterial pressure despite wide fluctuations exhibited in baroreceptor-denervated dogs.

Methods

Thirty-seven trained mongrel dogs, weighing an average of 19.8 ± 1.1 (SE) kg, were used for these studies. Twenty-four experiments were performed with dogs in the normal state, and 17 were performed after elimination of the sino-aortic baroreceptor reflexes. All experiments were performed on unanesthetized dogs.

PREPARATION OF DOGS

All dogs were equipped with chronic indwelling polyvinyl catheters, one introduced through a femoral artery and placed in the abdominal aorta at a site distal to the renal artery, and another placed in the inferior vena cava through the ipsilateral femoral vein. In seven dogs another venous catheter was introduced through an external jugular vein and placed at the level of the right heart for determinations of cardiac output by dye dilution. The catheters were tunneled subcutaneously to the cephalic portion of the back and were kept filled with a heparin solution of 1,000 U.S.P. units/ml when not in use.

Twenty-four dogs were prepared solely with chronically implanted catheters and are designated as "intact dogs." The baroreceptors of 17 dogs were denervated prior to the experimental period and are referred to as "denervated dogs."

Sino-aortic baroreceptor denervation was accomplished by performing a left thoracotomy followed by denervation of the aortic arch area and complete transection of the left cervical sympathetic-depressor-vagal nerve and the right sympathetic-depressor fibers. The carotid sinus areas were denervated bilaterally through a midline incision in the neck. Sodium pentobarbital anesthesia (30 mg/kg) was used for all surgical procedures. Details of the surgical procedures and the methods used to verify the completeness of the denervation procedure used in our laboratory have been published previously.⁸⁻¹⁰

In two denervated and two intact dogs, electromagnetic flow transducers (Biotronix, series 400) were placed on the ascending aorta at the time of denervation or at the time of chronic implantation of the catheter. Calibration of the flow transducers was performed *in vitro* before and, if possible, after termination of the experiment by using calf aortas perfused with blood that had been diluted with saline to a hematocrit of 40%.

EXPERIMENTAL PROTOCOL

Experiments generally were begun 3 weeks after the final surgical procedure. After the dogs were placed in the recording pen they were maintained on a fixed intake of sodium (40 mEq/day) and potassium (40 mEq/day), with free access to drinking water for a period of 5-7 days. The following two experimental protocols then were carried out.

Short-term Angiotensin Dose-Response Studies

We determined the changes in mean arterial pressure that resulted from intravenous infusions of angiotensin for

1 hour in seven intact dogs and in 10 denervated dogs. The experimental protocol for these studies on pressure sensitivity consisted of a 1-hour control period followed by a 1-hour infusion period and then finally a 1-hour postinfusion period. At high doses of angiotensin, when mean arterial pressures of 175 to 200 mm Hg were reached, shorter infusion periods of 20 minutes were used. Because the infusions were associated with rapid onset and offset transients in arterial pressure, the steady state pre- and postinfusion arterial pressures were averaged and considered to be the baseline control arterial pressure level.

Angiotensin II amide (Hypertensin, CIBA) was diluted to a concentration of 2.5 μ g/ml in 0.9% saline and infused intravenously at rates between 0.01 and 1.0 ml/min using a calibrated Harvard infusion pump (model 944). Saline alone infused at these rates caused no alteration of arterial pressure in either normal or denervated dogs.

Chronic Administration of A II to Intact and Denervated Dogs

Thirty-one experiments, each lasting 2-3 weeks, were performed on 20 dogs. Seventeen of these dogs were studied as intact animals and seven were studied after sino-aortic baroreceptor denervation. Four of the latter group had first been studied in the intact state. All dogs were placed in a specially designed chronic recording pen for 1 week prior to angiotensin infusion and maintained on a sodium intake of 40 mEq/day. Continuous recording of arterial pressure was started 24 hours prior to the start of angiotensin infusion and continued until 24-48 hours following the end of infusion. A II was infused at a constant rate for 2-3 weeks at 5.0 ng/kg per min.

NORMAL SODIUM INTAKE

Eleven experiments, each lasting 2-3 weeks, were performed on six intact dogs and five denervated dogs maintained on a sodium intake of 40 mEq/day before and throughout the period of angiotensin infusion. Angiotensin was diluted to a concentration of 12 μ g/ml of isotonic sodium chloride, and infused at 5.0 ng/kg per min. This resulted in the addition of 1.2-1.8 mEq of sodium per day with less than 10 ml of additional water.

HIGH SODIUM INTAKE

Ten experiments, each lasting 2-3 weeks, were performed on five intact and five denervated dogs. Angiotensin was diluted in isotonic sodium chloride and administered with a Sigmamotor pump (model TM 20) at rates selected to deliver 80 mEq/day. The total sodium received by each dog was 120 mEq/day (40 mEq by diet and 80 mEq by infusion).

PRESSURE-MONITORING AND ANALYTICAL TECHNIQUES

Chronic baroreceptor denervation is characterized by extreme lability of arterial pressure; this makes it very difficult to quantify accurately small experimentally induced changes in pressure.¹¹ It therefore was necessary to use special computerized averaging techniques to quantify the hemodynamic changes. The techniques for both the continuous data collection system and computerized anal-

ysis have been described in detail in a previous publication,⁹ therefore only a brief summary of these procedures is given. (1) To minimize the large fluctuations in arterial pressure seen with only minor laboratory disturbances, experiments were performed in a quiet room with restricted entry. Also, the dogs were housed in a large pen in which their movement was relatively unrestricted. (2) Arterial pressure was measured by using a pressure transducer (Statham, model P23Ac) built into a plaster of Paris jacket which fitted over the dog's back. The cables from the pressure and flow transducers were brought out of the top of the pen through a protective flexible tubing attached to the top of the plaster jacket. (3) Hemodynamic data were recorded continuously 24 hours a day on Grass model 7 polygraph charts run at 10.0 mm/min. These later were evaluated using a fiberoptic curve scanning system¹² and A-D converter that permitted conversion of the analog records to digital form for storage and analysis by a PDP-9 digital computer. The polygraph charts were digitized to about 1,500 data points per hour of recorded time. The standard deviation and error of the mean were computed simultaneously for every hour of collected data. Individual data points stored in the computer memory were used to generate frequency distribution curves of mean arterial pressure over any desired time period (see Fig. 1). The analytical techniques permitted very accurate computation of the average hourly mean arterial pressure.

OTHER MEASUREMENTS

Plasma volume was measured by spectrophotometry using samples collected 20, 40, and 80 minutes after injection of Evans blue dye. Cardiac output was determined in the long-term experiments in seven intact dogs by dye dilution using indocyanine green (Cardio-Green) dye as an indicator and a Gilford model 103-IR cuvette densitometer and a Gilford model 105 constant flow system for withdrawal of arterial blood at a constant rate. Each determination of cardiac output was obtained by averaging four to five dilution curves in which 2.5 mg of indocyanine green dye in 1 ml of solvent were injected into the dog's right atrium via the jugular vein catheter. Aortic electromagnetic flow transducers were used to measure cardiac

output in the short-term, 1-hour dose-response studies. The great lability of circulatory hemodynamics of denervated dogs prevented reliable use of dye-dilution techniques in this group of dogs. Water intake and urine output were determined daily throughout the course of the experiment.

Unless otherwise stated, all values are expressed as means \pm SE. Statistical significance was accepted for *P* values less than 0.05.

Results

SHORT-TERM INFUSIONS OF A II IN INTACT AND DENERVATED DOGS

Frequency Distribution Analysis of Variable Pressure Data

Figure 1 illustrates typical examples of the computerized analysis of the records that enabled the measurement of small changes in arterial pressure in denervated dogs. The graphs represent the frequencies of occurrence of different arterial pressures during the control and experimental periods. The distribution curves were determined from the total accumulated pressure values obtained during the designated time periods. They clearly demonstrate the lability of arterial pressure typically observed in denervated dogs, which in this case ranged from 50 to 200 mm Hg even during the control periods. Despite the fact that the random pressure variations were greater than the small elevations caused by low-dose angiotensin infusions, an analysis of distribution permitted quantification of the change.

The curves in Figure 1 also show the pressure changes resulting from two different rates of angiotensin infusion for periods of 1 hour to a denervated dog. The curve on the left of each pair represents the distribution of control pressures obtained by combining the 1 hour preceding and the 1 hour following the angiotensin infusion. The curve on the right in each graph represents the distribution of pressures during the infusion period. Figure 1A illustrates the shift of the distribution curve resulting from angiotensin infusion at a rate of 3.2 ng/kg per min. The control pressures were distributed around a mean of 112.5 ± 26.2 (SD) mm Hg and were elevated to 136.9 ± 26.9 mm Hg

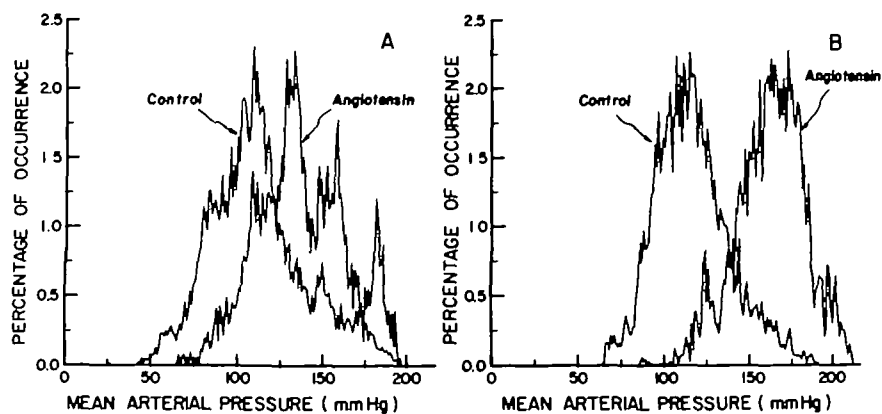


FIGURE 1 Examples of frequency distribution analyses of arterial pressure changes during two 1-hour infusions of angiotensin II (A II) to a conscious baroreceptor-denervated dog. An analysis of distribution such as this permitted small pressure elevations to be quantified in denervated dogs despite the fact that random pressure variations were often greater than the experimentally induced change. Panel A illustrates the rightward shift of the distribution curve from control which resulted from infusion of A II at 3.2 ng/kg per min in contrast to 7.6 ng/kg per min shown in panel B.

during the 1-hour infusion period ($P < 0.05$). Figure 1B illustrates the change brought about by infusion of angiotensin at a rate of 7.6 ng/kg per min. In this case, the control pressures were distributed around a mean of 116.9 ± 21.1 mm Hg and were elevated to 162.4 ± 22.7 mm Hg during the infusion period ($P < 0.05$).

All of the angiotensin results reported in this paper were analyzed in this manner, and a statistical comparison was made between each control period and its corresponding infusion period (Student's t -distribution). Since nearly 1,500 sample data points were collected per hour, the technique permitted fine discrimination between control and infusion periods.

Average Arterial Pressure Responses to Short-term Angiotensin Infusions in Intact and Denervated Dogs

The regression analysis seen in Figure 2 shows that the average arterial pressure response to a given dose of angiotensin was nearly twice as great in denervated dogs as in intact dogs. An analysis of covariance in a two-way classification comparing the slopes of the regression equations (F -test) yielded a high degree of significance ($P < 0.001$).

Average Heart Rate Response to Angiotensin Infusions of Intact and Denervated Dogs

Figure 3 illustrates that, in intact dogs, heart rate varied inversely with the changes in mean arterial pressure during acute angiotensin infusions; a direct correlation was obtained in denervated dogs.

A correlation was also observed between changes in heart rate and the rate of angiotensin infusion with intact dogs exhibiting a negative correlation between the rate of infusion and the change in heart rate ($Y = -15.4 \log X + 3.0$; $r = -0.41$) and denervated dogs a direct correlation ($Y = 23.6 \log X - 1.5$; $r = 0.42$).

CHRONIC INFUSION OF A II

Average Arterial Pressure Changes in Intact and Denervated Dogs Receiving Angiotensin (5.0 ng/kg per min) and High Sodium Intake

The average changes in mean arterial pressure obtained during continuous infusion of angiotensin II at 5.0 ng/kg per min in intact and denervated dogs are summarized in Figure 4. Pressures are expressed as percent of each dog's 24-hour control values. The dogs used in these experiments were maintained on a sodium intake of 120 mEq/day throughout the infusion periods.

Five significant features can be seen in these graphs: (1) During the first several hours the arterial pressure of the intact dogs rose to only about half the level attained at this same time by the denervated dogs. This difference in response between the intact and denervated dogs had been predicted from the short-term 1-hour dose-response curves shown in Figure 2. (2) By the 2nd day of infusion, in both the intact and the denervated dogs, arterial pressure reached nearly the same level. This pressure represented nearly 70% of the increase in pressure that was to be achieved later. (3) Arterial pressure continued to rise slowly in both groups until about the 7th day. (4) The steady state level of hypertension attained after 7 days of

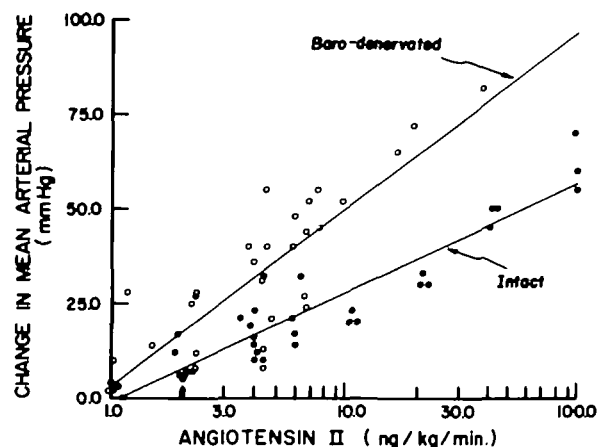


FIGURE 2 Dose-response analysis of mean arterial pressure changes in response to 1-hour infusions of angiotensin II to conscious intact dogs (38 infusions to seven dogs) and baroreceptor-denervated dogs (30 infusions to 10 dogs). The semilog regression analysis yields the equation $Y = 26.7 \log X - 1.7$ ($r = 0.94$) for intact dogs; denervated dogs yielded $Y = 46.7 \log X + 3.5$ ($r = 0.83$). Control pressures averaged 101.7 ± 3.0 mm Hg for intact dogs and 105.5 ± 5.1 mm Hg for denervated dogs.

infusion was nearly the same for both groups of dogs. In intact dogs the pressure averaged $137 \pm 9\%$ of control compared to $135 \pm 11\%$ for the denervated dogs. (5) When the angiotensin infusion was stopped, the pressure of the intact dogs fell halfway to control values during the first 2 hours and returned to the control over the following 12 hours. In contrast, the pressure of the denervated dogs quickly fell nearly to and sometimes below control values within 30 minutes after stopping the angiotensin infusion. (6) Finally, although not seen in the average hourly values, intact dogs consistently exhibited an increasing lability of arterial pressure after the first 5 days of angiotensin infusion.

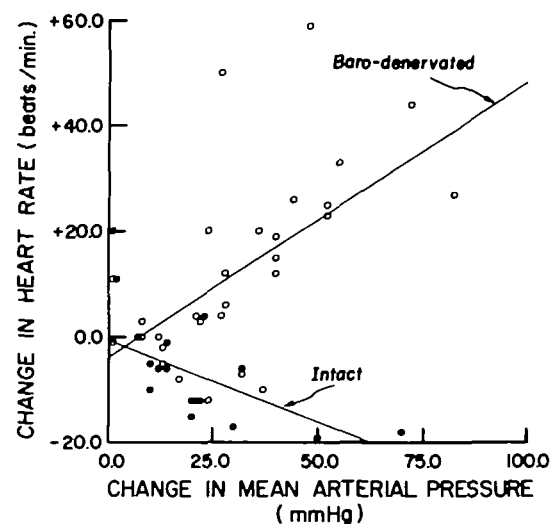


FIGURE 3 An inverse relationship between changes in mean arterial pressure and heart rate during infusions of angiotensin II was obtained in intact dogs (\oplus); $Y = 0.31 X - 0.6$ ($r = -0.69$). In contrast, a direct relationship was observed in denervated dogs (\circ); $Y = 0.52 X - 4.0$ ($r = 0.60$).

On- and Off-Transient Arterial Pressure Responses of Intact and Denervated Dogs during Chronic Infusion of Angiotensin

Figure 5 represents the experiments shown in Figure 4 on an expanded time scale so that the mean arterial pressure changes during the first 48 hours of infusion can be more clearly observed. It is seen that intact dogs exhibited an immediate rise in pressure to $112 \pm 6.3\%$ of control in

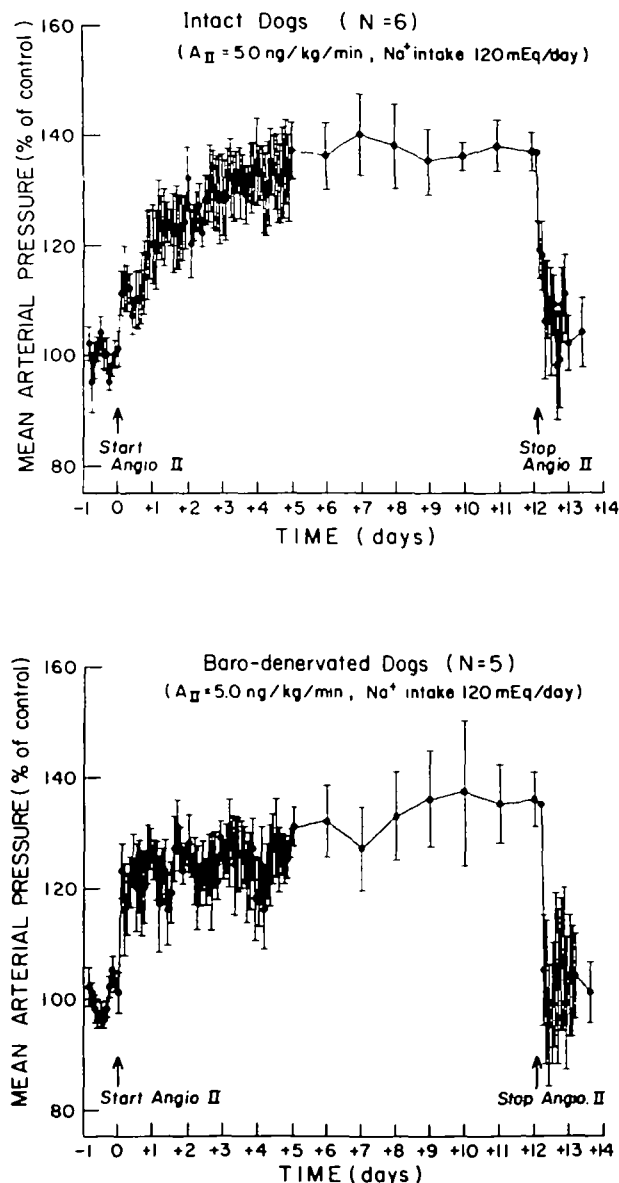


FIGURE 4 Average arterial pressure changes obtained during continuous intravenous infusion of angiotensin II at 4–6 ng/kg per min in dogs maintained on sodium intake of 120 mEq/day throughout the infusion. Pressure responses of intact dogs are shown in top panel and of denervated dogs in bottom panel. Each point represents an average of 2 hours (\pm SEM) throughout the first 5 days of infusion, and during the off-transient response. The remaining points represent an average 24-hour mean arterial pressure (\pm SEM). Pressures are expressed as percent of each dog's 24-hour control values, which for this group averaged 101.3 ± 4.3 mm Hg in intact dogs and 112.3 ± 6.4 mm Hg in denervated dogs.

contrast to the greater value ($124 \pm 7.1\%$) observed in denervated dogs ($P < 0.05$). These changes were evident during the first 15 minutes of infusion. It is also more clearly evident in this graph that after the first 28 hours of infusion, baroreceptor activity had little influence on the mean level of arterial pressure as shown by the superimposition of data for the two groups at this time.

Figure 6 summarizes the off-transient changes in arterial pressure obtained in the same group of dogs when the angiotensin infusion was abruptly stopped. For the first 2 hours after infusion the arterial pressure of intact dogs averaged 50% above control value. Denervated dogs exhibited a significantly greater fall in pressure ($P < 0.05$), which averaged 15% above control values. By the 4th hour, denervated dogs had returned to control levels. Intact dogs returned to control levels by the 14th hour after infusion.

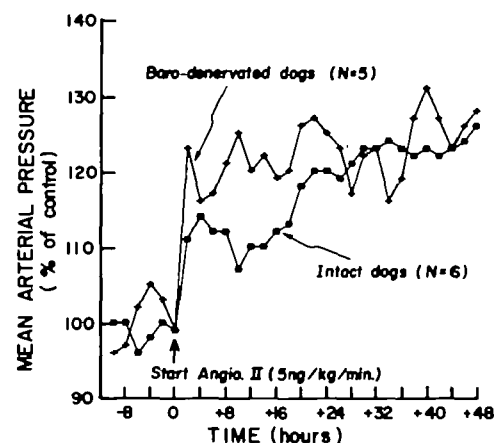


FIGURE 5 Mean arterial pressure changes during the first 48 hours of angiotensin II infusion (4–6 ng/kg per min) to intact and denervated dogs receiving sodium, 120 mEq/day. The data represent those summarized in Figure 4 using an expanded time scale so that the on-transient response can be more clearly observed.

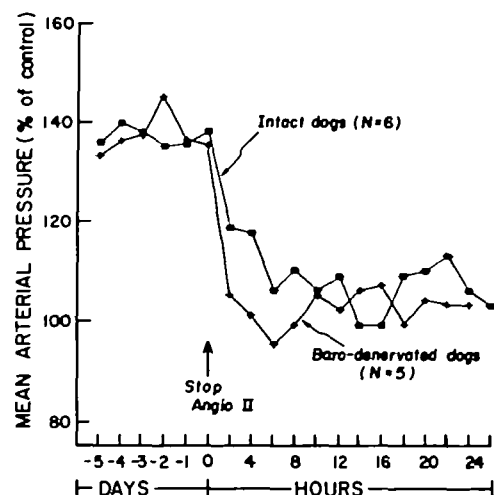


FIGURE 6 Mean arterial pressure changes during the first 24 hours following the end of the chronic infusion of angiotensin II (4–6 ng/kg per min) to intact and denervated dogs. Data were obtained from the same experiments shown in Figure 4.

Average Arterial Pressure Changes in Intact and Denervated Dogs Receiving Angiotensin (5.0 ng/kg per min) and a Normal Sodium Intake

Figure 7 summarizes the changes in arterial pressure attained during the infusion of angiotensin (5.0 ng/kg per min) to five intact (top) and five denervated (bottom) dogs maintained on a daily salt intake of 40 mEq/day. The same major features are apparent in these dogs as seen in the dogs that received 120 mEq/day (Fig. 4). The difference in salt intake, however, resulted in a significant difference ($P < 0.05$) in the degree of hypertension achieved. The denervated dogs at both levels of salt intake exhibited

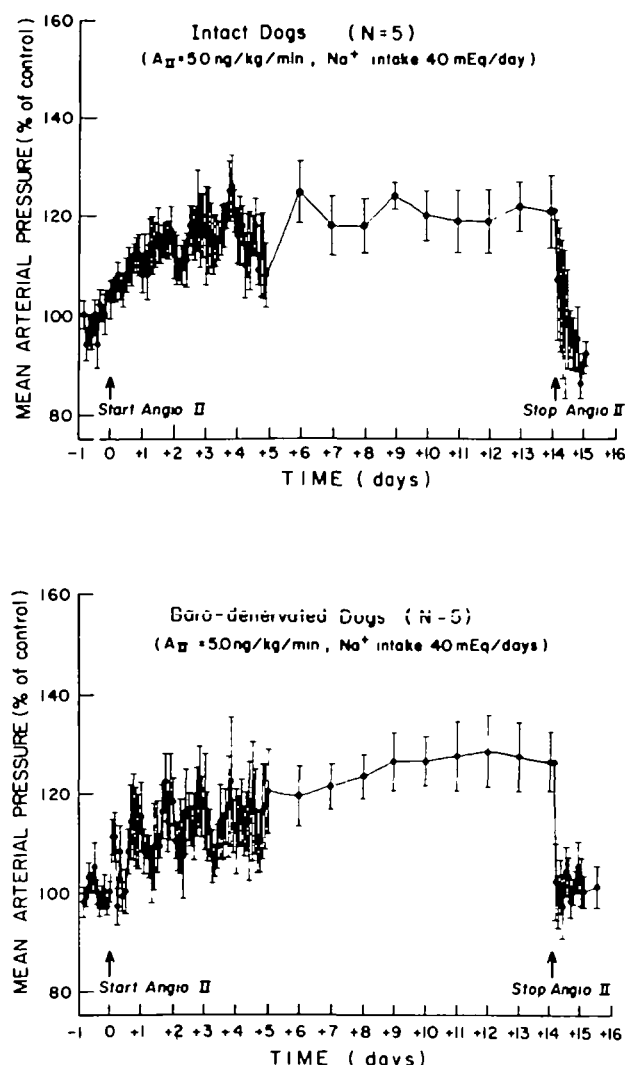


FIGURE 7 Average arterial pressure changes obtained during continuous intravenous infusion of angiotensin II (A II) (4–6 ng/kg per min) in dogs maintained on a sodium intake of 40 mEq/day throughout the infusion. Pressure responses of intact dogs are shown in top panel and of denervated dogs in bottom panel. Each point represents an average of 2 hours (\pm SEM) throughout the first 5 days of infusion and during the off-transient response. The remaining points represent an average 24-hour mean arterial pressure (\pm SEM). Pressures are expressed as percent of each dog's 24-hour control values, which for this group averaged 107.3 ± 3.0 mm Hg in intact dogs and 109.4 ± 4.5 mm Hg in denervated dogs.

diurnal variations of arterial pressure. This effect emerged more clearly in the group on the lower salt intake, where it represents a greater fraction of the overall pressure change (Fig. 7).

Cardiac Output and Blood Volume Changes during Chronic Infusion of Angiotensin in Intact Dogs

Cardiac output was determined by dye dilution in seven intact dogs and by electromagnetic flowmeter in two intact dogs while each received an infusion of angiotensin of 5.0 ng/kg per min and was maintained on a normal or high salt intake. Each dye-dilution determination consisted of an average of four to five dilution curves determined over a period of 1 hour. In two of the seven dogs flow was measured by dye dilution during the first 48 hours of infusion, and in two additional dogs it was measured by previously implanted electromagnetic flowmeters. Cardiac output was decreased in each of the four dogs by an average of 14.4% of control values. In contrast, when measured in seven dogs by dye dilution during the 4th to 5th day of angiotensin infusion, cardiac output was elevated by nearly 40% from an average control of 2.29 ± 0.34 liters/min to 3.22 ± 0.9 liters/min ($P < 0.05$, determined by analysis of paired variance). Dye-dilution techniques were not used to study the denervated dogs because of the inherent hemodynamic instability of these dogs, but cardiac output determined by implanted electromagnetic flowmeters in two denervated dogs also yielded an average decrease of nearly 12% during the first 12 hours of infusion. Technical difficulties prohibited prolonged study of these two dogs.

Blood volume in five of the same intact dogs averaged 88.7 ± 9.0 ml/kg during the control period. Determined at the 4th or 5th day of infusion, it averaged 79.0 ± 8.3 ml/kg ($P < 0.2$ by paired analysis).

Fluid Intake and Urine Output

Intact and denervated dogs maintained on a normal sodium intake (40 mEq/day) throughout the experiment showed no significant change ($P < 0.10$) in daily water intake or urine excretion throughout the period of infusion when angiotensin was administered at 5.0 ng/kg per min. If extraordinary sources of water loss remained constant throughout the experiment, it could be assumed that there was no significant retention or loss of body fluids in this group of dogs. Daily determination of hematocrit and plasma osmolality showed no significant change during the infusion.

In contrast, there was an average increase of 128% (45 ml/kg per day) in water intake of dogs that received an additional 80 mEq of Na^+ per day during infusion of angiotensin at 5.0 ng/kg per min. This additional sodium intake required a water intake of only 28.5 ml/kg per day to maintain isotonicity, so that the 45 ml/kg per day intake was in excess of the expected drinking in these dogs on the basis of sodium intake alone. Urine excretion during this period increased by only 65% (15.8 ml/kg) above control excretion rates. Hematocrit significantly fell from $35.5 \pm 1.9\%$ to $30.6 \pm 2.1\%$ by the end of 24 hours of infusion ($P < 0.05$), as did the plasma osmolality. Both were

nearly normal by the 3rd day of infusion. The data suggest, therefore, that in this group of dogs there was a net retention of water when angiotensin was administered with excess sodium.

Heart Rate Response during the Long-Term Angiotensin Infusion

After a slight decrease in heart rate (8 beats/min) during the first 24 hours of infusion in intact dogs (see Fig. 3), heart rate thereafter remained within normal limits in both groups of dogs. During the 24 hours preceding the infusion, control heart rates of intact dogs averaged 87.9 ± 3.4 beats/min and rates of denervated dogs averaged 97.0 ± 4.7 beats/min. When angiotensin was abruptly discontinued after more than 1 week of infusion, heart rate changes indicated that the baroreceptors had been reset to a new and elevated hypertensive level; that is, the intact dogs exhibited an average increase in heart rate of 1.28 beats/min for each 1.0 mm Hg decrease in arterial pressure. In contrast, essentially no change (-0.08 beat/min) was observed in denervated dogs.

Discussion

In previous studies, investigators have been unable to explain the mechanism or mechanisms for the gradual development of hypertension which is observed when small doses of angiotensin are administered continuously over prolonged periods of time. The present data suggest at least a partial answer for this phenomenon. The results indicate that a sustained angiotensin pressor effect, together with gradual adaptation (resetting) of the baroreceptors, can account for nearly 70% of the observed eventual increase in arterial pressure during prolonged infusions of A II. This gradual rise in arterial pressure can be described as negative exponential in form, with most of the hypertension developing during the 1st day of infusion. After the initial 28 hours of infusion, resetting of the baroreceptor reflexes does not appear to influence any further the rate of development of the level of hypertension. The following observations led to these conclusions.

PREDICTABLE DOSE-RESPONSE RELATIONSHIPS

The arterial pressure increase attained by intact dogs after 28 hours of angiotensin infusion (5.0 ng/kg per min) was nearly the same as that obtained in baroreceptor-denervated dogs during the 1st hour of infusion. This is the response that would be predicted from the short-term dose-response curves seen in Figure 2 if the baroreceptors had been reset by this time. In effect, the lower curve would adapt upward to the denervated (top) curve. Since the only difference between the two groups of dogs was the presence or absence of the baroreceptor reflexes, we conclude that during the 1st day the gradual pressure rise in the intact dogs was caused by resetting of the baroreceptors.

Although baroreceptor resetting seems to be one of the primary mechanisms involved in the gradual rise of pressure, it was not the sole mechanism. The immediate pressure changes observed during the 1st day (112% of control) represent about 35% of the final steady state level of hypertension, and an additional 35% rise (to 124% of

control) resulted from baroreceptor resetting. The remaining 30% rise will be discussed later.

HEART RATE RESPONSES IN TRANSIENT AND STEADY STATE PERIODS OF A II INFUSION

An index of baroreceptor activity can be obtained from changes in heart rate.¹¹ Comparison of the changes in heart rate that resulted from 1-hour infusions of A II in intact dogs with those attained after 1–2 weeks of A II infusion provided further evidence that the baroreceptors had reset to a higher operating level. For example, the heart rate was consistently slowed during short-term infusions of A II (Fig. 3), indicating reflex depression. After several days of continuous infusion, heart rate returned to normal and stayed within normal limits throughout the remainder of the infusion. In contrast, no slowing of heart rate was observed in the denervated dogs during the first few days of infusion. When the angiotensin infusion into intact dogs was stopped after more than 1 week of infusion, the heart rate increased by 1.28 beats/min for each 1.0 mm Hg fall in arterial pressure, indicating that the reflexes were attempting to maintain pressure at the new elevated level of pressure. Denervated dogs showed no significant change in heart rate at the end of infusion. These data indicate that the baroreceptors had become reset to the elevated level of blood pressure attained during prolonged angiotensin infusion.

OFF-TRANSIENT PRESSURE RESPONSES

Previous investigators have observed that the arterial pressure falls rapidly toward normal at the termination of a chronic infusion of A II.^{6, 13, 14} It has been concluded that this provides evidence against the concept of baroreceptor reflex resetting to explain the gradual rise of pressure. However, results of the present study, along with the basic physiological characteristics of the baroreceptors, predict that a rapid fall in pressure should occur despite reflex adaptation. A portion of the rapid decline in pressure is predicted on the basis of the inability of the reflexes to compensate totally for a step change in pressure. Reported values for static open-loop feedback gain average -1 to -2 ^{15–17} and indicate that the intact overall reflex system would restore a fall in pressure to about 50–60% of the original high level. Thus, in the presence of normal baroreceptors operating at maximum gain, the pressure would be expected to fall immediately 35–50% of the way back toward normal. An additional immediate fall in pressure could be accounted for by the suppression of endogenous renin secretion during the angiotensin infusion, a consistent observation in our laboratory during prolonged infusion of angiotensin at doses ranging from 5.0 to 23.0 ng/kg per min. Renin secretion remains suppressed for several days after the termination of prolonged infusion of angiotensin.⁷ Although it has been shown that the circulating levels of A II in animals maintained on a normal salt diet are responsible at most for only 10% of the normal total peripheral resistance,¹⁸ complete suppression of the endogenous renin secretion for several days after the end of A II infusion could account for an additional fall in arterial pressure of 10–15% when the infusion ended. Renin suppression and the intrinsic gain of the reflex system

could thus account for a rapid fall in pressure of greater than 50% of the way to normal in intact dogs and of 85–100% in denervated dogs.

RETURN OF ARTERIAL PRESSURE TO PREVIOUS HYPERTENSIVE STATES AFTER BRIEF CESSATION OF A II INFUSION

The infusion of A II was stopped for brief periods lasting from 15 to 30 minutes about every other day so that the dogs could be exercised. Following this period, and before restarting the A II, it was observed routinely that the arterial pressure of intact dogs (steady state hypertensive dogs) had fallen about halfway toward normal. But, when A II infusion was resumed, the pressure rose within 10 minutes to the same hypertensive level that initially had required nearly a week to achieve. This provides further support for the conclusion that the baroreceptors were operating at the new higher level of arterial pressure and indicates that this mechanism was at least in part responsible for the observed gradual rise.

POSSIBLE SEQUENCE OF EVENTS LEADING TO ANGIOTENSIN-INDUCED HYPERTENSION

The present study suggests, as did others before it, that the events leading to the gradual rise of arterial pressure during the chronic administration of small doses of angiotensin are very complex, and result from more than just several events related to arterial vasoconstriction.^{1–6} The small arterial pressure rise initially obtained at the onset of the angiotensin infusion at 5.0 ng/kg per min was most likely a result of direct arteriolar vasoconstriction, some of which may have been mediated through central nervous system actions of angiotensin,^{18–22} the sympathetic ganglia,²³ or stimulation of the postsynaptic nerve endings^{1, 24} and the adrenal medulla.²⁵ The data indicate that any greater rise in arterial pressure was effectively buffered by baroreceptor-induced reflex dilation, so that acute pressure responses observed at low rates of infusion were only mildly apparent (see Figs. 2, 4, and 7).

The results of the present study reinforce the previous observations on the rabbit by Dickinson and Yu,^{13, 14} who presented evidence that the slow rise in blood pressure during continuous infusion of small amounts of angiotensin was mediated through the sympathetic nervous system. The neurogenic vascular tone in their experiments was assessed by the fall in pressure resulting from an injection of trimethaphan. Our present study indicates that the apparent withdrawal of sympathetic activity seen by these investigators during the first day of infusion was a reflection of baroreceptor reflex activity attempting to restrain the pressor effects of angiotensin.

Since the pressure increases in the intact and the denervated dogs were the same after the 28th hour, it is probable that the baroreceptors no longer influenced the rate of development of hypertension thereafter. At the 28th hour, the arterial pressure had reached nearly 70% of the final steady state level of hypertension. The remaining 30% gradual rise in pressure during the 2nd to 10th days of infusion occurred at a rate that was slower than any previously reported rates for baroreceptor adaptation.^{26, 27} Therefore, the reflexes had little influence on this final pressure rise.

Although baroreceptor resetting appears to be responsible for a major portion of the “increase in sensitivity” to angiotensin, other factors apparently are at work and are responsible for a remaining 30% of the pressure rise. The present study provides no information to determine whether the final 30% rise in arterial pressure after baroreceptor adaptation is a result of the reported central nervous system actions of angiotensin.^{18–22} The hemodynamic results suggest that the rise in cardiac output observed by the 5th day of infusion is in some way associated with and perhaps responsible for the final rise in pressure. Gradual elevation of cardiac output previously was obtained by Olmstead and Page²⁸ by infusing gradually increasing (15–150 ng/kg per min) doses of angiotensin over a 30-day period. The mechanism for this rise in cardiac output following an initial decrease during the first several days is unclear, but the observed changes could have been mediated by changes in arterial or venous resistance, or both, either by direct actions of angiotensin or through the central nervous system.

It also is possible that the cardiac output changes could have resulted from angiotensin-mediated changes in vascular compliances causing redistribution of body fluid volumes. The blood volume was not significantly altered in these studies, and in dogs receiving a normal sodium intake throughout the infusion there was no net retention of water. However, an angiotensin-induced reduction either in the venous capacitance or in the unstressed vascular volume of the system could have resulted in an increase in the “effective blood volume.” Thus, angiotensin may exert subtle influences on the venous side of the circulation. The idea is supported by unpublished experiments in our laboratory by Manning, who has observed a rise of 1–2 mm Hg in the mean circulatory filling pressure in dogs infused with A II (23.0 ng/kg per min) for 2 weeks.

It could be argued that the sustained hypertension observed in these studies, regardless of the initial transient changes in resistance, capacitance, and cardiac output, is related to angiotensin-induced changes in renal function. If sodium and water excretion by the kidney can be depressed by small doses of A II, as has been reported by Waugh,²⁹ a higher arterial perfusion pressure would have been required to maintain the animal in fluid volume balance. The arterial pressure needs to rise high enough to achieve body fluid balance during A II infusion so that the kidneys retain enough salt and water to raise the pressure to the level necessary to maintain adequate renal function. These concepts have been developed theoretically in a previous publication.³⁰ Salt and water retention could have resulted from increased aldosterone secretion during the first 24 hours of infusion, but, as reported by Cowley and McCaa,⁷ aldosterone levels were nearly normal by the 24th hour of infusion, therefore the steady state changes in arterial pressure and cardiac output are difficult to explain on the basis of aldosterone secretion.

FACTORS DETERMINING THE DEGREE OF BARORECEPTOR INFLUENCE ON THE RATE OF DEVELOPMENT OF HYPERTENSION

The present study, considered together with other experiments recently performed in our laboratory, demonstrates that the influence of the baroreceptor reflex system

on the rate of development of hypertension depends on how rapidly the arterial pressure is attempting to change. For this reason the influence of the baroreceptor reflexes on the rate of onset of hypertension in this particular model could not be predicted from studies on other models of hypertension previously examined in this laboratory.^{10,31} For example, when the stimulus for pressure change was slowly applied in a model of salt-loading hypertension, the time required for intact dogs to reach a steady state level of hypertension was 3 times as great as in baroreceptor-denervated dogs when both received saline at 190 ml/kg per day.¹⁰ In contrast, when a rapid stimulus for pressure change was applied, as in the present experiments, baroreceptor-denervated dogs attained within 10 minutes the same pressure attained by the intact dogs over a 24-hour period; this is a 144-fold difference. A mixture of both the slow and rapid pressure responses was observed in dogs with an externally applied renal artery occluder.³¹ This results in immediate release of renin (the rapid phase), followed by a gradual expansion of body fluid volumes over a period of several days (the slow phase). In this instance, in baroreceptor-denervated dogs the pressure rose within the 1st hour to a level that was about 75% of the maximum value attained by the 24th hour after constriction. Intact dogs required 48 hours to attain the same maximum level of pressure. Thus, even with a single time constant for the baroreceptor adaptation mechanism, variable times can be required for the hypertension to reach a fixed steady state. This concept has been demonstrated in a mathematical computer simulation in which a single time constant (1,080 minutes) for baroreceptor resetting was compatible with the onset transients of these three experimental models of hypertension.

These studies demonstrate that the apparent degree of baroreceptor influence on the rate of development of hypertension is greatly influenced by the method used to produce hypertension. If the baroreceptors can adapt at the same speed as, or even more rapidly than, the rate of increase of the hypertension, then the reflexes will not influence the rate at which hypertension develops. This could explain why Alexander and DeCuir³² reported no significant difference in the rate of onset of hypertension in intact and denervated rabbits with latex-wrapped kidneys. It also explains the differences in the apparent degree of baroreceptor influence on the rate of development of hypertension in the three models of hypertension studied in our laboratory.

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