Renin response and angiotensinogen control during graded hemorrhage and shock in the dog

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THE RELEASE of renal renin stimulated by hemorrhage has been reported to be a normal compensatory response to the decreased blood volume and pressure through reflex mechanisms responsible for maintaining normal blood pressure (17, 18, 21). Later investigations (10, 11, 13) suggested that during prolonged hemorrhagic hypotension the action of the enzyme renin on angiotensinogen resulted in a decreased plasma angiotensinogen concentration as well as a secondary fall in plasma renin activity. However, in 1944, Middleton (25) reported that after the development of hemorrhagic shock and the reinfusion of the shed blood, the circulatory responsiveness to injected renin or angiotensin II was not depressed. He concluded that the mechanisms involved in the vascular response to the injected renin were not implicated in the development of hemorrhagic shock.

Recently it has been suggested that angiotensin II can act directly as a positive feedback in the isolated perfused liver to stimulate the synthesis and release of angiotensinogen (26). Earlier investigators have reported decreases in the vascular response of hepatectomized animals to injections of renin (4, 14, 27), and decreases in the normal plasma concentration of angiotensinogen in dogs with chemically destroyed livers (27) or in patients with cirrhosis and ascites (16).

The present studies have attempted to describe the response of the renin angiotensin-angiotensinogen system and its contribution to the regulation of systemic blood pressure during graded hemorrhagic hypotension and circulatory shock. Also, particular attention was directed toward any changes in plasma renin activity that might give credence to the previously reported pathophysiologic role for the renin-angiotensin system in the development of "irreversible" shock.

In addition, the role of angiotensin II in the mechanism of the control of plasma angiotensinogen was examined by blocking the hypothesized hepatic angiotensin II receptor with the angiotensin II antagonist, [Sar1, Ala8]angiotensin II, during graded hemorrhagic hypotension, and by stimulating the hypothesized hepatic angiotensin II receptor with synthetic angiotensin II ([Asp1, Val3]angiotensin II) infused continuously during hypovolemic hypotension and shock.

MATERIALS AND METHODS

Twenty-three mongrel dogs of either sex weighing from 15 to 20 kg were used for this study. Prior to anesthetization with sodium pentobarbital, 30 mg/kg (Beecham-Massengill Pharmaceuticals, Bristol, Tenn.), the dogs were fasted for 12 h. Supplemental anesthesia was administered intramuscularly and intravenously when necessary. The animals were permitted a 30-min equilibration period following the administration of the supplemental anesthesia.

Both femoral arteries were isolated and cannulated with polyvinyl chloride tubing (pv-c-105-10; Alpha Wire Co., Chicago) filled with heparinized saline. One cannula was connected to a Statham pressure transducer (P23A, no. 13947; Statham Instruments, Inc., Oxnard, Calif.) for systemic pressure monitoring. The dogs were permitted to hemorrhage from the other cannula into an overhead reservoir, chilled to retard the formation of angiotensin II in the shed blood. The blood reservoir was
initially located at a height above the dog such that the column of blood provided a hydrostatic pressure opposing further volume loss when the dog's mean blood pressure reached 125 mmHg. Subsequent pressure decreases were accomplished by lowering the hemorrhage bottle. This maneuver decreased the hydrostatic pressure opposing the dog's blood pressure and resulted in a further loss of blood volume and a decrease in blood pressure. The initial hemorrhage to 125 mmHg took approximately 45 min and was accomplished by opening the dog's arterial system to atmospheric pressure for periods of no longer than 10 s. In addition, systemic arterial blood samples were collected from this femoral arterial cannula. The femoral veins were cannulated for the infusion of the sustaining solution and for the return of the shed blood at the appropriate time.

The left kidney was exposed through a retroperitoneal approach. The left renal artery was exposed and carefully dissected free from the surrounding tissue for a length of 1.0-2.0 cm. A 10-mm, noncannulating, electromagnetic flow probe (Carolina Medical Electronics, Inc., King, N.C.) was placed on the left renal artery for the measurement of total renal blood flow. The gonadal vein was identified and its junction with the renal vein was carefully cleaned of the surrounding tissue. The left renal vein was then cannulated through the gonadal vein with a 16-gauge indwelling needle (Angiocath, Deseret Pharmaceutical Co., Sundy, Utah), appropriately bent to facilitate the cannulation. Once securely tied, the catheter was connected to a heparinized saline-filled polyvinyl chloride tube and brought to the outside of the incision. The left ureter was cannulated for collection of urine. Following the surgical preparation, the dogs were permitted to equilibrate for 30 min prior to beginning the experiment.

The sustaining solution was infused at 1 ml/min by a Holter roller pump (RL 175, Extracorporeal Medical Specialties, Inc., King of Prussia, Pa.). Each liter contained 1.2 g para-aminohippuric acid, 3 g creatinine, 4.5 g sodium chloride, 25 g dextrose, and 25 g mannitol.

Prior to hemorrhage, each dog received 500 U/kg sodium heparin injection. Each subsequent hour the dog received a supplemental dose consisting of half the original dose (2).

**Experimental Procedure**

In 10 dogs, a modified Wiggers shock protocol (35) was followed. The typical experimental protocol is illustrated in Fig. 1. It consisted of graded hemorrhagic pressure reductions from a control mean blood pressure of 140, to 125, 115, 100, 85, 75, and 50 mmHg. The dogs were then followed until they had taken back spontaneously 30% of their shed blood. At that time, the chilled blood was warmed to body temperature, filtered, and rapidly reinfused into the venous side of the circulation. Following reinfusion, the dogs were monitored during the following shock phases: normovolemic normotension and normovolemic hypotension. When the dogs could no longer maintain a blood pressure greater than or equal to 50 mmHg, the experiment was terminated.

Following graded hemorrhagic hypotension and circulatory shock in the control group of 10 dogs, a second group of 7 dogs was subjected to only the graded hemorrhagic pressure reductions during the intravenous administration of the angiotensin II antagonist, [Sar',Ala']angiotensin II, 5 μg/kg per min (Norwich Pharmacal Company, Norwich, N.Y.). A third group of six dogs was subjected to the same experimental shock protocol with the addition of intravenous angiotensin II...
infusion, 100 ng/kg per min (Hypertensin-CIBA) after the blood pressure first reached 50 mmHg. The infusion rate was 0.5 ml/min and was continued until termination of the experiment.

In these series of experiments, femoral artery and renal vein blood samples were collected in chilled tubes containing tetrasodium EDTA to produce a final concentration of 1 mg/ml; they were then centrifuged at 1,600 rpm for 30 min in an International refrigerated centrifuge. Two milliliters of plasma from each sample were frozen for later determination of renin activity and angiotensinogen concentration.

**Renin Activity**

Renin activity was determined by radioimmunoassay for angiotensin I (E. R. Squibb & Sons, Princeton, N.J.). The procedure was modified to maximize the production of angiotensin I during a 1-h incubation period. The raw plasma was adjusted to a pH of 5.5–6.0 with 0.5 N acetate buffer at pH 4.0. The procedure consisted first of a rapid gross adjustment to pH 5.5. After a 1-h equilibration period, the pH was checked for drifting and finally adjusted to a pH of 5.5–6.0. During this time, the samples were kept at 1-2°C with an ice water bath. The pH adjusted samples were then incubated for 1 h in a water bath at 38°C. Following incubation, the samples were returned to the ice water bath.

Angiotensinase and converting enzyme activities were minimized by the pH adjustment, the addition of sodium EDTA, and the reduced temperature.

**Renin Substrate, Angiotensinogen**

The determination of plasma angiotensinogen concentration involved a simple biochemical process; that is, to exhaust all of the plasma angiotensinogen with excess exogenous renin. Once this had been accomplished, the previously described radioimmunoassay method for angiotensin I was applicable. It must be recognized that prior to sampling, the incubated plasma samples must be diluted to bring the unknown samples within the range of the angiotensin I radioimmunoassay.

The amount and type of exogenous renin used in this assay was ascertained from the dose-response curve for the production of angiotensin I from normal dog plasma by either hog or dog renin. This reaction was maximum at 0.1 Goldblatt unit of either type of renin. Therefore, in this laboratory, angiotensinogen was assayed by exhausting the endogenous angiotensinogen with hog renin, the more concentrated of the two renins. The following reactants were added to the reaction tube prior to the 1-h incubation at 38°C: 0.1 ml EDTA-treated plasma sample, pH 5.5; and 0.1 Goldblatt unit of hog renin. After incubation, each sample was diluted to 2 ml with normal saline. At this point, the previously described radioimmunoassay for angiotensin I was conducted. Final calculation of angiotensinogen was corrected for the 1:20 dilution employed after incubation.

**Statistical Evaluation**

The overall effects of graded hemorrhagic hypotension and shock on the variables monitored were evaluated statistically by a treatment-times-subject analysis of variance design (24). This test examined the effect of graded hemorrhagic hypotension and shock on the measured variables, and showed statistically whether any of the physiological variables changed significantly during the experimental protocol. The following higher statistical designs: the Lindquist type-I analysis of variance design (24) and the three-dimensional factorial analysis of variance design (24), with the third dimension being replication, were used to evaluate the overall effects of shock and intravenous angiotensin II infusion, as well as the overall effects of graded hemorrhagic hypotension, and the intravenous administration of the angiotensin II antagonist. These tests permit the investigator to attribute more of the observed variance to a main effect and less to error variance or biological "noise," and to evaluate the simultaneous effect that more than one independent variable might have on the observed variables.

All calculations were performed by a digital PDP 11/20 computer (Digital Equipment Corporation, Marlboro, Mass.) and on a Wang electronic calculator, model 360 K (Wang Laboratories, Inc., Tewksbury, Mass.).

**RESULTS**

The results of this study will be presented in three major categories. The first category will consist of the patterns of peripheral renin activity and plasma angiotensinogen concentration during graded hemorrhagic hypotension (hypovolemic hypotension) and shock (normovolemic normotension and normovolemic hypotension). The second category will consist of the effect of the angiotensin II antagonist, [Sar1,Ala8]angiotensin II (5 μg/kg per min) on peripheral renin activity, renin release, and angiotensinogen concentration during graded hemorrhagic hypotension. The third category will consist of the effect of intravenous angiotensin II (100 ng/kg per min) on peripheral renin activity, renin release, and plasma angiotensinogen concentration during shock.

**Renin, Angiotensinogen Response to Hemorrhagic Hypotension and Shock**

**Peripheral renin activity.** Peripheral renin activity is defined for this study as the nanograms of angiotensin I produced during a 1-h incubation of a sample of peripheral arterial plasma at 38°C. Differences in activity are dependent upon the concentration of the circulating enzyme renin. Figure 2 illustrates the changes in peripheral renin activity during graded hemorrhagic hypotension and shock. As the systemic pressure was gradually reduced to 50 mmHg, there was a significant stepwise increase in peripheral renin activity (P < 0.001). Once the systemic pressure reached 50 mmHg, peripheral renin activity did not change significantly throughout the remainder of the experiment.

**Angiotensinogen concentration.** The angiotensinogen concentration represents the available substrate to the enzyme renin for the production of angiotensin I. Figure 3 illustrates the changes in the concentration of angi-
Renin, Angiotensinogen Response to Graded Hemorrhagic Hypotension During Angiotensin II Blockade With [Sar1, Ala8]angiotensin II

The renal and systemic hemodynamic effects of the vasoconstrictor angiotensin II (1 μg, iv) were completely blocked by the intravenous administration of the angiotensin II antagonist, [Sar1, Ala8]angiotensin II (5 μg/kg per min).

Peripheral renin activity. Figure 4 illustrates the patterns of peripheral renin activity during graded hemorrhagic hypotension in two groups of experimental animals. One group received no drugs; the other received intravenously the angiotensin II antagonist. The drug had a significant effect on the pattern of peripheral renin activity during the graded hemorrhagic pressure reduction ($P < 0.05$). Also, with respect to peripheral renin activity, both groups responded in a significantly different manner to the pressure changes ($P < 0.001$). In the group not receiving the drug, peripheral renin activity increased stepwise until the blood pressure reached 50 mmHg. In the group receiving the angiotensin II antagonist, peripheral renin activity increased stepwise to 100 mmHg, then decreased stepwise toward control from 100 to 50 mmHg.

Renin release. Renin release is defined as the difference between the peripheral arterial and renal vein renin activity multiplied by the total renal plasma flow per gram of kidney. Renin release increased significantly with the decrease in systemic blood pressure to 50 mmHg ($P < 0.005$). However, during the shock phase, postreinfusion, there was no change in the elevated renin release in these experimental animals.

Figure 5 depicts the patterns of renin release with and without the intravenous administration of the angiotensin II antagonist during graded hemorrhagic hypotension. There was no significant effect of the drug on renin release during graded hemorrhagic hypotension; however, there was a significant difference in the way each
group responded to the graded systemic pressure reduction ($P < 0.005$). In the group receiving the angiotensin II antagonist, renin release appeared depressed in the $85 - 50$ mmHg pressure range compared to the stepwise increase of renin release in the group not receiving the drug.

**Angiotensinogen.** As shown in Fig. 6, angiotensinogen concentration in the dogs receiving the angiotensin II antagonist intravenously decreased progressively as the systemic blood pressure was lowered stepwise by hemorrhage. The drug, $[\text{Sar}^1, \text{Ala}^8]$angiotensin II, allowed the concentration of angiotensinogen to decrease significantly ($P < 0.001$). Also, there was a significant pressure interaction ($P < 0.001$). In other words, both groups of dogs responded in a significantly different manner to the graded hemorrhagic pressure reductions. Angiotensinogen concentration decreased significantly only in the group receiving the angiotensin II antagonist.

**Renin, Angiotensinogen Response to Hemorrhagic Shock During Intravenous Angiotensin II**

In this study, the effect of intravenous angiotensin II during shock was evaluated as compared to the effect of only shock on the experimental animals. Drug-related changes in angiotensinogen concentration were analyzed by a three-dimensional analysis of variance design with the third dimension being replication (24).

**Peripheral renin activity.** Figure 7 displays the patterns of peripheral renin activity at various pressure levels during shock with and without intravenous angiotensin II. The response of peripheral renin activity was not significantly altered by the intravenous administration of angiotensin II. Also, the two groups of experimental animals responded in a similar manner at each pressure level. Therefore, there was no significant interaction between the response of each group to the pressure changes.

**Renin release.** Figure 8 illustrates the patterns of renin release during shock with and without intravenous angiotensin II. Statistically, there was no significant effect of the drug on the response of renin release. However, the two groups responded to the pressure changes during shock in a significantly different manner ($P < 0.025$). Renin release in the group receiving angiotensin II was depressed at $115$ mmHg and elevated at $50$ mmHg, while in the group not receiving the drug,
renin release was elevated at 115 mmHg and depressed at 50 mmHg.

Angiotensinogen. Figure 9 depicts the effect of intravenous angiotensin II on plasma angiotensinogen concentration during shock compared to the group not receiving the drug. Both groups of experimental animals responded in like fashion, with respect to this parameter, to the change in blood pressure. However, intravenous angiotensin II significantly increased the plasma concentration of angiotensinogen during shock ($P < 0.001$).

**Discussion**

Hemorrhage, hypotension, and shock are severe stresses for the regulatory mechanisms of the circulatory system to balance. Reflex vasoconstriction and circulating humoral agents reduce the circulatory capacity available to the remaining volume of blood, stimulate pump activity, and set into action mechanisms to restore the lost volume. The renin-angiotensin system is capable of contributing to both peripheral vasoconstriction and restoration of blood volume. Most reports in the literature (6, 8, 20, 31) agree that both normotensive hemorrhage and hypotensive hemorrhage stimulate the release of renal renin. There is some question, however, as to the role of the renin angiotensin system in the development of irreversible hemorrhagic shock, that point during prolonged hemorrhagic hypotension when the organism can no longer maintain the lowered blood pressure with the volume of blood present in the circulatory system.

The present studies suggest that the response of the renin-angiotensin system during graded hemorrhage reflects both the degree of local arterial pressure reductions and the graded withdrawal of vasomotor inhibition traveling from carotid baro- and cardiopulmonary receptors (29). In addition, the presence of a significantly elevated plasma renin activity as well as an adequate plasma concentration of angiotensinogen at 50 mmHg systemic blood pressure and during shock implicates a mechanism other than decreased plasma renin activity as a major contributor to the initiation of irreversible shock.

The response of the renin-angiotensin system depends not only upon the stimuli for renin release, but also upon the simultaneous negative feedback effects of angiotensin II on the continued release of renin (3, 9, 12, 30, 33, 34). Once the enzyme renin is released into the circulation, however, it reacts with its substrate, angiotensinogen, to release angiotensin I. It follows that as the circulating concentration of the enzyme renin increases during graded hemorrhagic hypotension, angiotensinogen concentration should decrease unless some mechanism exists to prevent a serious reduction in the circulating concentration of angiotensinogen.

It has been suggested recently that angiotensin II can directly stimulate the synthesis and release of angiotensin from the perfused rat liver (26). To evaluate the possibility that this proposed positive feedback mechanism for the maintenance of angiotensinogen might function in vivo during hemorrhagic hypotension, a competitive antagonist of angiotensin II [Sar$^1$,8-Ala$^2$] angiotensin II was infused intravenously (5 μg/kg per min) prior to and during graded hemorrhagic hypoten-

![Figure 8](image_url)

**Figure 8.** Renin release during shock with and without intravenous angiotensin II (100 ng/kg per min). Units are nanograms of angiotensin I produced per minute per gram of kidney. PR stands for pre-reinfusion period. Bars, $x \pm SE$.

![Figure 9](image_url)

**Figure 9.** Angiotensinogen concentrations during shock with and without intravenous angiotensin II (100 ng/kg per min). Units are nanograms of angiotensin I per milliliter. PR stands for pre-reinfusion period. Bars, $x \pm SE$. 

The development of this highly specific, competitive angiotensin II antagonist as well as some of its physiologic actions were described by Pals et al. (28) in 1971. They concluded that the drug competitively antagonized the systemic pressure effects of angiotensin II and exhibited no effect of its own. Subsequently, Johnson and Davis (23) reported that this angiotensin II antagonist (6 μg/kg per min) blocked not only the vasoconstrictive, but also the steriodogenic effects of angiotensin II.

The fact that the angiotensinogen concentration progressively decreased during graded hemorrhagic pressure reductions during the intravenous administration of the angiotensin II antagonist strongly suggests that endogenously produced angiotensin II plays a significant homeostatic role in the maintenance of plasma angiotensinogen concentration. However, the possibility should be considered that this observed positive feedback effect of angiotensin II on the site responsible for maintaining the normal concentration of plasma angiotensinogen might be secondary to a primary change in adrenocorticosteroid secretion. Slater et al. (32) have reported data suggesting that in addition to its vasoactive properties, angiotensin II can also augment the secretion of cortisol. If the animal is then treated with the angiotensin II antagonist, [Sar1,Ala8]angiotensin II, the secretion of cortisol induced by angiotensin II will be attenuated (22). Hasegawa et al. (19) have demonstrated that in the isolated perfused rat liver, cortisol can directly increase the synthesis and release of angiotensinogen. These authors (19) also suggest that rather than a primary individual effect of either angiotensin II or cortisol, the two substances might, instead, act synergistically to stimulate the further synthesis and release of angiotensinogen. These data presented by these different investigators as well as the recent direct positive-feedback data of angiotensin II on angiotensinogen synthesis and release reported by Nasjletti and Masson (26) suggest that the in vivo control of angiotensinogen might involve either or all of the following possibilities: a) a direct positive-feedback effect of angiotensin II on the hepatic site for synthesis and release of angiotensinogen, b) a secondary effect of angiotensin II acting through a primary effect on the secretion of adrenocorticosteroids, or c) a direct synergistic effect of both angiotensin II and adrenocorticoids on the hepatic site for synthesis and release of angiotensinogen.

A meaningful interpretation of the changes in renin activity and subsequently renin release during graded hemorrhagic hypotension in the presence of the angiotensin II antagonist is complicated not only by the significant reduction in the concentration of angiotensinogen, but also by the fact that the local negative-feedback control of renin release is eliminated. Together, however, these data collected during the continuous infusion of the angiotensin II antagonist give additional credence to the hypothesis of an angiotensin II-sensitive positive-feedback system for the maintenance of the plasma concentration of angiotensinogen.

Intravenous angiotensin II (100 ng/kg per min) was initially employed to test the viability and responsiveness of the renin-angiotensin system during shock. During post-reinfusion shock, renin release and peripheral renin activity did not change but remained significantly elevated. It was possible that the observed "leveling off" of peripheral renin activity and renin release during the shock phase of the protocol might be due to the modulating effect of endogenous angiotensin II on the further release of renin. The expected response to the intravenous angiotensin II was an inhibition of renin release, provided the stimulation of the negative-feedback control of renin release was not already maximal. The concentration of the infused drug was chosen, based on the published data of other investigators, as a dose that would demonstrate the negative-feedback control. In addition, it was a dose reported by Brown et al. (5) as not precipitating acute renal failure. If the system were still operative, exogenous angiotensin II should reduce the release of renin, to some extent, through further stimulation of the negative-feedback control mechanism. However, peripheral renin activity was not significantly affected by the intravenous angiotensin II, although visual inspection of these data suggests that the negative feedback effect of angiotensin II might still be functional. Renin release during shock in the dogs receiving intravenous angiotensin II responded to the pressure changes in a significantly different manner from renin release during shock in the dogs not receiving the drug. Examination of these data at each pressure level suggested that the significant interaction might be the result of an apparently depressed release of renin at 115 mmHg post-reinfusion. This decreased release of renin at 115 mmHg might be attributed to a more effective angiotensin II negative-feedback effect on renin release at this pressure level. However, at pressures below 100 mmHg the reflex release of renin as well as the local negative-feedback control of renin release is overshadowed by the dominant local mechanisms (15). Examination of the peripheral renin activity data at 115 mmHg post-reinfusion reflected the decrease in renin release seen during shock in the presence of intravenous angiotensin II.

During shock, the plasma concentration of angiotensinogen was increased significantly by the presence of exogenous angiotensin II. These data suggest for the first time in vivo, as was previously reported in the isolated perfused rat liver preparation (26), that angiotensin II might act as positive feedback on the liver for the synthesis and release of angiotensinogen.

In addition, these data suggest that under the conditions of these experiments angiotensin II is most likely responsible for the maintenance of plasma angiotensinogen concentration, as previously reported (1), during

![Diagram](http://ajplegacy.physiology.org/Downloadedfrom/10.2203/3.18.22.72)
hemorrhagic hypotension and shock. The primary feedback control of the renin-angiotensin-angiotensinogen system is illustrated in Fig. 10. When this system is stressed by graded hemorrhagic hypotension and shock, angiotensin II, the end product of the renin reaction, appears to control the excessive release of renin from the kidney and to prevent a decrease in the concentration of plasma angiotensinogen.

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