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Arterial Pressure Regulation during Hemorrhage: Homeostatic Role of Angiotensin II (38735)

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Recent studies indicate that angiotensin II plays an important role in the regulation of arterial pressure and renal blood flow in dogs with thoracic inferior vena cava constriction or sodium depletion (1–3). Infusion of a specific competitive antagonist of angiotensin II, 1-sarcosine-8-alanine-angiotensin II, significantly depressed mean arterial pressure and aldosterone secretion (1, 2) while plasma renin activity (1, 2) and renal blood flow (3) increased. Infusion of the antagonist also decreased arterial pressure in dogs with malignant hypertension secondary to renal arterial stenosis, characterized by elevated plasma renin activity (4, 5); however, infusion of 1-sarcosine-8-alanine-angiotensin II had no appreciable influence on arterial pressure in normal dogs (1, 2) or in dogs with chronic renal hypertension, with a normal plasma renin activity (4, 5). Thus, angiotensin II plays an important functional role in the maintenance and control of arterial pressure and renal blood flow in several experimental situations with clinical counterparts.

The experiments reported here were designed to evaluate the role of angiotensin II in another important experimental situation, namely, in the short-term, minute-by-minute regulation of arterial pressure in conscious dogs subjected to hemorrhage. Renin release is controlled, in part, by an intrarenal vascular receptor which is sensitive to small changes in mean arterial pressure (6, 7). Since hemorrhage increases plasma renin activity (6, 8, 9), one might anticipate a negative feedback relationship between arterial pressure and the renin–angiotensin system operating to counteract the hypotension induced by hemorrhage (8).

In the present study, it was hypothesized that during hemorrhage endogenous angiotensin II combines with its receptor sites in the smooth muscle of the peripheral arterioles to increase peripheral resistance and help maintain arterial pressure in the face of a reduced cardiac output. To evaluate this hypothesis, the competitive antagonist of angiotensin II, 1-sarcosine-8-alanine-angiotensin II, was given intravenously to conscious dogs which had established a new level of arterial pressure following hemorrhage.

Materials and Methods. Ten female hounds weighing 14–27 kg were used in this study. All animals were maintained on a diet which provided approximately 65 mEq of sodium and 55 mEq of potassium daily for at least 4 days prior to the day of the experiment; water was available *ad libitum*.

On the day prior to the acute experiment, the dogs were anesthetized with either Innovar (1.0 mg/20 lbs., intramuscularly; Pittman-Moore, Inc.) or sodium pentobarbital (30 mg/kg) and a catheter (Fr. 8 polyvinyl) was inserted under sterile conditions into the carotid artery which was ligated to retain the catheter. This catheter was used for recording arterial pressure and bleeding during the acute experiment. On the day of the acute experiment, the animals were brought to the laboratory and a catheter (PE 50) was inserted percutaneously into the saphenous vein for infusion of solutions. All experiments were performed on conscious animals; during each experiment the dog was lying quietly on the floor of the laboratory restrained only by a rope loosely fastened around the neck.

Control arterial pressure recordings were obtained for an hour via a pressure transducer attached to the carotid catheter, and arterial blood samples were obtained via the carotid catheter for determination of control plasma renin activity. The animal was hemor-

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rhaged 20 ml/kg of body wt over a period of less than 20 min from the carotid artery, and arterial pressure recording was continued for a period of 45 minutes; additional blood samples were obtained at the end of this 45-min posthemorrhage period. In four of six dogs, the pressor response to 1.0 μg of exogenous angiotensin II (Hypertensin, Ciba) was observed at 45–50 min after hemorrhage and prior to the infusion of the angiotensin II antagonist. After observing the pressor response to exogenous angiotensin II, an intravenous infusion of 1-sarcosine-8-alanine-angiotensin II[2] was begun at a rate of 6.0 μg/kg per min (in isotonic saline, 0.6 ml/min) and was continued for 30 min; additional blood samples for determination of plasma renin activity were obtained at the end of this infusion period, and the pressor response to 1.0 μg of exogenous angiotensin II was observed again. Recovery observations were made 60 min after the infusion of the angiotensin II antagonist was discontinued. As a control experiment the other four animals were not given 1-sarcosine-8-alanine-angiotensin II following hemorrhage; the time course of arterial pressure was monitored for 2 hr after hemorrhage in these four dogs.

The procedures for determination of plasma renin activity have been reported previously (10). Briefly, 10 ml samples of arterial blood were collected in 0.1 ml of 10% EDTA during each control, experimental and recovery period. The samples were cooled to 4° and the plasma removed after centrifugation. After the samples were prepared for the assay of renin by the method of Schneider *et al.* (10), they were assayed by the pressor response in the pentobarbital–anesthetized, pentolinium-blocked rat, with angiotensin II (Hypertensin, Ciba) as the standard. The presence of the angiotensin II antagonist in plasma does not influence the bioassay because the octapeptide is dialyzed out of the sample during preparation for incubation. Plasma renin activity is expressed as nanograms of angiotensin II per ml per 3 hr of incubation.

Student's *t* test for paired observations was used for statistical analysis of the data. Values are presented as means ± SEM.

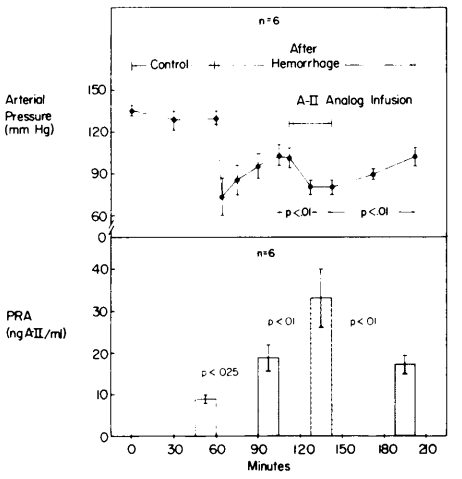

FIG. 1. Changes in arterial pressure and plasma renin activity (PRA) before and after hemorrhage over a period of less than 20 min (20 ml/kg body wt). The angiotensin II analog, 1-sar-8-ala-angiotensin II, was given intravenously at a rate of 6.0 μg/min per kg for 30 min. Values are means ± SEM. PRA increased significantly after hemorrhage ($P < 0.025$) and still further during A-II analog infusion ($P < 0.01$) and then returned to the preinfusion level which was significantly higher than the control ($P < 0.025$).

Results. The data for the six dogs which were given 1-sarcosine-8-alanine-angiotensin II following hemorrhage are summarized in Fig. 1. Hemorrhage (20 ml/kg of body wt) decreased the mean arterial pressure from 130 ± 4 mmHg to 73 ± 13 mmHg ($P < 0.005$); however, compensatory mechanisms partially restored the mean arterial pressure to 103 ± 7 mmHg ($P < 0.01$ compared to the prehemorrhage control pressure) during the 45 min following hemorrhage. During this same 45-min period, plasma renin activity increased from the prehemorrhage control of 8.8 ± 1.0 ng angiotensin II/ml to 18.8 ± 3.6 ng/ml ($P < 0.025$). At this time, the pressor response to 1.0 μg of exogenous angiotensin II averaged 22 ± 3 mmHg (range 12–27 mmHg) in four of the six dogs. Initially, infusion of 1-sarcosine-8-alanine-angiotensin II at 6.0 μg/kg min^{-1} resulted in a variable (range 0–25 mmHg) and transient pressure rise of 13 ± 8 mmHg ($P < 0.01$) which returned to the baseline control pressure within 3–5 min. The angio-

[2] Generously supplied by Norwich Pharmacal Company, Norwich, New York.

tensin II analog then decreased the mean posthemorrhage arterial pressure from 102 ± 7 mmHg to 80 ± 6 and 80 ± 6 mmHg after 15 and 30 min of infusion ($P < 0.01$ for both values). During the infusion period, the plasma renin activity increased from its posthemorrhage value of 18.8 ± 3.6 ng angiotensin II/ml to 32.9 ± 6.9 ng/ml ($P < 0.01$). Immediately following the infusion period, the pressor response to 1.0 μg of exogenous angiotensin II was completely obliterated. Sixty minutes after cessation of the infusion of the angiotensin II antagonist, the mean arterial pressure had recovered to 102 ± 6 mmHg which was not significantly different from the posthemorrhage, preinfusion arterial pressure of 102 ± 7 mmHg; during the same 60 min recovery period, plasma renin activity returned to 16.9 ± 2.3 ng angiotensin II per ml which also was not different ($P > 0.2$) from the pre-infusion value of 18.8 ± 3.6 ng/ml.

In the control experiment, four dogs did not receive the angiotensin II antagonist; hemorrhage decreased the mean arterial pressure from 111 ± 6 mmHg to 50 ± 3 mmHg ($P < 0.01$). However, mean arterial pressure was rapidly restored to 74 ± 4 mmHg during the 15 min following hemorrhage and continued to increase slowly during the remaining 105 min of the experiment, reaching a mean of 92 ± 6 mmHg at 120 min after hemorrhage. Plasma renin activity increased from 6.3 ± 0.7 to 9.9 ± 0.5, 11.1 ± 1.2 and 13.0 ± 2.2 ng/ml at 60, 90 and 120 min after hemorrhage ($P <$ 0.05 for all three values). No consistent behavioral changes were observed in response to hemorrhage in either experiment.

Discussion. Analyses of short-term arterial pressure regulation during hemorrhage have stressed the importance of the carotid baroreceptors and reflex activation of the sympathetic nervous system as a compensatory mechanism (11). Although understood less completely, the kidney's role in arterial pressure maintenance during hemorrhage has been described also (8, 9). Sapirstein *et al.* (8) postulated that during hemorrhage a renal humoral mechanism, possibly the renin–angiotensin system, operates to help preserve an adequate arterial pressure. However, no conclusive evidence was presented to identify clearly this renal humoral mechanism as the renin–angiotensin system.

This study presents new data which strongly support the concept of the renin-angiotensin system as an important determinant of arterial pressure during hemorrhage. Hemorrhage of conscious dogs (20 ml/kg of body wt) decreased their mean arterial pressure significantly, but the arterial pressure was partially restored following hemorrhage (Fig. 1). During this same period of time, plasma renin activity increased significantly (Fig. 1) and the subsequent infusion of a specific competitive antagonist of the vascular action of angiotensin II, 1-sarcosine-8-alanine-angiotensin II, resulted in a significant decrease of the mean arterial presure after 15 and 30 min of infusion (Fig. 1). Within 60 min after the infusion of the angiotensin II antagonist was stopped, the mean arterial pressure had recovered to the preinfusion, posthemorrhage arterial pressure level. Since the infusion of 1-sarcosine-8-alanine angiotensin II into normal dogs for a comparable period of time (45 min) had no appreciable influence on arterial pressure (1, 2), the present data suggest strongly that increased plasma levels of angiotensin II play an important role in the regulation and maintenance of arterial pressure during hemorrhage.

Initially, infusion of the angiotensin II analog resulted in a small (13 ± 8 mmHg), variable pressure rise which returned to the baseline pressure within 3–5 min. This slight pressor activity of the analog has been observed in other studies (1, 2, 5) and probably reflects a direct action of the analog on the vascular smooth muscle. This initial pressor response to the angiotensin II analog does not obscure the striking drop in arterial pressure that occurred throughout most of the infusion period and which reflected the antagonism of angiotensin II at the arteriolar level.

In a recent study, Jakschik *et al.* (12) presented similar results obtained with different specific inhibitors of the renin–angiotensin system; however, unlike the present study, these investigators studied the role of angiotensin II during hemorrhage in the anesthetized dog which was bled to and

maintained at a fixed arterial pressure of 45–50 mmHg. Under these experimental conditions, Jakschik *et al.* (12) reported that endogenous angiotensin II blockade resulted in an arterial pressure fall of about 20 mmHg.

Infusions of exogenous angiotensin II into the renal artery have been shown to decrease renin release in dogs (13). Thus, the observed rise in plasma renin activity during infusion of 1-sarcosine-8-alanine-angiotensin II may have resulted either from blockade of the angiotensin II feedback inhibition of renin release (13) or from activation of the renal vascular receptor secondary to the fall in arterial pressure (6, 7). The elevation of plasma renin activity during infusion of 1-sarcosine-8-alanine-angiotensin II has been observed in other studies (1, 2).

Summary. The role of the renin-angiotensin system in the maintenance of arterial pressure following hemorrhage was studied in conscious dogs. Hemorrhage (20 ml/kg body wt) decreased the mean arterial pressure, but compensatory mechanisms partially restored the arterial pressure toward normal. Plasma renin activity increased more than twofold following hemorrhage. To evaluate the role of endogenous angiotensin II in this compensatory response, a specific competitive antagonist of angiotensin II, 1-sarcosine-8-alanine-angiotensin II, was infused intravenously at 6.0 μg/kg min^{-1} for 30 min; the mean posthemorrhage arterial pressure decreased from 102 $\pm$ 7 mmHg to 80 $\pm$ 6 mmHg after 15 and 30 min of analog infusion ($P < 0.01$ for both values). After a recovery period of 60 min, arterial pressure returned to pre-infusion levels. These results suggest that angiotensin II plays an important role in the short-term maintenance of arterial pressure following hemorrhage in the conscious animal.

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