Changes in catecholamine and angiotensin levels in the cat and dog during hemorrhage

RODNEY C. HALL AND ROBERT L. HODGE
Department of Human Physiology and Pharmacology, University of Adelaide, Adelaide, South Australia 5001

Several studies have been carried out to determine separately the effects of hemorrhage on the circulating levels of catecholamines, renin, or angiotensin. The role of the sympathetic nervous system in hemorrhage has been reviewed recently (4). It is well established in the dog that hemorrhage severe enough to cause systemic hypotension is associated with a rise in catecholamine levels (5, 8, 19, 20, 22). Hemorrhage, in these studies, was sufficient to cause blood pressure to fall to 40-50 mm Hg. Moderate falls in blood pressure were also associated with a rise in catecholamine levels (9). In a study on the effects of slow hemorrhage in the dog (21), a gradual rise in catecholamine levels was observed commencing after the onset of hypotension.

The renin-angiotensin system of the dog during hemorrhage has been studied by several workers. Severe hemorrhage is consistently associated with a rise in angiotensin levels (17) or renin levels (2). Hemorrhage insufficient to cause a fall in arterial pressure was also associated with a rise in angiotensin levels (12, 15). A concurrent estimation of renin and angiotensin levels showed that a rise in both occurred during hemorrhage in the absence of any fall in systemic blood pressure (3).

The response of the cat to hemorrhage has been studied less extensively. A rise in catecholamines in adrenal vein blood was observed following severe hypotension (13). These workers also observed a rise in catecholamine levels during slow, intermittent hemorrhage before blood pressure had fallen significantly. They suggested that these results may provide evidence that adrenomedullary stimulation might occur in the cat before the onset of hypotension. No studies have been reported on changes in angiotensin levels in the cat during hemorrhage.

A previous study (11) showed a species difference between the cat and dog in the humoral responses associated with the cardiovascular effects of endotoxins. However, in these two species, endotoxins have different hemodynamic effects which may secondarily modify other stimuli to the release of catecholamines and angiotensin; thus, the cat shows a marked initial rise in central venous pressure, while the dog shows a progressive fall. These observations cannot, therefore, establish a definite species difference.

The present study was carried out to determine the changes in circulating levels of angiotensin and catecholamines in these species during a similar and reproducible cardiovascular stress, hemorrhage. By using a continuous superfusion technique (18), a simultaneous estimation of both catecholamines and angiotensin was carried out, and the timing of the changes in angiotensin and catecholamines in relation to changes in two cardiovascular parameters, central venous pressure and arterial pressure, was determined.

METHODS

Cats of either sex were anesthetized with chloralose 80 mg/kg iv after induction with ether and halothane. Dogs were anesthetized with chloralose 100 mg/kg iv after induction with thiopentone 25 mg/kg iv. Tracheostomy was carried out on all animals.

All dogs were respired artificially by intermittent positive-pressure ventilation. Cats breathed spontaneously.

Arterial pressure was recorded from the carotid or femoral artery using a Statham P23 AC pressure transducer. Central venous pressure was recorded using a Statham P23 BC transducer and a catheter passed into the thoracic cavity via the external jugular vein. The position of the catheter was determined during the experiment by recording venous waves and, in many cases, right ventricular traces were obtained. In these cases, the catheter was withdrawn a few millimeters. Finally, at the end of the experiment, the position of the catheter was ascertained at post-mortem.
Superfusion Circuit

Animals were heparinized (1,000 IU/kg iv), and blood was taken from a carotid or femoral artery and pumped over the assay tissues at a constant rate by a roller pump at 10 and 15 ml/min in cats and dogs, respectively. Before superfusing the tissues, the blood passed through a water jacket heated to 38 C. Having superfused the tissues, the blood was collected in a reservoir and returned to the animal by gravity via a femoral or external jugular vein. To minimize trauma to the blood polyethylene and silicone tubing was used throughout the extracorporeal circuit. The volume of the extracorporeal circuit was 8-10 ml for cats and 15-25 ml for dogs.

Bioassay Tissues

The tissues selected for this study were the rat colon and the rat stomach strip. At physiological concentrations, the former is virtually specific for angiotensin (18), to which it responds by contracting. The rat colon preparation responds to changes in angiotensin levels of the order of 0.3-0.6 ng/ml. The inhibitory effects of catecholamines on the rat colon were blocked by the intraluminal infusion of propranolol 250 μg/min (12). The rat stomach strip is preferentially sensitive to catecholamines and responds by relaxation. No other vasoactive substance causes a relaxation of the rat stomach strip. Using these tissues, the superfusion technique offers a means of continuously assaying changes in the circulating levels of catecholamines. In our hands, the sensitivity of the assay method compares favorably with chemical methods (6, 7), being able to detect changes in levels of the order of 0.5-1.0 ng/ml. Furthermore, apart from the initial priming volume of the extracorporeal circuit, no further blood need be removed, in contrast to the chemical methods which require from 10 to 20 ml of blood for each estimation.

Angiotensin causes a variable contraction of the rat stomach strip. Thus, generation of angiotensin may interfere with the relaxation of the rat stomach strip if catecholamines were released simultaneously. However, the degree of interference in each experiment was measured during the calibration of the tissues with intravenous angiotensin. Thus, the dose of angiotensin which would cause a contraction of the stomach strip was precisely determined. Furthermore, the response of the rat stomach strip to catecholamines is more rapid than the response to angiotensin so that a change in catecholamine levels is recorded before any interference can occur. Control experiments carried out in a previous study (1) established that for the levels of catecholamines and angiotensin observed in the present study, the sensitivity of the rat stomach strip and blocked rat colon to catecholamines and angiotensin, either alone or together, did not change.

The tissues were calibrated by intravenous infusions of epinephrine or angiotensin. This assay technique measures only changes in the circulating levels of hormones; such changes are therefore expressed in terms of equivalent responses from intravenous infusions of the appropriate hormone. Since the rat stomach strip was used, differentiation between epinephrine and norepinephrine was not possible (18). The response of the rat colon measures the rate of generation of angiotensin which is secondary to a release of renin (3).

Drugs

Sodium thiopentone (intraval sodium, May and Baker, Ltd.) was prepared in 0.15 M saline. α Chloralose (British Drug Houses, Ltd.) was prepared as a 1% solution in 0.15 M saline.

Heparin (Commonwealth Serum Laboratories), 5,000 IU/ml, was administered intravenously in a dose of 1,000 IU/kg.

Epinephrine (Sigma) was prepared in 0.15 M saline adjusted to pH 5.6 with ascorbic acid.

Angiotensin (β-asparaginyl amide, Hypertensin, Ciba) was prepared in 0.15 M saline.

Experimental Groups

Effect of rate of hemorrhage (7 cats, 5 dogs). Each animal was hemorrhaged at two rates—a mean of 0.5 ml/kg per min (slow hemorrhage) and 3.0 ml/kg per min (fast hemorrhage). The mean volume of blood removed for both rates was 8.6 ml/kg. After each hemorrhage the blood removed was kept in siliconized containers at 38 C prior to retransfusion 10-15 min later. The interval between successive hemorrhages was 20-40 min. The sequence of hemorrhages was varied from animal to animal.

Fast hemorrhage from both species was from an artery, as was slow hemorrhage in dogs. Slow hemorrhage in cats was carried out from a vein for technical reasons.

Effect of volume of hemorrhage. Five additional dogs were subjected to continuous hemorrhage at a mean rate of 1.0 ml/kg per min until 25 ml/kg had been removed.

RESULTS

Group 1. Rate of Hemorrhage

The results obtained from a single experiment on a cat are shown in Fig. 1. Both slow and fast hemorrhage induced a fall in the base line of the rat stomach strip and of the unblocked rat colon, indicating a rise in circulating catecholamines, and a contraction of the blocked rat colon, indicating a generation of angiotensin. In this experiment also, the dose of epinephrine required to overcome the competitive β-blockade by propranolol of the rat colon is 0.5-1.0 μg/min. This is in excess of the levels of catecholamines estimated during hemorrhage which therefore would not be expected to interfere with the assay of angiotensin. A similar degree of β-blockade was established in all other experiments.

The changes in blood pressure, central venous pressure, angiotensin, and catecholamine levels in cats and dogs are shown in Fig. 2. The mean changes ± 1 se are plotted for both slow and fast rates of hemorrhage. The statistical treatment of the results, including changes within species and between species, is shown in Table 1.

In all experiments, the return of the shed blood was associated with a return of the tissues to the prehemorrhage baseline, indicating a return of circulating hormone levels to
CATECHOLAMINE AND ANGIOTENSIN LEVELS IN HEMORRHAGE

Effect of Hemorrhage on Cats

Slow hemorrhage. During slow hemorrhage there was no significant change in arterial pressure. The change in central venous pressure was variable. Thus, there was a rise in one cat, no change in two, and a fall in four. The mean change was a fall of 5 mm Hg; this was not significant. However, hemorrhage was associated with a significant elevation in both catecholamine and angiotensin levels. The mean rise in secretion rate of catecholamines was equivalent to 81 ng/kg per min (P < .01), and that for angiotensin was 17.6 ng/kg per min (P < 0.001).

Fast hemorrhage. During fast hemorrhage there was a significant fall in arterial pressure (P < 0.01) but no significant fall in central venous pressure. The levels of angiotensin generated during fast hemorrhage were the same as those during slow hemorrhage. There was now, however, a

control values. However, the time course of the return of the tissues to the control levels varied for the rat stomach strip and the rat colon, the former occurring within 5 min, while the latter took 15–30 min. This is presumably a reflection of the half-lives of epinephrine and renin in the circulation.

Effect of Hemorrhage on Dogs

Slow hemorrhage. During slow hemorrhage there was no significant change in arterial pressure. The change in central venous pressure was variable. Thus, there was a rise in one cat, no change in two, and a fall in four. The mean change was a fall of 5 mm Hg; this was not significant. However, hemorrhage was associated with a significant elevation in both catecholamine and angiotensin levels. The mean rise in secretion rate of catecholamines was equivalent to 81 ng/kg per min (P < .01), and that for angiotensin was 17.6 ng/kg per min (P < 0.001).

Fast hemorrhage. During fast hemorrhage there was a significant fall in arterial pressure (P < 0.01) but no significant fall in central venous pressure. The levels of angiotensin generated during fast hemorrhage were the same as those during slow hemorrhage. There was now, however, a
rise in catecholamine levels equivalent to 156 ng/kg per min above control levels. This was significantly greater than those observed during slow hemorrhage \((P < 0.05)\). The failure of central venous pressure to fall during fast hemorrhage may be due to the associated relatively high circulating levels of catecholamines. If this dose of epinephrine is administered intravenously to a nonhemorrhaged cat, there is invariably a rise in central venous pressure of the order of 5–10 mm H\(_2\)O.

**Effect of Hemorrhage in Dog**

*Slow hemorrhage.* Slow hemorrhage was associated with a small but significant fall in central venous pressure \((P < 0.01)\), but no significant change in arterial pressure. There was a significant rise in the circulating levels of angiotensin \((P < 0.001)\) equivalent to a generation rate of 15.5 ng/kg per min. There was no rise in catecholamine levels observed in any dog.

*Fast hemorrhage.* Fast hemorrhage caused a significant fall in both central venous pressure \((P < 0.001)\) and arterial pressure \((P < 0.01)\). The fall in central venous pressure was not greater than that observed during slow hemorrhage. There was no significant difference between the angiotensin generated during fast as compared with slow hemorrhage. However, there was now a significant elevation of catecholamine levels during fast hemorrhage \((P < 0.01)\), being equivalent to 72.8 ng/kg per min.

**Comparison of Cats and Dogs**

There was no difference between species in the change in arterial pressure during slow or fast hemorrhage. There was a more marked fall in central venous pressure in dogs, as compared with cats, at both rates of hemorrhage. This was, however, not significant \((\text{slow hemorrhage } 0.5 > P > 0.4; \text{ fast hemorrhage } 0.1 > P > 0.05)\). There was no difference between the angiotensin levels generated in either species at the slow or fast rate of hemorrhage. Slow hemorrhage was not associated with a rise in catecholamine levels in the dog, while there was a significant rise in cats. This species difference also showed up at fast rates of hemorrhage when the dog now produced catecholamine levels of about 73 ng/kg per min, but the cat produced 156 ng/kg per min.

**Group 2. Progressive Hemorrhage**

Animals in this group were subjected to a slow, continuous hemorrhage. The mean changes \((\pm 1\, \text{SE})\) in arterial pressure, central venous pressure, angiotensin, and catecholamine levels are shown in Fig. 3.

The first parameter to change was central venous pressure, which fell significantly after the removal of 5 ml/kg. This was associated with a rise in the circulating levels of angiotensin which was significant \((P < 0.05)\) after 10 ml/kg had been removed. There was, at this time, no fall in arterial pressure. Further, angiotensin was generated as hemorrhage progressed until a mean generation rate of 17 ng/kg per min was reached. At this stage, 15 ml/kg of blood had been removed, and the fall in arterial pressure was 9 mm Hg from a mean control level of 135 mm Hg \((\pm 4.2)\). With continuing hemorrhage to 25 ml/kg, the arterial pressure fell to 80 mm Hg \((\pm 12)\), but the level of angiotensin showed no further significant rise.

There was no rise in the circulating levels of catecholamines until the arterial pressure had fallen to 105 mm Hg \((\pm 10.7)\). As arterial pressure continued to fall, there was a further rise in catecholamine levels, which reached 94 ng/kg per min \((P < 0.05)\), at which time 25 ml/kg of blood had been removed and the arterial pressure was 80 mm Hg. If epinephrine is administered to nonhemorrhaged dogs in a dose of 84 ng/kg per min, there is a small pressor response ranging from 8 to 15 mm Hg and an invariable rise in central venous pressure of the order of 5–10 mm H\(_2\)O.

**DISCUSSION**

The results obtained by the simultaneous estimation of circulating levels of catecholamines and angiotensin are, in general, in agreement with the results of previous workers who considered each hormone separately. Since a continuous estimation was carried out, it is possible not only to relate the renin-angiotensin and sympathoadrenal systems to the severity of hemorrhage, but also to establish the time
relationship of the release of each hormone. Our results show that the relative importance of the renin-angiotensin and sympathoadrenal systems in response to a given stress cannot be assessed if the stress imposed is severe, since a fall in arterial pressure to 40–50 mm Hg will activate both systems simultaneously. It is thus difficult to interpret the results obtained from animals in which severe hypotension is administered as an intravenous infusion to a non-hemorrhaged dog, it has a minimal pressor effect. This raises, then, the question of the role of angiotensin in circulatory homeostasis, and it certainly appears that its effect on blood vessels is of minor importance, unless one postulates a selective action on a specific vascular bed, such as the kidney. In this respect, it is interesting to note that in the few studies in which low doses of angiotensin have been used, the renal response is an antinatriuresis and a fall in glomerular filtration rate (10, 14, 16). The well-documented stimulation of aldosterone release by angiotensin (10) could serve as a longer term homeostatic mechanism provided cardiovascular disturbances were not excessive. Furthermore, the demonstration of release of antidiuretic hormone by low doses of angiotensin (1) raises the possibility that the physiological role of angiotensin may also extend to water balance.

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