Effect of Angiotensin-Converting Enzyme Inhibitor (SQ 20881) on the Plasma Concentration of Angiotensin I, Angiotensin II, and Arginine Vasopressin in the Dog during Hemorrhagic Shock

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SUMMARY The effect of an angiotensin-converting enzyme inhibitor on the circulating levels of angiotensin I, angiotensin II, and arginine vasopressin was studied in dogs subjected to hypotensive hemorrhagic shock. In dogs subjected to hemorrhage but not given the inhibitor, angiotensin II rose 20-fold (from 69 to 1,343 pg/ml of plasma), whereas in dogs subjected to hemorrhage but pretreated with the inhibitor, angiotensin II rose only 2-fold (from 92 to 171 pg/ml of plasma). In the pretreated dogs angiotensin I rose 30-fold (from 108 to 3,232 pg/ml of plasma). There was no statistically significant difference between the vasopressin levels found in the untreated dogs and the levels found in dogs given the inhibitor (1,016 and 1,095 pg/ml of plasma). Of the 15 dogs in the untreated group, five died before retransfusion was completed (four of cardiac failure and one of cardiac arrhythmia); none of the 10 dogs in the inhibitor-treated group died. These observations suggest that the very high levels of angiotensin II observed following severe hemorrhage do not contribute significantly to the increased secretion of vasopressin and that the inhibitor protects against death, possibly by suppressing the very high blood levels of angiotensin II observed following this type of experimental hemorrhagic shock.

IT IS WELL established that hypotensive hemorrhage in the dogs causes a rise in plasma renin concentration,1 renin activity,2 angiotensin3-5 and vasopressin6-7. Angiotensin II and vasopressin are both powerful vasoconstrictor peptides and a role for them in the development of irreversible hemorrhagic shock has been suggested: mesenteric vasoconstriction following hemorrhage in the cat was markedly reduced after nephrectomy and hypophysectomy.8 More recently Errington and Rocha e Silva9-11 and Erdös et al.12 have demonstrated increased survival following hemorrhage in dogs with experimental diabetes insipidus and in dogs treated with angiotensin-converting enzyme inhibitor SQ 20881.

Methods used to measure angiotensin and vasopressin in hemorrhage experiments have for the most part been based on bioassay techniques. Scornik and Paladini3 used a bioassay for angiotensin which is incapable of distinguishing angiotensin I from angiotensin II. Changes in blood angiotensin II concentrations were measured by Regoli and Vane4 and Jakschik et al.2 by the blood-bathed organ technique. This has the advantage of continuous monitoring of changes in angiotensin concentrations, but cannot measure basal levels. To date there have been no studies reporting specific measurements of angiotensin I and angiotensin II as well as vasopressin in experimental hemorrhage in dogs. This paper describes the measurement by radioimmunoassay of all three peptides in dogs subjected to hemorrhagic shock, using a modified Wiggers preparation, with and without prior treatment with the nonapeptide angiotensin-converting enzyme inhibitor (SQ 20881). The effectiveness of the inhibitor to suppress angiotensin II levels and to improve survival rates is demonstrated.

Methods

MATERIALS

Angiotensin-converting enzyme inhibitor nonapeptide, pGlu-Trp-Pro-Arg-Pro-Glu-Ile-Pro-Pro-OH (SQ 20881) was obtained from the Squibb Institute for Medical Research, Princeton, New Jersey. [Asp',Ile5]angiotensin I, Research Standard A (71/328), and [Asp',Ile5]angiotensin II, Research Standard A (71/302), were both obtained from the Medical Research Council Institute for Biological Standards and Control, Holly Hill, Hampstead, London. [Asn',Val5]angiotensin II was Hypertensin, obtained from Ciba Laboratories, Horsham, Sussex, England. [Arg8]vasopressin (150 IU/ml) was a synthetic preparation supplied by A.B. Ferring, Malmo, Sweden. This preparation yielded only a single detectable spot following high voltage electrophoresis using Whatman No. 1 paper and a buffer system of pyridine-acetic acid-water (2.3:0.6:97 vol/vol) pH 5.6. The peptide was detected with 1% ninhydrin in acetone, 1% cadmium acetate in 50% acetic acid (85:15 vol/vol). Vasopressin standard was stored at −20°C in acetic acid (0.05 M), pH 3–3.5, at 1 μg/ml in plastic containers. Dilutions were made in the appropriate radioimmunoassay buffer just before use. Chemically pure [Arg8]vasopressin has been shown to contain 400 IU/mg;13 thus 1 μU is equivalent to 2.5 pg.
**ANIMAL EXPERIMENTS**

Experiments were performed on 30 healthy adult grayhounds weighing 25.6 ± 0.8 kg and pretreated with an elemental diet for 72 hours. The diet consisted of a fluid mixture of amino acids, fat, carbohydrate, electrolytes, and vitamins, made up to a volume of 1 liter/day for an average 30-kg dog. This preparation has been shown to minimize gastrointestinal hemorrhage following canine hemorrhagic shock.\(^{14,15}\) The precise mechanism of this protective role remains uncertain. Anesthesia was induced with sodium thiopental (20 mg/kg) by intravenous administration. Auffed endotracheal tube was passed and anesthesia was maintained with halothane delivered by Fluotec vaporizer, set at 0.5–1.0%. Oxygen at a flow rate of 4 liters/min was passed through the vaporizer as the carrier gas, and nitrous oxide at a flow rate of 6 liters/min was added distal to the vaporizer to dilute the halothane to a maximum of 0.4% and to provide an inspired oxygen concentration of 40%. Polyethylene catheters were inserted into the right femoral artery and vein. A large metal cannula was inserted into the left femoral artery and connected via tubing to an overhanging siliconized glass reservoir. Immediately thereafter, heparin (2,500 U) was administered intravenously and this was repeated at 2-hour intervals until the end of the experiment. Blood pressure was measured with a capacitance transducer and recorded on an ink-writing recorder.

After completion of instrumentation and systemic heparinization, an average period of 45 minutes was allowed for stabilization before the first basal blood sample was taken for hormone assay. After the first basal sample, 300 ml of blood was removed from all dogs, including the controls. This was used as a fluid load to test left ventricular function before and after shock. The second basal sample was taken just before the larger hypotensive hemorrhage. Hypotensive hemorrhage was induced by bleeding from the left femoral arterial cannula into the overhanging sealed glass reservoir containing 300 ml of acid-citrate-dextrose (ACD) or citrate-phosphate-dextrose (CPD) anticoagulant solution in saline. The mean arterial blood pressure was reduced to 35–40 mm Hg over a period of 2.5 hours by alteration in the height of the reservoir which was in full communication with the femoral artery. Retransfusion of the shed blood was achieved over a period of 15–30 minutes. Retransfusion proceeded until the right atrial pressure (used as an index of the adequacy of intravascular fluid volume replacement) had attained levels equivalent to those present immediately before hemorrhage.

Dogs which survived retransfusion were killed, under anesthesia, after a 2-hour period by an intravenous injection of potassium chloride, and postmortem examination was performed.

Three groups of dogs were studied.

**Group 1.** This was a control group and consisted of five dogs which were subjected to all the anesthetic and surgical procedures described but which were not bled to 35 mm Hg. Blood samples for angiotensin II and AVP measurement only were taken from the right femoral artery at hourly intervals, starting at least 45 minutes after the surgical procedures were completed (Fig. 1a).

**Group 2.** The 15 dogs in this group were subjected to the complete hemorrhagic shock procedure. Blood samples for angiotensin II and AVP only were taken at intervals throughout the experiment, as shown in Figure 1b.

**Group 3.** A total of 10 dogs in this group also were bled as described, but immediately before and following completion of the hemorrhage, when blood pressure had settled at 35 mm Hg, the dogs were given the angiotensin-converting enzyme inhibitor (SQ 20881) via the femoral artery. A total dose of 1 mg/kg in 10 ml of dextrose was injected, 5 ml on each occasion. Again blood for the estimation of angiotensin II, AVP, and also, in this group, angiotensin I was taken at intervals indicated in Figure 1c.

**BLOOD SAMPLING AND ASSAYS**

Angiotensin II was measured by the radioimmunoassay technique of Dusterdieck and McElwee.\(^{14}\) The angiotensin II antiserum cross-reacted only 0.6% with angiotensin I. Results quoted are corrected for a mean recovery of 85% (range, 75–93%). The recovery was determined on six separate occasions by adding 1,000 pg of [Ile\(^5\)]angiotensin II to duplicate samples of dog blood (10 ml).

Angiotensin I was measured by a modification\(^{17}\) of the radioimmunoassay method of Waite.\(^{18}\) The angiotensin I antiserum used had an undetectable cross-reaction with [Ile\(^5\)]angiotensin II (< 0.0001%). Blood concentrations

![Diagram of blood sampling protocol](http://circres.ahajournals.org/)

**FIGURE 1.** Diagram of blood sampling protocol. a: six samples taken from the control group of dogs (group 1) at hourly intervals, the first taken at least 45 minutes after completion of surgery. b: six samples taken from the untreated bled group (group 2). 1, basal; 2, at 5 minutes before SQ 20881 injection; 2a, 5 minutes post-SQ 20881 and prehemorrhage; 3, end of hemorrhage; 4–6, as above.
of angiotensin I were converted to plasma concentrations by an adjustment based on the hematocrit value for each sample.

The mean recovery of angiotensin I (5,000 pg) added to blood samples on six separate occasions was 67% (range, 56–77%). All plasma concentrations are given corrected for a recovery of 67%.

Arginine vasopressin was measured by the radioimmunoassay as described for dog plasma.19 Plasma concentrations are given corrected for a mean recovery of 60% (range, 55–67%; n = 7).

**ANGIOTENSIN II IN DOG BLOOD: Val⁵ OR Ile⁵**

A 10-ml arterial blood sample from one dog in the untreated group taken at the end of the shock period, was extracted in the usual way for angiotensin II. This extract then was chromatographed on Whatman 3-mm paper using a solvent of butanol, acetic acid, water, pyridine (15:3:12:10 vol/vol), which separates angiotensin II from its C-terminal hepta-, hexa-, and pentapeptide fragments.20–22 The paper was dried, and the region containing the angiotensin II was identified relative to marker angiotensin II run on adjacent lanes. This area of paper was cut out and eluted with 10% methanolic ammonia. The methanolic ammonia eluate was dried, dissolved in 0.05 M tris(hydroxymethyl)aminomethane (Tris), pH 7.5 (1 ml), and duplicate 100-μl samples were removed and assayed for angiotensin II using an antiserum which cross-reacted 100% with both the Val⁵ and Ile⁵ analogues (antiserum A). The remainder of the extract was reanalyzed with a different antiserum which cross-reacted less than 10% with the Ile⁵ analogue (antiserum B). The value for the percentage bound at each dilution was plotted against the angiotensin II content as found using antiserum A. This curve was compared with those obtained for standard Ile⁵- and Val⁵-angiotensin II, also constructed using antiserum B.

**ANGIOTENSIN II AND ITS C-TERMINAL METABOLITES**

A further sample of blood (10 ml) taken at the end of the shock period was extracted for angiotensin II and subjected to chromatography as described above. On this occasion the chromatogram was dried and the lane containing the plasma extract cut into 1-cm strips which were eluted separately with methanolic ammonia. The eluates were dried and assayed using an antiserum which cross-reacted 100% with the octapeptide and its C-terminal hepta-, hexa-, and pentapeptide fragments.

**GEL FILTRATION OF VASOPRESSIN EXTRACT**

A vasopressin extract from a blood sample obtained at the end of the hemorrhage was passed through a Sephadex G-10 column (30 × 1 cm). The column was equilibrated and eluted with 0.02 M acetic acid, and 2-ml fractions were collected. From each fraction 200 μl were dried and assayed for arginine vasopressin. The elution profile obtained was compared with that obtained for standard arginine vasopressin eluted from the same column under similar conditions.

**STATISTICAL ANALYSIS**

Repeated measurements in the same subject were compared by paired t-test, and comparison of means by unpaired t-test. Mortality between groups was compared by chi-square test.

**Results**

**ANGIOTENSIN II IN DOG BLOOD**

As can be seen from Figure 2, when the angiotensin II antiserum which discriminated between Ile⁵- and Val⁵-angiotensin (antiserum B) was used in the radioimmunoassay, the dilution curve for the dog plasma extract closely followed that obtained for the Ile⁵ standard, being displaced to the right compared to the curve obtained for the Val⁵ analogue. This more detailed investigation confirms the findings of a similar immunological study21 in which the same two antisera were used but in which prior chromatographic removal of any interfering metabolites was not performed. It strongly suggests, but does not prove, that angiotensin in dog blood has isoleucine as the amino acid in position 5.

**ANGIOTENSIN II AND ITS C-TERMINAL METABOLITES**

Although all blood samples used were arterial, the possible presence of significant amounts of C-terminal metabolites, particularly during periods when angiotensin II levels were elevated, could be important. Most angiotensin II antisera cross-react to a lesser or greater degree with these C-terminal metabolites and their presence in plasma results in falsely high measured levels of angiotensin II. The angiotensin II antiserum used in our radioimmunooassay...
cross-reacts 100% with the C-terminal hepta-, hexa-, and pentapeptide fragments and it was considered essential to check for their presence in the arterial blood samples. Figure 3 shows the chromatographic profile obtained on separating the octapeptide from the hepta-, hexa-, and pentapeptide fragments. As a percentage of the total immunoreactive material present the octapeptide made up 84%, the hepta- 7%, and the hexa- and pentapeptides 9%. It would appear from this that even following an acute and very powerful stimulus such as hemorrhage, arterial blood contains only a very small percentage of the C-terminal metabolites and that the immunoassay used is, in fact, measuring angiotensin II and not fragments reacting as angiotensin II.

GEL FILTRATION OF VASOPRESSIN EXTRACTS

Figure 4 shows the elution profiles of the plasma vasopressin extract and standard arginine vasopressin. The major vasopressin immunoreactive peak from the plasma extracts made up between 80% and 90% of the total immunoreactive material, and coincided with the standard vasopressin peak. From this and its immunological properties, the plasma extracts are indistinguishable from standard arginine vasopressin.

CHANGES IN ANGIOTENSIN II AND VASOPRESSIN IN GROUP 1

Changes in plasma angiotensin II and vasopressin over a 6-hour period in this control group are shown in Figure 5a and b. Although the plasma concentration of both peptides clearly increased between the 1- and 2-hour periods, the changes did not achieve statistical significance. Following this a gradual fall in concentration during the remaining 4 hours took place. This initial rise is probably a result of the removal of 300 ml of blood. This blood was removed between the first and second basal samples. The mean plasma angiotensin II concentration of 123 pg/ml (range, 77-160) and vasopressin concentration of 48 pg/ml (range, 26-72) found in anesthetized animals subjected to surgery are clearly elevated when compared to levels found in normal unanesthetized dogs.21-23

CHANGES IN ANGIOTENSIN II AND VASOPRESSIN IN GROUP 2

The changes in mean plasma angiotensin II concentration for the 15 dogs subjected to the unmodified hemorrhagic shock are shown in Figure 5a. Within 5 minutes after the completion of the hemorrhage the basal plasma concentration rose from 69 ± 8 pg/ml to 447 ± 79 pg/ml (P < 0.001) and at the end of the shock period it had risen further to 1,343 ± 218 pg/ml, a 20-fold increase overall. The plasma concentration at the end of the shock period was significantly greater than that at the end of hemorrhage (P < 0.001). In those dogs which survived until the end of the retransfusion the plasma concentration fell to 70 ± 16 pg/ml and was not significantly different from the prehemorrhage basal concentra-
Plasma Angiotensin (pg/ml)

Plasma AVP (pg/ml)

Sample No. Group 1

Sample No. Group 2

Figure 5 Changes in the plasma concentration of (a) angiotensin II (A II) and (b) arginine vasopressin (AVP) in the control group of dogs (group 1) (●) and the untreated bled group of dogs (group 2) (○). Bars indicate ± SEM. Ordinate values are plotted logarithmically.

Changes in Angiotensin II, Angiotensin I, and Vasopressin in Group 3

In the first five dogs of this group the required degree of hypotension was achieved as in the untreated group, but the bled volume proved to be significantly less (47.3 ± 1.0 ml/kg as compared to 54.2 ± 1.8 ml/kg, P < 0.005). This difference in bled volume was presumably a consequence of the vasodilating effect of the converting enzyme inhibitor, acting through its suppression of angiotensin II. For this reason the other five dogs in group 3 were bled to give a final bled volume (60.2 ± 3.5 ml/kg, P < 0.01) greater than that in group 2. Although the bled volume in these five dogs was greater, there was no significant difference between the final mean blood pressure when compared, either to that for the first five dogs in this group or to that for the 15 dogs in group 2. The physiological explanation for this is, as yet, uncertain. The final bled volume for all 10 dogs in group 3 (53.8 ± 2.8 ml/kg) was not significantly different from that in Group 2.

Regardless of the different maximum bled volumes, there was no significant difference, at any point, in the plasma concentrations of either angiotensin I, angiotensin II, or vasopressin between these two subgroups of five dogs. Consequently, for the purposes of comparison, all 10 dogs in group 3 were considered together. Figure 7a shows the changes in plasma concentration of angiotensin II for all 10 dogs in this group which were pretreated with the converting enzyme inhibitor prior to hemorrhage. Five minutes following the first injection there was a sharp fall in angiotensin II concentration from 92 ± 23 to 15 ± 2 pg/ml (P < 0.001). Also within 5 minutes after completion of the hemorrhage the concentration had risen to only 79 ± 25 pg/ml (P < 0.01) (as compared to 447 ± 79 pg/ml in the untreated group). The second inhibitor injection was given 10 minutes after completion of hemorrhage, and at the end of shock the angiotensin II concentration had risen to only 171 ± 38 pg/ml (as compared to 1,343 ± 218 pg/ml in the untreated group), less than a 2-fold increase (as compared to a 20-fold increase in the untreated group). Unlike the concentration after hemorrhage the concentration of angiotensin II at the end of the shock period was
That the suppression of the rise in angiotensin II was due to the inhibition of the conversion of angiotensin I to angiotensin II was confirmed by the profile for angiotensin I (Fig. 7b). Following the first injection of inhibitor, angiotensin I rose from 108 ± 30 pg/ml to 426 ± 163 pg/ml (P < 0.02). This corresponded to a sharp fall in angiotensin II. Further rises in concentration following hemorrhage (1,139 ± 440 pg/ml, P < 0.01 compared to the basal concentration) and shock (3,232 ± 670 pg/ml, P < 0.001 compared to the basal concentration) were qualitatively similar to the changes in the angiotensin II concentration in the untreated group but quantitatively they were of a much greater magnitude (a 30-fold rise from basal). This would be expected if renin secretion were still active as a result of the hemorrhagic stimulus and a buildup in angiotensin I had taken place due to an enzyme block in its conversion to angiotensin II. Following retransfusion the angiotensin I concentration, while tending to fall, was still very high (1,347 ± 361 pg/ml) up to 1 hour after retransfusion was completed.

Unlike angiotensin II the converting enzyme inhibitor appeared to have little or no effect on the changes in vasopressin concentration in this group (Fig. 7c) as compared to the untreated group. The mean concentration after hemorrhage was 1,095 ± 261 pg/ml (compared to 1,016 ± 157 pg/ml in the untreated group) and before retransfusion was 456 ± 107 pg/ml (compared to 401 ± 64 pg/ml). The mean changes between the basal concentration and the concentration at the end of hemorrhage and between the basal concentration and the concentration at the end of the shock period for the treated and the untreated group were not significantly different.

FIGURE 7 Changes in the plasma concentration of (a) angiotensin II, (b) angiotensin I, and (c) arginine vasopressin (AVP) in the converting enzyme inhibitor-treated group of dogs (group 3) (—). Broken line (— — —) indicates the profiles obtained in the untreated group. Bars indicate ± SEM. Ordinate values are plotted logarithmically.

significantly greater (P < 0.05) than the prehemorrhage basal level. Following retransfusion the concentration fell to prehemorrhage levels.

As mentioned, the angiotensin II antiserum used in the radioimmunoassay cross-reacted 0.6% with angiotensin I. Also, angiotensin I is extracted from plasma in the method used for the extraction of angiotensin II. Because of the low cross-reaction this does not normally present any problem. However, where the plasma levels of angiotensin I are very high (see below), this may result in the measurement of falsely high levels of angiotensin II. On the basis of the above percentage cross-reaction the levels of angiotensin I found in this group of dogs would result in measured plasma concentration of angiotensin II 5-10 pg/ml and 15-25 pg/ml of plasma higher than they should be at the end of hemorrhage and at the end of shock, respectively. In other words the converting enzyme inhibitor was suppressing the angiotensin II to an even greater extent than is apparent.

As described in Methods, all dogs in all groups were bled 300 ml (12 ml/kg of body weight) between the first and second basal samples. This, in itself, represents a substantial hemorrhage, in the region of 15% of total blood volume. It was therefore of interest to compare plasma concentrations of the three peptides before and after this bleed.

In group 1 consisting of only 5 control dogs, there was a distinct rise in both angiotensin II and vasopressin across this bleeding procedure. However in neither instance did the changes reach significance. Angiotensin II and vasopressin also rose between the two basal samples in the 15 dogs in group 2; only the change in angiotensin was significant (36 ± 6 pg/ml to 69 ± 8 pg/ml, P < 0.01). Similarly, in group 3 consisting of 10 dogs there was a significant rise in angiotensin II (64 ± 17 pg/ml to 92 ± 23 pg/ml, P < 0.05). Angiotensin I also rose significantly (67 ± 19 pg/ml to 108 ± 30 pg/ml, P < 0.05). Although plasma vasopressin again rose, the change was not significant.

As all dogs in the three groups were treated exactly the same up to, and including, the second basal blood sample, it was considered justifiable for the purposes of comparing the two basal values to group them together. When this was done, for all 30 dogs, the changes for both angiotensin II and vasopressin were highly significant (54 ± 7 pg/ml to...
93 ± 11 pg/ml,  P < 0.001; and 25.5 ± 5 pg/ml to 40.2 ± 7 pg/ml,  P < 0.01, respectively). The mean arterial blood pressure at the time of the first basal blood sample was 156.6 ± 2.8 mm Hg, and after removal of the 300 ml of blood the pressure fell to 141.3 ± 4.2 mm Hg. This fall in pressure was significant (paired t-test,  P < 0.001).

MORTALITY AND CARDIOVASCULAR EFFECTS

The lethal consequences of hemorrhagic shock of this type in dogs are well documented.9-12 Also it has been shown that inhibition of the rise of angiotensin II by the converting enzyme inhibitor protects against this. The present study would appear to support this observation. Five of 15 dogs in the untreated group died before retransfusion was completed, as compared to none of 10 in the group given converting enzyme inhibitor (  \chi^2 = 4.1,  P < 0.05). Hemorrhagic shock also produces changes in cardiac function,14 and of the five dogs which died in this study, four did so of acute cardiac failure and one of arrhythmia.

Discussion

The specificity of the angiotensin II radioimmunoassay used in the present study is of great importance.25 Arterial blood was used throughout, and it is clear from the chromatographic studies performed that gross changes in tissue perfusion and hemodynamics, which could conceivably have changed the rate and mode of destruction of angiotensin II to its immunoreactive fragments, have not altered the observation23 that between 80% and 90% of the immunoreactive material is in the form of angiotensin II octapeptide.

The profile of angiotensin II in the untreated dogs (group 2) is very similar to that found for renin activity by Jakshik et al.,2 using a radioimmunoassay for angiotensin I. Certainly the high plasma angiotensin II concentrations of 447-1,343 pg/ml plasma at the beginning and end of shock would be expected to have a strong vasoconstrictor effect since there is evidence in the normal dog that much lower levels are within the vasoconstrictor range.21 Also it has been shown by Brough et al.,26 that in dogs with intact kidneys there is a 65% compensation of blood pressure following hypotensive hemorrhage, as compared to nephrectomized dogs and dogs treated with the converting enzyme inhibitor (SQ 20881) which showed only a 24% compensation. This indicates a strong vasoconstrictor effect of the renin-angiotensin system in hemorrhage. These facts would support the suggestion by Errington and Rocha e Silva10,11 that vasoconstriction and reduced tissue perfusion may be one mechanism by which angiotensin II promotes the development of irreversible shock in the experimental situation.

Preventing the rise of angiotensin II following hemorrhage by prior administration of the angiotensin-converting enzyme inhibitor (SQ 20881) has resulted in reduced mortality and improved performance.10-12 However, in these experiments plasma angiotensin II was not measured and the degree to which it was suppressed is not known. The dose of 1 mg/kg of body weight used by us was highly effective in suppressing the rise in angiotensin II following hemorrhage. Since both Errington and Rocha e Silva11 and Erdös et al.12 used doses of inhibitor similar to ours it is probable that they also achieved equally effective suppression.

The plasma concentrations of vasopressin found by us following hemorrhage are similar to those found by Errington and Rocha e Silva.9 Also the observation of Weinstein et al.27 that the initial rise after hemorrhage is not maintained with time also is confirmed in the present study. The reason for the secretion of such massive quantities of vasopressin in response to hemorrhage is not known, but certainly it is far in excess of that required to produce maximal fluid retention as a means of counteracting volume loss. While we have no direct evidence that vasopressin, as well as angiotensin, is acting to maintain blood pressure via vasoconstriction, this possibility has been suggested by Rocha e Silva and Rosenberg7 and by Cowley et al.28

Unlike angiotensin II the converting enzyme inhibitor had no effect on the plasma concentration of vasopressin as compared to that in the untreated dogs. This is of interest in view of the suggestion that angiotensin II has a stimulatory effect on vasopressin secretion.29-31 We were unable to find any significant difference in the concentration of vasopressin following hemorrhage in inhibitor-treated dogs as compared to the untreated dogs when there was a 5- to 6-fold difference in the concentration of angiotensin II. Similarly there was no significant difference in vasopressin concentration between the two groups just prior to retransfusion when there was an 8-fold difference in the angiotensin II concentration. In fact, in absolute values the concentration of vasopressin tended to be higher both after hemorrhage and before retransfusion in the treated group than in the untreated group. These results agree with the findings of Claybaugh and Share,32 who demonstrated a marked increase in vasopressin concentration following hemorrhage in dogs even when renin secretion was prevented. In our opinion this and other evidence33-34 strongly suggests that angiotensin II has no significant part to play in the potentiation of vasopressin secretion following severe hemorrhage in the dog. However, the results do not disprove the possibility of an angiotensin II-mediated potentiation of vasopressin secretion in unanesthetized dogs under more physiological conditions. Although considered unlikely, the possibility that the very high plasma concentrations of angiotensin I may stimulate arginine vasopressin (AVP) secretion has to be borne in mind. As far as we are aware no one has examined this question.

The increase in angiotensin II and vasopressin following the hemorrhage of 12 ml/kg is of interest. Claybaugh and Share32 reported an increase in plasma vasopressin and renin following a bleed of 12 ml/kg which did not cause a fall in mean arterial pressure. In contrast, in the present study, removal of 12 ml/kg led to a small but highly significant fall in mean arterial pressure.

The significant reduction in mortality in the inhibitor-treated group of dogs as compared to the untreated group is compatible with previous findings that the converting enzyme inhibitor is capable of protecting against death following hemorrhagic shock. The observations reported in this paper and elsewhere would suggest that this may be...
achieved by suppressing the very large rise in angiotensin II which occurs following hemorrhage. Also that four of the five dogs which died in the untreated group did so as a result of acute cardiac failure is of interest in view of the work of Gavras et al.30 who demonstrated the presence of multifocal myocardial lesions in rabbits following the infu-

sion of angiotensin II.

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