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THE EFFECTS OF ALTERATION OF BLOOD-VOLUME ON THE CONCENTRATION OF CIRCULATING ANGIOTENSIN IN ANAESTHETIZED DOGS

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SUMMARY

1. The blood-bathed organ technique was used to assay the concentration of angiotensin in the blood of anaesthetized dogs.

2. Alterations of blood volume caused inverse changes of angiotensin concentration owing to changes in the rate of generation of angiotensin which are probably due to changes of the rate of renin secretion.

3. Haemorrhage of 14–26 ml. blood/kg caused an increase of 0·25–1·5 μg/min in the rate of generation and an increase of 0·1–0·33 ng/ml. in the blood concentration of angiotensin.

4. The changes of angiotensin generation rate were not due to changes of renal arterial or venous pressure. They were abolished by blocking the renal nerves with lignocaine; they showed a consistent inverse correlation with central venous pressure but not with systemic arterial pressure.

5. It is concluded that changes of blood volume bring about changes of the rate of generation of angiotensin by a reflex mechanism the efferent limb of which involves the renal nerves. The afferent pathway remains to be elucidated, but the systemic baroceptors do not appear to be of primary importance.

6. The renin-angiotensin system is important in the homeostatic response to changes of blood volume.

INTRODUCTION

Early experiments with the renin–angiotensin system (see Page & Bumpus, 1961; Peart, 1965) suggested that it is important in the maintenance of arterial blood pressure. Renin is liberated during severe haemorrhagic shock (Hamilton & Collins, 1942; Dexter, Frank, Haynes & Altschule, 1943) and the concentration of angiotensin in blood is increased

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during moderate haemorrhage (Scornik & Paladini, 1964; Regoli & Vane 1964*a*, 1966).

The demonstration that angiotensin is a potent stimulus for the secretion of aldosterone (Genest, Nowaczynski, Koiw, Sandor & Biron, 1960; Laragh, Angers, Kelly & Lieberman, 1960) suggests that the renin–angiotensin system is important in regulating sodium re-absorption and hence in maintaining blood volume. In this paper we describe experiments on anaesthetized dogs in which circulating angiotensin was assayed continuously by the blood-bathed organ technique (Vane, 1964; Regoli & Vane, 1964*b*). The results show that the concentration of angiotensin in the blood is modified by alterations of blood volume even when these are not accompanied by detectable changes of arterial pressure. Some of these results have been presented to the Physiological Society (Hodge, Lowe & Vane, 1965).

METHODS

In dogs weighing 12–30 kg (most of which were greyhounds), anaesthesia was induced with ether or halothane and maintained with chloralose (100 mg/kg), supplemented with pentobarbitone (60–120 mg, I.V.) 3–4 hr later and then every 30–120 min. Cannulae were inserted into a jugular vein, a carotid artery and the trachea. Each animal was ventilated mechanically and given heparin (1000 units/kg). Blood from a carotid artery was pumped at 15 ml./min by a roller pump through plastic tubing over three assay organs (Vane, 1964) before returning by gravity to the jugular vein. Mean arterial pressure was recorded on a kymograph with a mercury manometer from a side arm of the carotid arterial cannula. Catheters were inserted through a femoral vein and artery to measure central venous and arterial pressure with Statham P23Db pressure transducers and a photographic recorder. (The undamped natural frequency of the catheter-transducer system was 40 c/s and the damping coefficient was 0·7.)

In most experiments the bladder was catheterized and urine output recorded by a drop counter. To replace the excreted fluid an equal volume of 0·9 % sodium chloride (w/v) was injected intravenously by an automatic syringe regulated by the drop counter.

Assay techniques

The three assay organs were a rat stomach strip (Vane, 1957), a chick rectum (Armitage & Vane, 1964) and a rat colon. Their movements were recorded on a kymograph by auxotonic levers of 16:1 magnification with a resting load on the tissues of 1–3 g. The rat stomach strip relaxes in response to adrenaline or noradrenaline (10^{-10} g/ml.) and contracts in response to angiotensin (10^{-9} g/ml.). The chick rectum relaxes in response to adrenaline (10^{-10} g/ml.) and is relatively insensitive to noradrenaline and angiotensin.

The rat colon is highly sensitive to angiotensin and responds by contraction to concentrations as low as 10^{-10} g/ml. (Regoli & Vane, 1964*b*). Catecholamines relax the colon and this can interfere with its response to angiotensin. In some experiments, therefore, the chick rectum was replaced by a second rat colon which was perfused intraluminally with propranolol (10^{-3} g/ml.); this abolished the response to catecholamines without changing that to angiotensin.

The technique detected changes of angiotensin concentration occurring over periods of up to 40 min; but the effects of slower changes could not be distinguished from gradual drifts of the base lines of the assay organs. The absolute concentration of angiotensin at any moment could only be derived from the fall of base line which occurred after nephrectomy,

when it was assumed that the circulating angiotensin concentration had fallen to zero. The response of the assay organs to endogenous angiotensin could be calibrated either in terms of blood angiotensin concentration, by infusing angiotensin at a known rate into the blood passing over the assay organs, or in terms of the rate of angiotensin generation by infusing it intravenously at a known rate.

Specificity of assay

The specificity of this assay of angiotensin has been discussed elsewhere (Regoli & Vane, 1966); it depends on the differential responses of the assay organs and allows angiotensin to be distinguished from adrenaline, noradrenaline, 5-hydroxytryptamine, bradykinin, acetylcholine, adenosine derivatives, vasopressin, oxytocin, aldosterone, and other adrenal cortical hormones.

A response of the assay organs to a change of blood volume was accepted as being due to a change in the concentration of endogenous angiotensin concentration only when the following conditions were fulfilled:

(1) The differential response of the three assay organs was identical with the response to infused angiotensin. (Throughout this paper it is assumed that endogenous angiotensin has the same properties as synthetic angiotensin.)

(2) The response did not occur in animals whose kidneys were excluded from the circulation.

The following drugs were used, doses of salts being expressed in terms of base: (−)-adrenaline bitartrate (British Drug Houses); angiotensin amide (Hypertensin-Ciba); dextran, 6 % in 0·9 % sodium chloride w/v (Dextraven, Boots); heparin (Boots); lignocaine (Duncaine, Duncan Flockhart); (−)-noradrenaline bitartrate (British Drug Houses); renin (hog renin, Nutritional Biochemical Corporation).

RESULTS

Effect of increasing the blood volume

The blood volume was expanded by infusing Krebs solution (seven dogs), saline (five dogs), or dextran (four dogs) at a rate of 0·5–1·5 ml. kg^{-1} min^{-1} to total of 10–30 ml./kg. These infusions produced changes of tone of the assay organs characteristic of a fall of angiotensin concentration in the blood. Figure 1 illustrates such an experiment in which 480 ml. Krebs solution was infused into a dog. This caused gradual relaxation of the rat colon with no change of the rat stomach strip. In three control animals the abdomen was opened and the renal pedicles dissected but not clamped; expansion of the blood volume then had the usual effect, causing relaxation of the rat colon. In four other control animals the kidneys were excluded from the circulation by nephrectomy (one dog) or by clamping the renal pedicle (three dogs); subsequent expansion of the blood volume produced no response of the assay organs.

In order to dilute the circulating blood without changing its volume, dextran in saline was infused intravenously into four animals at 15 ml./min and blood was withdrawn at the same rate until 8–12 ml./kg blood had been withdrawn. This procedure had no effect on the assay organs, but when the blood was re-infused their responses were characteristic of a fall of angiotensin concentration. In three control animals in which the renal pedicles had been clamped, neither the exchange transfusion nor the

return of the blood had any effect on the assay organs. When, instead of returning the blood slowly, it was re-injected suddenly, the relaxation of the colon was still gradual with a time constant of about 20 min.

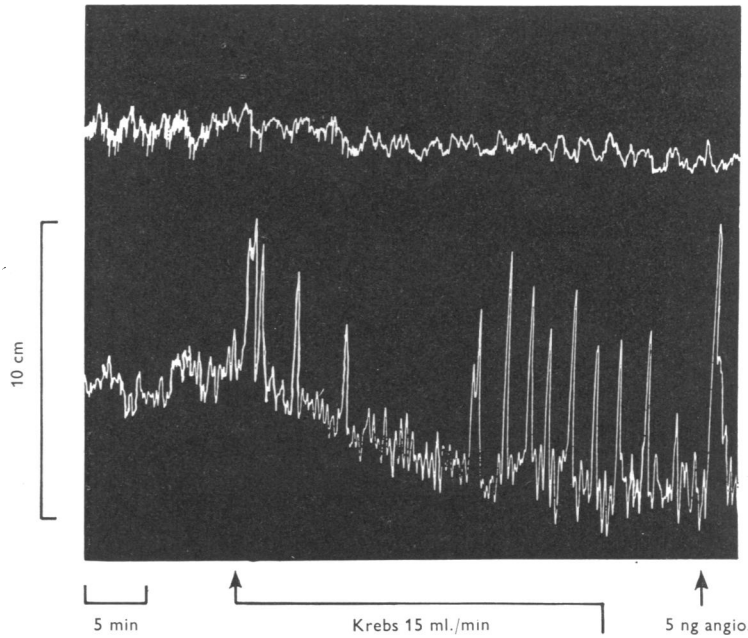

Fig. 1. Dog 18 kg, female. From top to bottom, a rat stomach and a rat colon superfused with carotid arterial blood. When Krebs solution was infused intravenously into the dog at 15 ml./min to a total volume of 480 ml. the rat colon slowly relaxed, and the rat stomach strip showed little or no change. Afterwards an injection of angiotensin (5 ng) given directly into the blood bathing the tissues shows that only the rat colon responds to this dose. Time scale 5 min, vertical scale 10 cm. The rat colon in this experiment showed high spontaneous activity.

These results are summarized in Fig. 2. Expansion of the blood volume caused relaxation of the rat colon in twenty out of twenty-two experiments. In nine experiments on control animals in which the kidneys were excluded from the circulation, expansion of blood volume produced no such change. From these results it was concluded that increasing the blood volume caused a fall of angiotensin concentration which was not due to dilution of the blood.

Effect of reducing the blood volume

The blood volume was reduced by removing 8–26 ml. blood/kg at 0·5–2·0 ml. kg^{-1} min^{-1}. This caused a gradual contraction of the rat colon with no effect on the other organs, characteristic of a rise of angiotensin concentration. On retransfusing the withdrawn blood, whether slowly or rapidly, the response of the assay organs was characteristic of a gradual

fall of the angiotensin concentration to its original level. In five animals, these effects were no longer seen after the renal pedicles were clamped. Haemorrhage and retransfusion caused no response of the assay organs in three other control animals whose renal pedicles were clamped at the start of the experiment. Figure 3 shows an experiment which illustrates some of these points. The responses of the organs were first calibrated by intravenous infusions of angiotensin (Fig. 3*a*); it should be noted that these

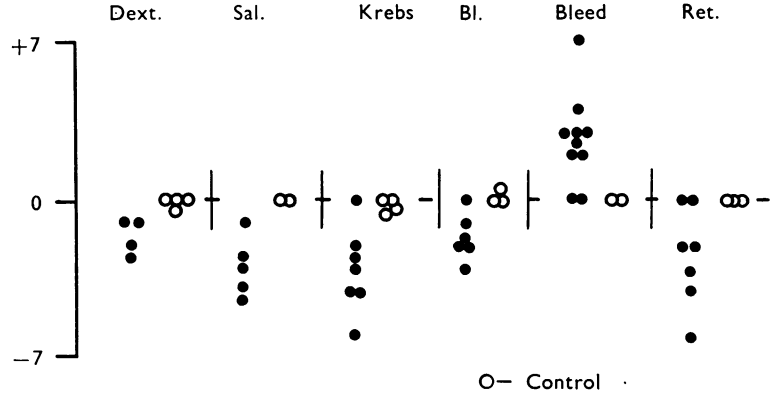

Fig. 2. Results in twenty-one dogs. Filled circles: animals with intact kidneys. Open circles: control animals after nephrectomy or excluding the kidneys from the circulation. The figure shows the changes (in cm) of base line of the rat colon induced by changing the blood volume in various ways. The blood volume was increased by infusion of: 6 % Dextran in 0·9 % sodium chloride solution (w/v) (Dext.); 0·9 % sodium chloride solution (Sal.); Krebs solution (Krebs); blood (Bl.); at 0·5–1·5 ml. kg^{-1} min^{-1}, to a total volume of 10–30 ml./kg. The blood volume was reduced by slow haemorrhage at 0·5–2·0 ml. kg^{-1} min^{-1}, to a total volume of 8–13 ml. kg (Bleed). In the same animals the blood was returned (Ret.) These procedures caused changes of base line of the rat colon only in the animals with intact kidneys.

infusions caused both contraction of the rat colon and an increase of arterial pressure. Blood was then withdrawn (780 ml. in 14 min, Fig. 3*b*) and this also resulted in contraction of the rat colon. When the blood was retransfused the colon relaxed again but not to its initial level; the calibration showed that the contraction of the rat colon at its maximum corresponded to an increase in the formation of angiotensin of more than 1 μg/min. The renal pedicles were then dissected, during which there was further contraction of the rat colon indicating a transient increase of blood angiotensin concentration. When the renal pedicles were clamped, the rat colon slowly relaxed until after 25 min a new base line was established. Calibration showed that before dissection of the renal pedicles the rate of generation of angiotensin had been more than 0·5 μg/min (Fig. 3*c*). Further removal of blood from the dog had no effect on the rat colon (Fig. 3*c*).

In two animals, after haemorrhage had resulted in a contraction of the rat colon, instead of retransfusing the blood the renal pedicles were clamped; this procedure had the same effect as retransfusion, causing

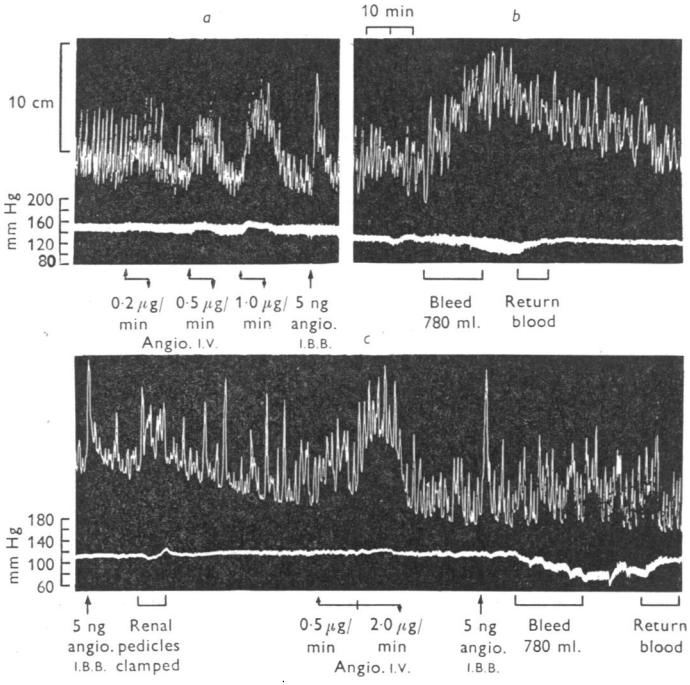

Fig. 3. Dog 29·5 kg, male. 295 ml. dextran in saline injected intravenously at beginning of experiment. Each panel shows a rat colon superfused with carotid arterial blood and the arterial pressure recorded by mercury manometer. Panel *a* shows the effects of intravenous infusions of angiotensin at 0·2, 0·5 and 1 μg/min; these doses contracted the rat colon and raised the arterial pressure. At the end of this panel is the effect of angiotensin (5 ng) injected into the blood bathing the tissue (I.B.B.). Panel *b* shows that when 780 ml. of blood was withdrawn the rat colon contracted and the arterial pressure fell only slightly; this contraction of the rat colon was equivalent to an increase of angiotensin generation rate of over 1 μg/min. On retransfusion of blood the colon relaxed again; note the long time course of this relaxation.

Panel *c* shows the effect of an angiotensin injection (5 ng) I.B.B. The renal pedicles were then dissected, causing a brief contraction of the rat colon. After clamping the renal pedicles, the rat colon relaxed slowly (note the similar time course of the relaxation in panel *b* and *c*). Angiotensin was then infused intravenously at 0·5 and 2·0 μg/min showing that before the renal pedicles were dissected the angiotensin generation rate in the blood had been more than 0·5 μg/min but less than 2·0 μg/min; note the rapid relaxation of the rat colon when the infusion was stopped. A further injection of angiotensin (5 ng I.B.B.) showed that the sensitivity of the rat colon had increased. Despite this, when the haemorrhage and retransfusion were repeated, there were no effects on the rat colon. Time scale 10 min. Vertical scales 10 cm and mm Hg. The chick rectum and rat stomach showed no changes throughout and have been omitted for clarity.

relaxation of the rat colon. Such an experiment is illustrated in Fig. 4 which also shows that retransfusion of the withdrawn blood had no effect after excluding the kidneys from circulation.

The effects of reducing the blood volume are summarized in Fig. 2. On eight out of ten occasions, reduction of the blood volume caused contraction of the rat colon which relaxed when the blood was returned.

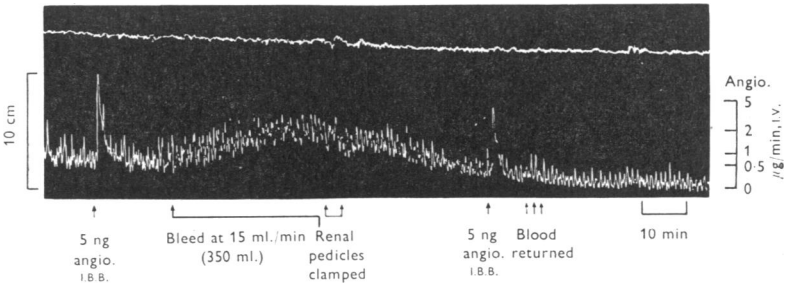

Fig. 4. Dog 28·5 kg, male. At the start of the experiment 300 ml. of dextran in saline had been infused intravenously. Top trace rat stomach strip, bottom trace rat colon, both superfused with carotid arterial blood. The record shows the different sensitivity of the two tissues to an injection of angiotensin (5 ng I.B.B.). Withdrawal of 350 ml. of blood at 15 ml./min caused contraction of the rat colon, which was reversed by excluding the kidneys from the circulation. Note the prolonged time course of the relaxation (cf. Fig. 3) and that the colon finally relaxed to a lower base line than before the haemorrhage. A further injection of angiotensin (5 ng I.B.B.) showed that the colon was still sensitive to angiotensin, but retransfusion of the 350 ml. of blood now had no effect on the rat colon. Time scale, 10 min. Angiotensin generation rate (right-hand scale) obtained from earlier calibrations. Vertical scale 10 cm.

Neither of these responses was obtained in five control animals in which the kidneys were excluded from the circulation. From all these results it was concluded that reduction of the blood volume caused a rise of the concentration of circulating angiotensin which was reversed by retransfusion. Even after rapid retransfusion the fall of angiotensin concentration was slow, with a time constant of about 20 min. A similar time course was observed after overtransfusion (see Fig. 1), after clamping the renal pedicles (Fig. 3), after removal of a partial occlusion of the aorta above the renal arteries (Regoli & Vane, 1966), or after the injection of hog renin intravenously. In contast, the fall of angiotension concentration after stopping an intravenous infusion of angiotensin was much faster (see Fig. 3) with a time constant of less than 3 min.

In five dogs the blood concentration of angiotensin during haemorrhage was estimated by infusing angiotensin into the blood superfusing the assay organs in amounts sufficient to match the response to haemorrhage; the results are shown in Table 1.

Cause of the changes of angiotensin concentration

The observed changes of angiotensin concentration could have been due to changes either in the rate of formation or in the rate of inactivation of angiotensin. To distinguish between these alternatives, in three dogs angiotensin was infused intravenously; the resulting contraction of the rat colon was then matched by infusing angiotensin into the blood bathing the organs. Since the rate of blood flow over the organs was known the increase of concentration of angiotensin in the blood passing over them could be calculated, and the same increase of blood concentration must have been produced by the intravenous infusion. These infusion procedures were repeated when haemorrhage had caused a rise of blood angiotensin concentration. The results are summarized in Table 2. A given rate of

TABLE 1. Change of blood angiotensin concentration produced by haemorrhage. At the beginning of these experiments, each animal was given approximately 10 ml. dextran in saline/kg, I.V.

Dog no.	Wt. (kg)	Volume of blood removed (ml./kg)	Rise of angiotensin concentration due to haemorrhage (ng/ml.)
47	28·0	18	0·2
48	24·0	33	0·3
50	33·2	26	0·1
51	29·0	20	0·33
52	31·0	19	0·17

TABLE 2. The steady-state blood concentration of angiotensin produced by intravenous infusions before and after haemorrhage

Dog no.	I.V. infusion rate of angiotensin (μg/min)	Blood concentration of angiotensin due to I.V. infusion (ng/ml.)	
		Before haemorrhage	After haemorrhage
48	0·5	0·33	< 0·33
49	0·5	0·1	0·1
50	1·5	0·33	< 0·33

intravenous infusion after haemorrhage caused a rise of angiotensin concentration the same as or smaller than the rise before haemorrhage. From this it was concluded that the rate of inactivation of infused angiotensin was either unchanged or increased. Therefore the rise in concentration of endogenous angiotensin during haemorrhage must have been due to an increase in its rate of formation.

In eight dogs the rate of angiotensin generation after haemorrhage was estimated by subsequent infusion of angiotensin intravenously in doses sufficient to match the response to haemorrhage (Table 3). These infusions often had detectable effects on arterial pressure.

TABLE 3. The increased rate of angiotensin generation occurring as a result of haemorrhage. At the beginning of these experiments, each animal was given approximately 10 ml. dextran in saline/kg, I.V.

Dog no.	Weight (kg)	Vol. of blood removed (ml./kg)	Increase of the rate of angiotensin generation (μg/min)	Rise of B.P. produced by infusion of angiotensin at the rate shown (mm Hg)
36	28·5	14	1·0	6
39	29·5	26	0·5	Not known
41	25·5	24	0·5	4
42	22·0	15	0·25	12
44	22·0	27	0·5	10
47	28·0	18	1·0	5
50	33·0	26	0·5	10
51	29·0	20	1·5	10

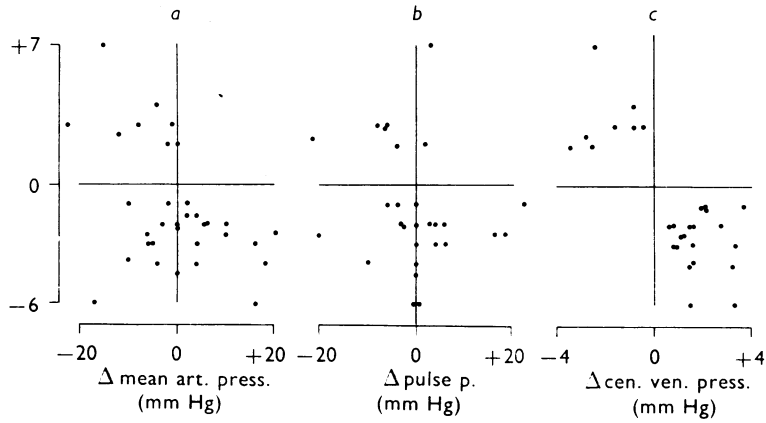

Fig. 5. The results of changes of blood volume in twenty-eight dogs. Changes of arterial mean pressure (*a*), arterial pulse pressure (*b*) and central venous pressure (*c*) are plotted against changes of angiotensin concentration in arterial blood, expressed as changes of the base line of the rat colon in cm. Note the consistent inverse correlation between central venous pressure and angiotensin concentration, and the absence of correlation in *a* and *b*.

Stimulus for changes of angiotensin generation

The haemodynamic effects of altering the blood volume include changes of arterial and venous pressures. Such changes could affect the kidneys both by direct local effects and indirectly through baro-receptors elsewhere which elicit reflex changes of renal vasomotor tone. Figure 5 shows that changes of angiotensin concentration bore no consistent relation to changes of arterial mean or pulse pressures; thus, during expansion of the blood volume, the angiotensin concentration decreased, whether the arterial pressure rose or fell. Moreover, changes of angiotensin concentration during haemorrhage often occurred before there were changes of

arterial pressure (Fig. 6). Even when reduction of blood volume caused a fall of arterial pressure (which ranged from 1 to 20 mm Hg), the increased rate of angiotensin formation could not have been due solely to the reduced renal arterial pressure because the same reduction of pressure brought about by inflating a balloon in the aorta above the renal arteries caused no increase of angiotensin concentration. To reproduce with an aortic balloon the rise of angiotensin concentration caused by haemorrhage, it was necessary to lower renal arterial pressure by 40–90 mm Hg (six dogs). Thus the changes of angiotensin concentration during haemorrhage were not primarily dependent on changes of systemic arterial pressure.

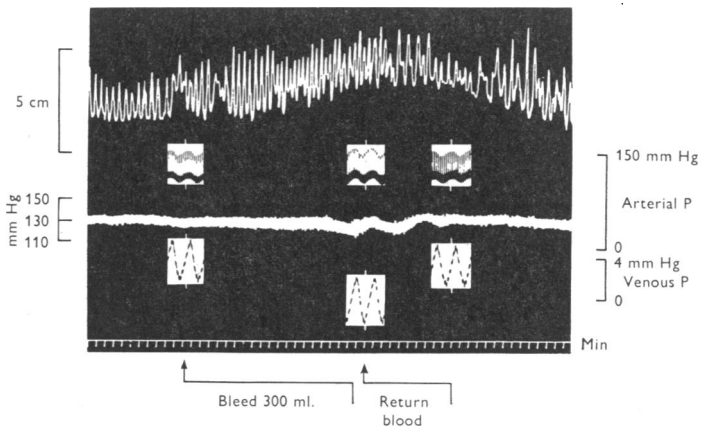

Fig. 6. Dog 21·0 kg., female. Upper trace—rat colon superfused with arterial blood. Lower trace—arterial pressure by mercury manometer. At the start of the experiment, 210 ml. dextran in saline had been infused intravenously. Removal of 300 ml. of blood at 15 ml./min caused a rise of angiotensin concentration registered by the rat colon. On retransfusion of the blood, the rise was reversed. The upper three insets show 6 sec records of arterial pressure at the time marked; the lower three insets show the central venous pressure with variations due to respiration. Note that the rise of angiotensin concentration preceded any detectable fall of mean arterial pressure and occurred in spite of a rise of arterial pulse pressure, but is associated with a fall of venous pressure. Time scale in min. Vertical scales—rat colon 5 cm; mean B.P. mm Hg; arterial pulse pressure 150 mm Hg; central venous pressure 4 mm Hg.

In contrast to arterial pressure, changes of central venous pressure showed a consistent inverse correlation with changes of angiotensin concentration (Fig. 5). The fall of angiotensin concentration during expansion of the blood volume could not have been due to an increase in renal venous pressure because inflation of a balloon in the inferior vena cava above the renal veins (raising the renal venous pressure by up to 12 mm Hg) did not lower the angiotensin concentration (three dogs) although expansion of the blood volume in these animals did so. If the

relation between central venous pressure and angiotensin generation rate is one of cause and effect, it seems probable that nervous reflexes are involved. Such reflexes could be initiated by changes of pressure in the great veins, in the atria or in the pulmonary circulation.

Whatever the afferent pathway, if the generation of angiotensin is under reflex control the efferent pathway is likely to be through the renal nerves. In fourteen dogs the generation of angiotensin in response to haemorrhage was tested before and after injection of lignocaine (5–10 ml. of 10^{-2} g/ml.) into each renal pedicle in order to anaesthetize the renal nerves. The injection itself caused a fall of blood pressure, probably due to abolition of renal vaso-constrictor tone. Immediately after the injection

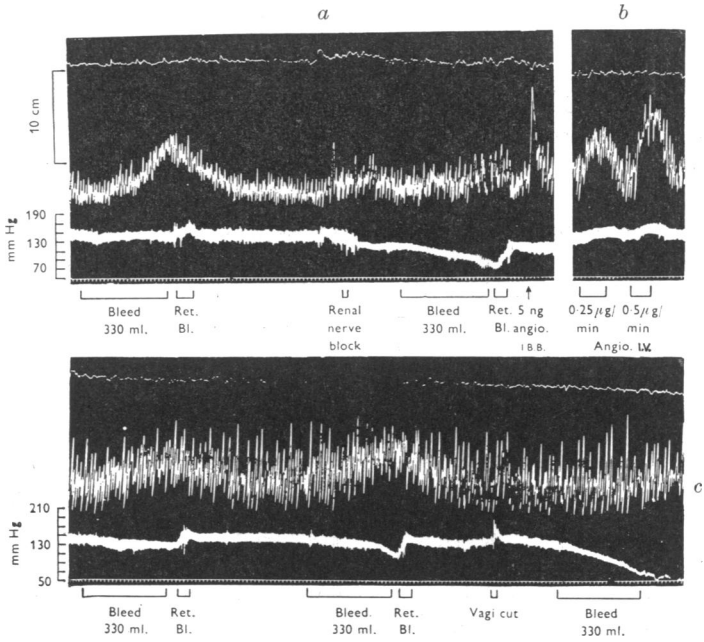

Fig. 7. Dog 22·0 kg, female. Upper trace—chick rectum, middle trace—rat colon: both superfused with carotid arterial blood. Lower trace—arterial pressure by mercury manometer. Removal of 330 ml. of blood at 15 ml./min caused a rise of angiotensin concentration registered by the rat colon. On retransfusion of the blood (Ret. Bl.) the rise was reversed. Next 10 ml. of 1 % lignocaine was injected into each renal pedicle around the renal artery, to block the renal nerves. Following this, bleeding and retransfusion had no effect on the blood angiotensin concentration. Panel *b* shows the differential response of the two tissues to angiotensin infused at 0·25 and 0·5 μg/min I.V. Panel *c* starts 2 hr after the lignocaine injection and shows that the changes of angiotensin concentration in response to haemorrhage and retransfusion were almost completely restored. (In this experiment, the changes were abolished again by vagotomy.) Time scale in min. Vertical scales, 10 cm and mm Hg.

haemorrhage caused no increase in the blood angiotensin concentration. After 1–3 hr the effects of lignocaine had disappeared and the response was restored. Such an experiment is illustrated in Fig. 7. The withdrawal of 350 ml. blood at a rate of 0·7 ml. kg^{-1} min^{-1} caused an increase of angiotensin concentration which slowly declined when the blood was returned (Fig. 7*a*). During the haemorrhage, there was only a slight fall in blood pressure. The renal nerves were then blocked with lignocaine; this in itself reduced the blood pressure. When the haemorrhage was repeated there was no increase of angiotensin concentration but a substantial fall in blood pressure. This showed that the renal nerves were an important factor in maintaining arterial pressure during haemorrhage. Figure 7*b* shows the responses of the rat colon and the blood pressure to infusions of 0·25 and 0·5 μg angiotensin/min I.V. Two hours after injecting lignocaine the haemorrhage was repeated; this now caused an increase of angiotensin concentration, which was reversed by retransfusion, and 30 min later haemorrhage had an even larger effect on the angiotensin concentration (Fig. 7*c*). The vagi were then cut in the neck. Haemorrhage no longer caused an increase of angiotensin concentration but the fall in blood pressure was now so great as to be irreversible.

In two control animals injections of saline into the renal pedicles and of lignocaine intravenously and intramuscularly did not abolish the effect of haemorrhage on the concentration of angiotensin.

DISCUSSION

These results show that when the blood-volume of the anaesthetized dog is changed there are reciprocal changes in the generation rate of angiotensin in the circulation. In analysing the relation between these two variables the influence of several others was considered. First, since changes in blood composition alone had no effect they could not have been the stimulus for the changes in the generation rate of angiotensin. Nor could the observed changes in the steady-state concentration of angiotensin in the blood have been due to alterations in its volume of distribution, since these changes were sustained for periods longer than the half-life of angiotensin in the circulation. They could therefore have been due only to changes in the rate of generation or in the rate of destruction. Secondly, since the changes of angiotensin concentration were not due to changes in the rate of destruction (Table 2) we conclude that they were due to changes in generation rate induced by the alterations in blood volume.

The most likely way in which blood-volume could affect the rate of angiotensin generation is by altering the output of renin from the kidney

and our experiments provide some evidence of this. Two of the procedures used, i.e. local reduction of renal arterial pressure and haemorrhage are known to increase the concentration of renin in blood (see Peart, 1965). After reversing these procedures the decline of the raised angiotensin concentration had a time course similar to that seen after increasing the blood volume, after excluding the kidneys from the circulation, or after injecting hog renin. Furthermore, the decline was always slower than after stopping an infusion of angiotensin. The simplest explanation of these results is that the changes in angiotensin generation rate are due to changes in the rate of secretion of renin into the circulation.

If changes in blood volume induce changes in renin secretion the question arises as to how this is brought about. Both renin output (Skinner, McCubbin & Page, 1964) and blood angiotensin concentration (Regoli & Vane, 1966) are increased when renal arterial pressure is decreased. However, our results confirm earlier observations (Scornik & Paladini, 1964) that for a given a fall of renal arterial pressure the rise in angiotensin concentration is much greater after haemorrhage than during application of an aortic clamp. Indeed, in many of our experiments the concentration increased without any alteration in renal arterial pressure. Furthermore, increases in renal venous pressure did not lower the angiotensin concentration. These findings show that the site at which changes of blood volume were detected and transduced lies outside the kidney and that the changes in the rate of renin secretion were brought about either by nervous reflexes or by humoral transmitters. Our results show that a nervous reflex is involved of which the efferent limb is the nerve supply to the kidney.

The nature of the link between renal nerve activity and the secretion of renin is still unknown. Renin is present in either the juxtaglomerular apparatus or the macula densa (see Peart, 1965). The innervation of these structures is not yet established; electron microscopy has shown non-myelinated nerves close to them (Barajas, 1964) whereas histochemical staining has shown adrenergic nerves associated only with blood vessels (Nilsson, 1965). There is no need to postulate that changes of renin secretion are controlled by specific nerves since the changes could be secondary to the effects of renal vasomotor nerves on renal arteriolar pressure, renal blood flow or glomerular filtration rate. Any physiological or pharmacological factor which alters renal sympathetic activity could then be expected to alter the rate of secretion of renin, even when the renal arterial pressure remains constant.

The afferent limb of the reflex controlling renin secretion is difficult to define. Since changes of angiotensin concentration bore no relation to changes of arterial mean or pulse pressure it is unlikely that systemic arterial baroceptors were primarily involved. However, the consistent

inverse relation between central venous pressure and angiotensin concentration suggests that changes of blood volume were detected by stretch receptors in the great veins, atria, right ventricle or pulmonary circulation. No direct evidence on this point is available.

Our results suggest that renin secretion is normally under reflex control and that in response to changes of blood volume the direct effects of renal arterial pressure are of lesser importance. The rate of generation of angiotensin in the circulation is sufficient to have significant effects both on the blood pressure (Table 3) and on the secretion of aldosterone (Genest *et al.* 1960, Laragh *et al.* 1960). The renin–angiotensin system therefore seems to be of importance in both short and long term homeostatic responses to changes in blood volume.

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