Significance of Blood Angiotensin Levels in Different Experimental Conditions

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ALTHOUGH a great deal of information on the nature and pharmacological action of angiotensin is available, we are still not certain about its physiological significance. A role has been attributed to this substance in renal hypertension, in the regulation of blood pressure and renal hemodynamics and, more recently, in sodium metabolism.

From what we know about its pharmacological action, it seems likely that angiotensin acts according to its concentration in the blood. The measurement of this concentration in different experimental conditions would therefore seem to be of particular relevance.

A procedure for the estimation of angiotensin in blood has been developed in our laboratory.[1] Fifty millilitres of blood is collected from the femoral artery directly into ethanol, and angiotensin is partially isolated through several extraction procedures with different solvents, isoelectric precipitation, and chromatography in a Dowex 50 column. The pressor activity of the samples is finally tested in small nephrectomized rats.

With this procedure it is possible to detect the small amounts of angiotensin present in the blood of normal dogs. About two-thirds of angiotensin added to the blood *in vitro* can be regularly recovered, the same result being obtained with angiotensin I or II, with valine or isoleucine in position 5, aspartic acid or asparagine in position 1, and also with impure human angiotensin. A marked increase in angiotensin blood levels was detected in dogs after the injection of hog or dog renin, and an excellent correlation was obtained between these levels and the increases in blood pressure. The pressor activity of the samples is identical with that of angiotensin; it disappears after the incubation of the samples with trypsin; and no activity was found in the blood of dogs in different conditions after nephrectomy.

For these reasons, the procedure seems to meet the requirements of sensitivity, accuracy and specificity for the estimation of angiotensin in the blood, at least from what we know about this substance at present.

The object of this report is to summarize the results obtained when angiotensin blood levels were measured under different experimental conditions. The pressor activity of the samples will be expressed as their equivalence in micrograms of asparaginyl 1, valyl 5, angiotensin II, with a correction for an average recovery of 63%.[2*]

RENAL HYPERTENSION[1]

0-0.27 μg. of angiotensin per litre was found in the blood of normal unanesthetized dogs (Fig. 1, column 1). Anesthesia may increase these figures considerably.[3] Column 2 of Fig. 1 shows 25 values from 14 unanesthetized malignant hypertensive dogs in which 0-2.1 μg. of angiotensin per litre of blood was found. Although a definite average increase over normal values could be calculated ($p < 0.01$), one-third of these figures fell within the normal range. The remaining values are lower than those obtained when similar increases in the blood pressure were obtained by the injection of renin.[1, 2] Column 3 of Fig. 1 shows that no activity could be found in the blood of seven of these dogs 24 hours after total nephrectomy.

Since in these malignant hypertensive animals the severe constriction of the renal arteries produced macroscopic renal lesions, the possibility must be considered that the irregular increases in angiotensin blood levels were the result of a nonspecific liberation of renin from the damaged renal cells.

More definite results were obtained in eight chronic benign hypertensive dogs. As shown in Fig. 1, column 4, 15 values obtained from these animals were identical with normal values.

From these results we have concluded[1, 2] that an increase in angiotensin blood level does not seem to be essential for the maintenance of renal hypertension. Is it possible that in spite of these negative results this hypertension is caused by the renin-angiotensin system?

Twenty years ago, Huidobro and Braun-Menéndez[4] and Collins and Hamilton[5] demonstrated increased renin activity in the plasma of dogs after a severe hemorrhage. The possibility was then considered that a decrease in blood pressure produces a release of renin from the kidney. If this is so, and if this release of renin plays a role in the regulation of blood pressure, it may be that the kidney whose renal artery has been constricted "regulates" blood pressure at a higher level, that is, that renal hypertension is maintained, at least in part,

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*In previous publications,[1, 2] the pressor activity of the samples was expressed in rat units per litre of blood. No correction was made for recovery lower than 100%. One rat unit has the same pressor activity as 0.02 μg. of asparaginyl 1, valyl 5, angiotensin II.

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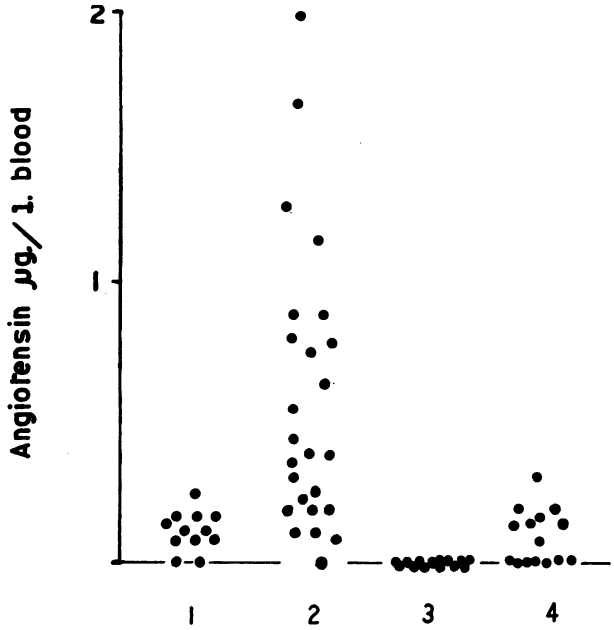

Fig. 1.—Angiotensin blood levels in: (1) normal dogs; (2) dogs made hypertensive by severe clamping of the renal arteries; (3) nephrectomized dogs, and (4) chronic renal hypertensive dogs.

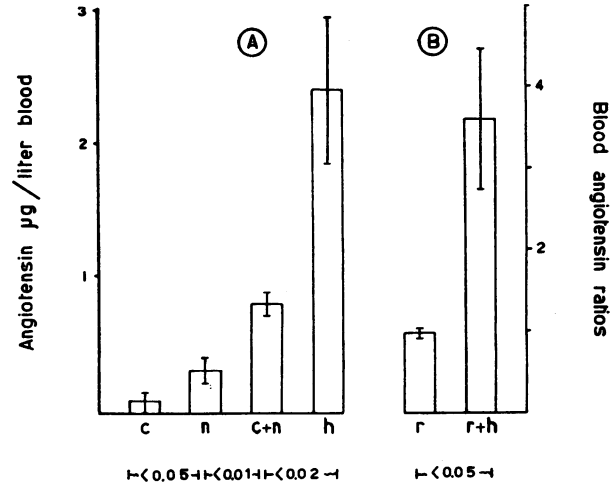

Fig. 3.—Variations in angiotensin blood levels following different experimental conditions: (A) Influence of constriction of the aorta (c), noradrenaline infusion (n), constriction of the aorta plus noradrenaline infusion (c + n), and hemorrhage (h). Columns represent average increases of final over control values. (B) Influence of bleeding on the effect of renin injection (r + h); control experiments (r). Columns represent average ratios: angiotensin blood levels after second renin injection over angiotensin blood levels after first renin injection. In all cases the range ± S.E. is indicated. Differences between groups were calculated by the t test and p values are recorded at the bottom.

by a discontinuous release of renin from the constricted kidney.

For this reason it was considered of interest to measure angiotensin in hemorrhagic hypotension and other related conditions.

HEMORRHAGIC HYPOTENSION AND RELATED CONDITIONS[3]

As shown in Fig. 2 (left), a marked and progressive increase in angiotensin blood levels was found in anesthetized dogs after a severe bleeding capable of decreasing the blood pressure to 80 mm. Hg or less. No activity was detected in the blood of nephrectomized dogs after this procedure. On the other hand, when a similar decrease in renal perfusion pressure was elicited by the constriction of the aorta above the renal arteries, no increase in angiotensin blood levels could be detected (Fig. 2, right). It was therefore concluded that the marked increases in angiotensin provoked by bleeding are not due solely to the decrease in the renal perfusion pressure. By what mechanism, then, are they produced?

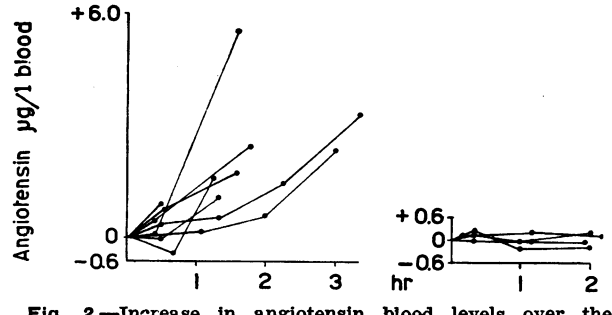

Fig. 2.—Increase in angiotensin blood levels over the control values (recorded as zero) at different times after hemorrhage (left) and constriction of the aorta (right).

Two possibilities were investigated: First, studies were carried out on the role of the well-known extreme reduction of renal blood flow after hemorrhage, which is due not only to the decrease in blood pressure, but also to a marked increase in renal vascular resistance.[6, 7] In an attempt to simulate this condition, a decrease in renal perfusion pressure was again elicited by constriction of the aorta, and, in addition, a highly vasoconstrictor dose of noradrenaline (2-4 µg./kg./min.) was infused. Although, as described above, no increase in angiotensin concentrations was observed after constriction alone (Fig. 3, c), and although the infusion of noradrenaline alone produced only a small increase (Fig. 3, n), the combination of both provoked a considerable increase in angiotensin blood levels (Fig. 3, c + n). This experiment suggests that the extreme reduction in renal blood flow may explain at least one part of the increases observed after bleeding (Fig. 3, h).

Secondly, the influence of hemorrhage on the effect of renin was also investigated. It may be predicted that liberated renin, being distributed in a smaller blood volume, reaches higher concentrations. It is also possible that disappearance of renin and/or angiotensin from the blood is slower in these extreme conditions. The effect of renin on angiotensin blood levels before and after bleeding was studied in dogs whose kidneys were removed four hours before, in order to prevent the endogenous liberation of renin. In each of these animals two injections of the same amount of dog renin were repeated, with an adequate interval between them. In Fig. 3 (B) the average ratios of angiotensin blood levels attained by both injections is recorded (a ratio of 1.0 means that the increase produced by the second injection is the same as

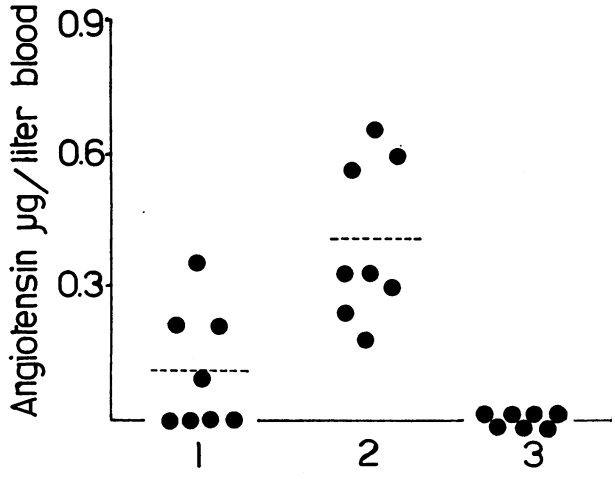

Fig. 4.—Angiotensin blood levels in: (1) normal dogs, (2) same dogs after sodium depletion, and (3) same dogs after total nephrectomy.

the increase produced by the first.) Column r illustrates control experiments in nephrectomized dogs. Column r + h shows a second group in which bleeding was performed between the first and the second injection. From a comparison of both groups it is evident that bleeding produced a marked increase on the effect of renin injections in angiotensin blood levels.

It seems, then, that the marked increase in angiotensin blood levels after bleeding is not due to the decreased renal perfusion pressure alone and may be explained by the extreme reduction in renal blood flow and/or a potentiation of the effect of liberated renin. The physiological significance of these increases in such extreme conditions is dubious.

SODIUM DEPLETION

Evidence has been accumulated in recent years which points to angiotensin as a physiological stimulus for the release of aldosterone by the adrenal cortex[8-10] (see also other contributions to this Symposium).

From the several experimental conditions in which increased secretion of aldosterone was demonstrated, sodium depletion seems to be the condition most directly related to the sodium-retaining action of the hormone. It was therefore considered of interest to investigate the concentrations of angiotensin in the blood of sodium-depleted dogs.

For this purpose, a synthetic diet was prepared, of which 80 calories, containing 2.6 mEq. of sodium and 26 mEq. of potassium, was administered daily for every 10 kg. body weight. During a one- to two-week control period, 33 mEq. of sodium chloride per 10 kg. was daily added to this diet. At the end of this control period, angiotensin blood levels, plasma sodium concentrations and the apparent volume of distribution of Na^{24} were measured. Salt was then suppressed from the diet, and 2 c.c. of meralluride was injected in each dog

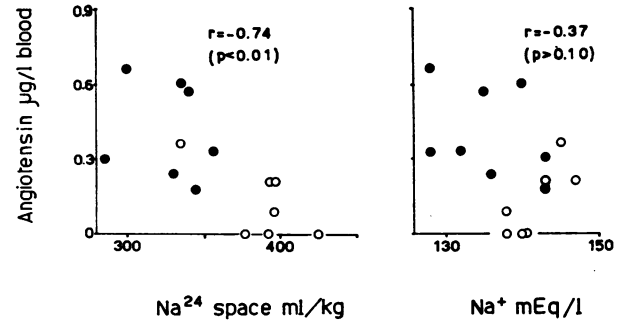

Fig. 5.—Correlation between angiotensin blood levels and the apparent volume of distribution of Na^{24} (left) or the plasma sodium concentration (right). The correlation index (r) and the probability that it is not different from zero (p) are recorded in each case.

at days 1 and 8 of this second period. At days 11 and 12 all of the measurements were repeated.

It should be recalled that this procedure is closely similar to that with which Davis, Ayers and Carpenter[11] were able to demonstrate a kidney-dependent increase in aldosterone secretion. The sodium-free diet and the mercurial diuretic produced in our dogs a sodium depletion estimated, on the average, as 18% of the total exchangeable sodium.

Under these conditions, angiotensin blood levels increased in eight dogs from 0.1 ± 0.05 to 0.4 ± 0.06 µg. per litre ($p < 0.01$) (Fig. 4, columns 1 and 2). The dogs were nephrectomized, and 24 hours later no pressor activity was found in their blood (Fig. 4, column 3). Moreover, a significant correlation was found between angiotensin blood levels and sodium space, but not between angiotensin and sodium plasma concentrations (Fig. 5).

These results fit with the hypothesis that angiotensin is the physiological stimulant for the release of aldosterone. The increases shown after severe sodium depletion were, however, moderate when compared with the effect of the injection of pressor doses of renin.[1, 2] More information about the sensitivity of the adrenal cortex to prolonged infusions of angiotensin in low doses, and simultaneous determinations of aldosterone and angiotensin concentrations in the blood in different conditions, will be necessary before a definite conclusion on this point can be reached.

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