ARTERIAL ANGIOTENSIN II AND VENOUS IMMUNOREACTIVE MATERIAL BEFORE AND DURING ANGIOTENSIN INFUSION IN MAN

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SUMMARY

1. Angiotensin II together with any immunoreactive fragments of angiotensin were measured in arterial and venous plasma before and during infusion of angiotensin II amide in five normotensive subjects, both in sodium-replete and sodium-depleted states. Because the antibody used in the radioimmunoassay cross-reacted with larger angiotensin breakdown products and because these fragments are present in higher concentrations in venous than arterial plasma, samples of both were assayed. Measurements on venous plasma are referred to as immunoreactive material (IM).

2. Arterial plasma angiotensin II concentration (PAC) and venous IM were compared in two additional subjects during infusion of angiotensin II amide and of synthetic human angiotensin II.

3. Arterial PAC and venous IM increased in linear relation to the dose of angiotensin infused, similar results being obtained with angiotensin II amide and of synthetic human angiotensin II.

4. Before infusion, venous IM and arterial PAC were comparable (A:V ratio = 0.99±0.16, n = 29). During infusion venous IM was consistently lower than arterial PAC; with angiotensin II amide the A:V ratio was 1.72±0.49 (n = 33), and with synthetic human angiotensin it was 1.63±0.2 (n = 6).

5. Results of an in vitro study suggest that the formation of new angiotensin in the vein is not sufficient to explain the lower A:V ratio of PAC before infusion.

6. It is proposed that arterial PAC rather than venous IM should be measured when the relationship between blood levels and biological effects of the peptide are the object of the study, particularly during angiotensin infusion or when rapid changes in circulating angiotensin occur.

Key words: infusion, angiotensin II, immunoreactive material, radioimmunoassay, arteriovenous ratios.

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The development of sensitive radioimmunoassay methods for the measurement of angiotensin II (e.g. Boyd, Landon & Peart, 1967; Catt, Cain & Coghlan, 1967; Valloton, Page & Haber, 1967) have provided an important additional tool for the evaluation of the renin-angiotensin system. Since some of the peptide fragments formed by degradation of angiotensin II exhibit a great affinity for antibodies raised against octapeptide angiotensin, falsely high results will be obtained when these fragments are present. Cain, Catt & Coghlan (1969) and Catt, Cain, Coghlan, Zimmet, Cran & Best (1970) found the apparent venous angiotensin II concentration in man to be about 20% lower than the arterial concentration. Most of the immunoreactive material in venous blood was the hexapeptide fragment (amino acids 3–8) of angiotensin, whereas 85% of the material in arterial blood was octapeptide.

Dusterdieck & McElwee (1971) showed that the difference between apparent arterial and venous angiotensin II levels was very small when the antibody used exhibited a high cross-reactivity with the hepta- and hexa-peptide fragments of angiotensin. However, we now report further studies involving infusion of angiotensin which showed that the arteriovenous ratio of immunoreactive material changed during the course of the infusion.

Because arterial plasma angiotensin II measured by radioimmunoassay closely reflects true angiotensin II (Cain et al., 1969) and since intravenously infused angiotensin II passes the pulmonary bed without significant destruction (Hodge, Ng & Vane, 1967; Biron, Meyer & Panisset, 1968) we shall use the term arterial plasma angiotensin II concentration (PAC). For venous levels, however, we shall use the term venous ‘immunoreactive material’ (IM). For the ratio of arterial plasma angiotensin II to venous immunoreactive material the abbreviation A:V ratio will be used.

METHODS

Subjects and infusion experiments

The various procedures were approved by the Ethical Supervisory Committee of the Western Infirmary, Glasgow, and informed consent was obtained from all the subjects participating in the study. In no instance was an arterial pressure of 135/95 mmHg exceeded during angiotensin infusion.

The first study was performed on the morning immediately following three full days on a fixed diet containing 150 mEq of sodium and 60 mEq of potassium per 24 h. Immediately after the first infusion, the sodium intake was reduced to 10 mEq/day (potassium remaining at 60 mEq), and an intravenous injection of frusemide (30–60 mg) was given to enhance sodium loss. This diet was continued for a further 3 days, the second study being performed on the morning of the fourth day. The cumulative loss of sodium in the urine exceeded the total sodium intake by 76–247 mEq (mean 143 mEq). Infusion studies started between 08.00 and 09.00 hours on the day immediately after the last day of each diet and after at least 8 h of recumbency. All subjects were fasted from 20.00 hours on the previous day.

Argyle ‘Medicut’ cannulae (20 G.A. or 18 G.A.) were introduced into an antecubital vein of each arm, one being used for infusion and the other for blood sampling. A similar cannula was placed in one femoral artery; 5% dextrose was then infused into one arm vein at 0.25 ml/min. Then 20 and 40 min later, arterial and venous blood samples (10 or 20 ml) for the immunoassay were drawn simultaneously and at the same speed into syringes containing a solution which inhibited angiotensinase and converting-enzyme activity (Düsterdieck & McElwee, 1971).
The veins of the arm were congested by inflating a blood-pressure cuff for a few seconds before the sampling started.

After the basal period angiotensin II amide (Hypertensin, Ciba, 0.5 µg/ml in 5% dextrose solution) was infused for three consecutive periods of 1 h each at 2, 4 and 8 ng min⁻¹ kg⁻¹ with a variable-speed infusion pump (Sage Instruments model 255-1). The delivery of the pump was checked in all experiments by repeated measurements of the position of the plunger. With one exception (see Fig. 2) the actual delivery was within 8% of the expected value and the variation was neglected. The blood-sampling procedures described above were repeated during the final minutes of each infusion. Preliminary experiments in two normal subjects had shown that venous immunoreactive material differed by less than 10% when measured in samples taken 30 and 60 min after the start of an angiotensin II amide infusion (6 ng min⁻¹ kg⁻¹).

Two further normal male volunteers (26 and 30 years of age) were infused with both angiotensin II amide ([Asn¹-Val⁵]-angiotensin II) and synthetic [Asp¹-Ile⁵]-angiotensin II (supplied by the M.R.C. Division of Biological Standards, Mill Hill, London, and passed through 0.22 µm M.F. millipore filters before use) on the fourth day of taking a fixed diet containing 150 mEq of sodium and 60 mEq of potassium; the interval between the infusions was 3 weeks in one subject and 6 weeks in the other. The experimental procedure was similar to that described before except that the final infusion period (8 ng min⁻¹ kg⁻¹) lasted 15 min.

Effect of delay in addition of inhibitor solution

A sample (60 ml) of venous blood was rapidly drawn from: (a) three patients with various endogenous angiotensin II levels; (b) two normal subjects 15 min after the start of the 2 ng min⁻¹ kg⁻¹ angiotensin II amide infusion; (c) the same two subjects 15 min after the start of 3 ng min⁻¹ kg⁻¹ [Asp¹-Ile⁵]-angiotensin II infusion.

The first 20 ml sample was immediately mixed with 1 ml of the angiotensinase and converting-enzyme inhibitor solution. The second and the third samples were added to the inhibitor after a delay of 2 and 4 min respectively. Immunoreactive material was then measured in all samples. The temperature of the blood in the syringe after 2 and 4 min was 34°C and 33°C respectively.

Radioimmunoassay of angiotensin II

Arterial PAC and venous IM were measured by the method of Dusterdieck & McElwee (1971). In this method angiotensin and its larger fragments are extracted from plasma on Dowex ion-exchange resin (H⁺ form), eluted with methanolic ammonia solution and assayed in serial dilutions in an incubation mixture containing antiserum to angiotensin II and ¹²⁵I-labelled angiotensin II amide (10 pg). With [Asn¹-Val⁵]-angiotensin II as reference, the cross-reactivity on a molar basis of the antiserum used is 100% to [Asp¹-Ile⁵]-angiotensin II, 0.6% to [Asp¹-Val⁵]-angiotensin I, 81% to the heptapeptide (amino acids 2-8) fragment, and 118% to the hexapeptide (amino acids 3-8) fragment (both fragments [Val⁵]-).

The mean recovery of [Asp¹-Ile⁵]-angiotensin II added to blood containing inhibitor was 83% (±11% SD; n = 44). The recovery of the heptapeptide and hexapeptide fragments added to blood was not significantly different from that of the octapeptide. The results are corrected for a 17% loss during extraction. The normal range of such immunoreactive material in peripheral venous blood of thirty healthy supine subjects taking an unrestricted diet, and with [Val⁵]-angiotensin II amide as standard, was 5–35 pg/ml, mean 16 pg/ml.
RESULTS

Changes of arterial plasma angiotensin II and venous immunoreactive material during infusion of angiotensin

Arterial PAC and venous IM increased in all subjects during infusion of the peptide. The increase of mean arterial PAC and venous IM were linear functions of the dose of angiotensin infused (Fig. 1). Before infusion, arterial PAC and venous IM were not significantly different. During infusion, however, venous levels of IM were consistently lower than arterial PAC.

Sodium depletion increased basal arterial PAC and venous IM (Fig. 1). The lines connecting mean arterial PAC and venous IM before and during angiotensin infusion in the sodium deplete state were approximately parallel to the corresponding lines in the sodium replete subjects.

![Graph showing changes in arterial PAC and venous IM during angiotensin infusion](image)

**Fig. 1.** Arterial plasma angiotensin concentration (---) and venous plasma levels of immunoreactive material (-----) before (basal) and during incremental infusions of angiotensin II amide in the sodium replete (●) and deplete (○) states. Points represent means of measurements in five subjects ± SEM.

Arterial PAC and venous IM attained by infusion of [Asn¹-Val⁵]-angiotensin II (angiotensin II amide) and [Asp¹-Ile⁵]-angiotensin II in the same subjects under identical conditions were similar (Fig. 2). As with the study of angiotensin II amide, venous IM was lower than arterial PAC during infusion of the [Asp¹-Ile⁵]-angiotensin.

The relationship between individual arterial PAC and venous IM before and during angiotensin infusion is shown in Fig. 3. Before infusion (Fig. 3a) the mean ratio (A:V) of twenty-nine pairs of arterial PAC and venous IM was 0.99 ± 0.16. This includes data from two patients with
Arterial and venous angiotensin raised, and two with normal angiotensin levels not under the dietary scheme. Since the mean ratio of endogenous arterial PAC to venous IM before (1.00 ± 0.18) and after (0.99 ± 0.13) sodium depletion was not significantly different, the regression of all values for endogenous arterial PAC (y) and venous IM (x) can be described by one equation: y = 0.94x + 1.

During infusions of angiotensin II amide the mean A:V ratio of all observations (thirty-five pairs) increased to 1.72 ± 0.49 (y = 1.92x - 28). The increase was statistically significant (P < 0.001). Again, there was no significant difference between A:V ratios in the sodium-replete (1.80 ± 0.51) and in the sodium-depleted state (1.58 ± 0.43).

The increase of the A:V ratio was not solely a feature of an abnormally high arterial PAC since an increased A:V ratio was apparent when only 2 ng min⁻¹ kg⁻¹ of angiotensin II were infused in the sodium-replete state (1.53 ± 0.33), and the arterial angiotensin levels (62–92 pg/ml, mean 73 pg/ml) were then similar to those in the sodium-depleted state before infusion (32–85 pg/ml, mean 60 pg/ml).

![Graph](image-url)
FIG. 3. Relationship between arterial plasma angiotensin concentration and venous plasma levels of immunoreactive material before (a) and during (b) infusion of angiotensin II. ●, Measurements in the sodium replete state; ○, measurements in the sodium deplete state; ×, measurements in four patients with different diseases not included in the infusion study; ▲, measurements during infusion of synthetic human angiotensin II; - - - indicates the hypothetical arteriovenous ratio (A:V ratio) of 1.0. The regression lines describe: (1) all A:V ratios included in (a); (2) all A:V ratios during infusion of angiotensin II amide (● and ○); (3) all A:V ratios during infusion of synthetic human angiotensin II (▲).

FIG. 4. Effect of delay in addition of inhibitor on % change of immunoreactive material in venous plasma. ●, Blood taken before angiotensin infusion (100% = 8 pg/ml, 26 pg/ml and 51 pg/ml respectively); □ and △, blood drawn during angiotensin II amide infusion (100% = 54 pg/ml and 91 pg/ml respectively); ■ and ▲, blood drawn during synthetic human angiotensin II infusion (100% = 178 pg/ml and 198 pg/ml respectively).
Arterial and venous angiotensin

There was no significant difference between the mean ratios of arterial PAC to venous IM during infusions of synthetic human angiotensin II (1.63 ± 0.2) and of angiotensin II amide in the sodium-replete state. The regression equation for arterial PAC and venous IM during infusion of synthetic human angiotensin was

\[ y = 1.80 - 11. \]

The correlation between arterial PAC and venous IM was highly significant before and during angiotensin II amide and synthetic human angiotensin II infusions (P<0.001, in all cases). The coefficient of variation of the A:V ratios, however, was greater during angiotensin II amide infusions (29%) than when endogenous arterial PAC and venous IM was measured (16%).

Effect of delay in the addition of inhibitor solutions

In basal conditions plasma angiotensin concentration is governed mainly by endogenous renin levels, but during angiotensin infusion this relationship is broken. In the latter circumstances circulating angiotensin consists of a large fraction of exogenous and a small fraction of endogenous angiotensin, both of which are subjected to inactivation in the capillary bed and in the plasma, whereas the generation of fresh angiotensin II in peripheral blood remains dependent on the relatively low renin levels. These considerations might be of importance in the development of the arteriovenous gradient of IM during angiotensin infusions, and in vitro experiments described above were done to investigate this possibility further.

The results are shown in Fig. 4. In samples of blood drawn before angiotensin infusion, delay in the addition of inhibitor led to an increase of venous IM. After 2 min of incubation IM had increased by 10–30% and after 4 min by 47–52%. During infusion, by contrast, the corresponding levels decreased by 3–12% and 10–20% respectively. By extrapolation of these data the average change of IM after 1 min of incubation was +11% in samples drawn without angiotensin infusion and −3% in samples taken during angiotensin infusion. The difference of one set of samples relative to another was approx. 14%/min.

DISCUSSION

Arteriovenous ratios

Our results confirm the observations of Cain et al. (1969) and Catt et al. (1970) that arterial angiotensin II levels and venous IM are similar when measured by radioimmunoassay using an antibody which exhibits nearly 100% cross-reactivity with the hepta- and hexa-peptide fragments of angiotensin. During angiotensin infusion, however, venous IM is much lower than the arterial PAC, and the A:V ratio becomes much more variable.

It is noteworthy that the A:V ratio during infusions of [Asp4-Ile5]-angiotensin II, which has been shown to be the human form of angiotensin (Arakawa, Nakatani, Minohara & Nakamura, 1967; Catt et al., 1970), followed the same pattern as that seen during the infusion of angiotensin II amide. This indicates that chemical differences between endogenous and infused angiotensin are not the cause of the change in A:V ratio during angiotensin infusion.

Intra-arterial infusions and injections of angiotensin II in animals have, in general, a smaller pressor effect than intravenous ones (Akinkugbe, Brown & Cranston, 1966; Hodge et al., 1967; Biron et al., 1968; Haas, Goldblatt, Lewis & Gipson, 1968).

In man also, the pressor effect of intra-arterial angiotensin II amide is greatly reduced (Berman, Roza, Mitra & Vertes, 1971). The latter authors found that this was partly due to a marked reduction of local blood flow after intra-arterial injections. Both these observations and
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the conclusion of Cain et al. (1969) that only 29% of the immunoreactive material in venous blood is octapeptide angiotensin, the remainder being largely made of pharmacologically less active metabolic fragments, are consistent with the concept (Hodge et al., 1967; Vane, 1969) that most of the arterial angiotensin II is extracted in the peripheral capillary bed. Venous angiotensin levels, however, when measured by radioimmunoassay, appear falsely high as the metabolic fragments of angiotensin II released into the venous blood are themselves immunoreactive.

As explained above, the development of an arteriovenous gradient of IM during angiotensin infusion was not solely a feature of an abnormally-high arterial PAC. This being so, it can be calculated from the ratios before and during angiotensin infusion (0.99 and 1.72 respectively), that for a given arterial PAC the levels of venous IM were on average 43% lower during than before infusion.

The question arises, therefore, whether our in vitro experiment can contribute to an explanation for this observation. Fig. 4 shows that in venous blood incubated in vitro without inhibitor the balance between the formation and the destruction of immunoreactive material is positive when angiotensin is endogenously formed, but is negative when it is infused. This observation is not, apparently, a consequence of different venous IM levels at zero time of in vitro incubation, as some of the IM levels at this time were similar in both sets of experiments. It is therefore unlikely that the change can be explained on a kinetic basis.

The in vitro incubation can be considered as a model, though not exact, of the in vivo incubation of venous blood between the capillary bed and the place of venepuncture. This change in venous IM in the in vitro experiments is therefore in the right direction, but not sufficient to account totally for the 43% difference in venous levels observed before and during infusion.

The only report in the literature, of which we are aware, on A :V ratios of angiotensin measured by radioimmunoassay during angiotensin infusions is by Cain, Catt, Coghlan & Blair-West (1970). These authors infused [Asp]\(^1\)-angiotensin II (10–40 \(\mu\)g/h) into a sheep and found the venous IM level during infusion to be approx. 50% of the arterial level. The apparent venous angiotensin level before infusion however, was only 60% of the arterial level. In this case, the A :V ratio of angiotensin II did not markedly change during infusion.

Angiotensin metabolism

More detailed studies with separate measurements of angiotensin II and its larger immunoreactive fragments in venous blood before and during angiotensin infusions would be required to show whether the metabolism of angiotensin II in the peripheral capillary bed changes during infusion. Our measurements of venous IM include angiotensin fragments and therefore allow no conclusion as to the extraction of angiotensin by peripheral tissues. The observation, however, that comparable arterial angiotensin II levels are attained by infusion of similar doses of angiotensin II amide and [Asp]\(^1\)-Ile\(^5\)]-angiotensin II seems to indicate that their plasma clearance rate is similar. This is in agreement with the observations of Hodge et al. (1967), in the dog, that the extraction of angiotensin II by peripheral tissues is more important than are plasma angiotensinases, which destroy angiotensin II amide much faster than angiotensins with aspartic acid in the N-terminal position (Brunner & Regoli, 1962; Nagutsu, Gillespie, Folk & Glenner, 1965; Zwanzig & Oelkers, 1969).

The results of our arterial plasma angiotensin measurements during angiotensin infusion in
the sodium-replete and sodium-depleted state allow no direct conclusions on the influence of sodium depletion on angiotensin metabolism. As shown in Fig. 1, the results during the infusions are approximately parallel in the sodium-replete and sodium-depleted states. The slight divergence of the curves at the highest dose level infused is not statistically significant.

The practical implications of these findings are that where basal angiotensin II levels are measured by radioimmunoassay, using an antibody exhibiting a complete cross-reaction with the hepta- and hexa-peptide fragments, the apparent venous angiotensin II levels (angiotensin II plus fragments) will closely reflect the arterial angiotensin II levels. This is important from a routine clinical point of view as venous samples are easier to obtain than arterial. However, during angiotensin infusion when the relationship between the blood level and the biological effect is the object of the study, and also in conditions when rapid changes in endogenous angiotensin II levels occur, then arterial levels should be measured.

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REFERENCES


