PRESSOR RESPONSE TO ANGIOTENSIN I AND ANGIOTENSIN II: THE SITE OF CONVERSION OF ANGIOTENSIN I

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(Received 12 June 1972)

SUMMARY

1. The pressor responses to angiotensin I were compared with those to angiotensin II after injections into the left ventricle and jugular vein in the sheep, dog and pig.
2. The ability of angiotensin I to raise the blood pressure was less than that of angiotensin II with both routes of injection, a difference which was more marked after ventricular injection.
3. When equipressor doses of the hormones were given there was a delay of 1–3 s in the onset of the pressor response to angiotensin I compared with angiotensin II after left-ventricular injections; the difference in the delay in onset was less apparent with intravenous injections.
4. The development of the pressor responses was similar with both hormones when equipressor doses were used but the rises in blood pressure were more prolonged with angiotensin I, especially when given by the left-ventricular route.
5. The in vitro rate of activation of angiotensin I by blood was much slower than the apparent in vivo formation of angiotensin II.

Key words: pressor response, angiotensin I conversion, angiotensin II, blood pressure.

Angiotensin occurs in two forms, a decapeptide, angiotensin I and an octapeptide angiotensin II (Skeggs, Marsh, Kahn & Shumway, 1954a, b; Helmer, 1955, 1957; Skeggs, Kahn & Shumway, 1956). The purpose of the present investigation was to compare the pressor effects of the two angiotensins after injection into the left ventricle and jugular vein and to relate the findings to in vitro studies with arterial and venous blood.

METHODS

Two kinds of angiotensin I were used. One, supplied by Ciba Ltd, Basle, Switzerland, was

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Asn$^1$-Val$^5$-angiotensin I, the angiotensin I equivalent of the synthetic [Asn$^1$-Val$^5$]-angiotensin II used (‘Hypertensin’, Ciba, Basle). The preparation, isolation and identification have previously been outlined (Osborn, Pickens, Willicombe, Pirie & Mahler, 1970; see also Montague, Riniker & Gross, 1966; Reindel & Hoppe, 1954; Weygand, Hoffmann & Wünsch, 1966).

The other angiotensin I employed was [Asp$^1$-Ile$^5$]-angiotensin I supplied by Schwarz BioResearch, Orangeburg, New York. It was synthesized using the solid-phase peptide synthesis technique (Merrifield, 1963). Purification was by gel chromatography and the purity was subsequently checked with thin layer chromatography. Amino acid analysis (Spackman, Stein & Moore, 1958) was also used to determine the nature of the material. These procedures established that the isolated material contained less than 5% impurities (Schwarz BioResearch, 1970; Dr W. C. Roberts, personal communication, 1970).

Preparation of the angiotensin solutions

Stock angiotensin I solutions of 20 μg/ml in 0.15 M-NaCl were made in polystyrene bottles; appropriate diluted saline solutions, also in polystyrene bottles, were made as required. Solutions of angiotensin II in 0.15 M-NaCl were made from stock solutions of 10 μg/ml, prepared on the day of the experiment and kept in glass vessels; storage of the diluted solutions was also in glass vessels except for those of 0.10 and 0.20 μg/ml which were stored in polystyrene bottles. There was no evidence of instability under these conditions.

Possible contamination of angiotensin I

The rat colon contracts weakly to angiotensin I compared with angiotensin II (Ng & Vane, 1967, 1968) and this assay method (Regoli & Vane, 1964) can be used to assess the degree of contamination of angiotensin I. Twelve experiments were made with the Ciba angiotensin I and six with the Schwarz angiotensin I.

The angiotensin II was used at concentrations of 0.05–10 ng/ml in the organ bath; 10–100 times as much angiotensin I produced the same maximum contractions in a particular preparation and on average about 25 times as much was required. The response usually developed more slowly with angiotensin I and the findings suggested that angiotensin I preparations were unlikely to be contaminated by more than 1% of angiotensin II, or material acting similarly.

Preparation of the experimental animals and injections of hormones

One hundred and forty-three Kerry Hill and Welsh Mountain ewes and wethers of average weight 34±8 kg (SD), four dogs and four pigs were used. The dogs averaged 20 kg and the pigs 39 kg. Anaesthesia and general preparation were as described previously (Osborn, Hughes, Pirie, Willicombe & Mahler, 1969). The first 30 s of the blood-pressure response was recorded at 5 mm/s and subsequent tracings at 0.25 mm/s when the onset of the response was required. All other recordings were made at a speed of 0.25 mm/s throughout. The animals tolerated the experiments well without signs of stress.

Serial intravenous injections of 5 or 10 μg of angiotensin II (in 5 ml of saline over 1–2 s followed by a rapid wash with 2 ml of saline) were made at the beginning of each experiment until good reproducibility was observed (i.e. pressor responses were consistently within ±10% of the mean value). Subsequent injections of the hormones were made at 6–8 min intervals so that 3–4 min elapsed between the time when the blood pressure returned to normal and the next injection.
Studies on angiotensin I conversion

**Estimation of the maximum rise in blood pressure and the onset of the response**

The maximum pressor response and the time elapsing between the start of an injection and the first discernible rise in blood pressure was independently estimated by two observers. The rise in blood pressure was estimated to the nearest mmHg and the onset to the nearest 0.2 s. The estimated maximum pressor response seldom differed by more than 1 mmHg between the two readings. When the pressor response was 20 mmHg or greater the two estimations of onset agreed within 0.2 s. The onset was appreciably more difficult to place with rises in blood pressure of 10 mmHg or less. The averages were taken to the nearest mmHg for the rises in pressure and to the nearest 0.2 s for the time of onset.

**TABLE 1. Replication of pressor response in sheep after serial injections of angiotensin I and of angiotensin II.** The results in columns (a), (b), (c), (d) and (e) refer to the average ratios and associated SD calculated by comparing a particular pressor response to the next, the next but one, the next but two, the next but three and the next but four respectively. JV represents jugular vein injection and LV represents left-ventricular injection.

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**Replication of the pressor responses**

Experiments were carried out in four sheep of about average sensitivity and reproducibility in which thirty successive injections were made to determine the replication of the procedure. The doses of angiotensin I (Schwarz preparation) were 20 µg when the hormone was injected into the left ventricle in one experiment and 12 µg when injected intravenously in another. Similar studies were made with 4.0 µg of angiotensin II. The replication of the pressor response was established by calculating the ratio of a particular rise in blood pressure to the next, the next but one, the next but two, the next but three and the next but four. The results are reported in Table 1.

**Dose–response relationship**

This relationship was established in twelve sheep, three dogs and three pigs using doses of 1.2–24 µg of angiotensin I (both preparations in each experiment) and of 0.5–10 µg of angiotensin II. The doses were chosen at random and one injection of each was made by either route in every case.
Experiments were also made in six other sheep to establish the relationship between the onset of the pressor response and its height. Three investigations were performed with 1·2–24 \( \mu \)g of angiotensin I (Schwarz preparation) and three with doses of 0·5–10 \( \mu \)g of angiotensin II. Three series of injections, with doses given in random order, were made in each case.

**Use of single doses of angiotensin I**

Experiments were made in eight sheep, three dogs and three pigs, in which five series of injections of both preparations of angiotensin I were made into the left ventricle and jugular vein in each case. The doses were chosen to give pressor responses of about 20 mmHg.

Similar experiments were made in five sheep in which the Ciba angiotensin I was used and in five others with the Schwarz material.

**Use of equipressor doses**

The dose–response relationship had shown that approximately equipressor responses to the three hormones (usually in the range 15–30 mmHg) occurred with injections of 20 and 12 \( \mu \)g of angiotensin I into the left ventricle and jugular vein respectively and of 4·0 \( \mu \)g of angiotensin II by either route. Further investigations were made to study in detail the rate of onset, development and duration of the pressor response with these doses. Three series of injections were made in each of sixty-five experiments, the injections within a series being made randomly.

**Variation of pressor response with injection and infusion of angiotensin II over different times**

Angiotensin II (10 \( \mu \)g) was injected into the left ventricle over 3, 10 and 30 s in fifteen sheep (three series in each case) to determine the effect of changing the speed of introduction into the circulation on the height and duration of the blood-pressure response.

**Estimation of circulation times**

Radioactive sodium pertechnetate \((^{99mTc} \text{pertechnetate})\) was injected as a bolus into the femoral artery in six sheep; its progress around the circulation was monitored by detectors placed at right-angles to the rib cage on either side of it near the heart and by one detector scanning the hind-limb contralateral to the injected one. The circulation time between the hind-limbs was usually between 18 and 20 s. The radioactivity travelled from the right to left side of the heart in 4–6 s and reached the hind-limb after a further 4–6 s. These findings indicated that when angiotensin was injected into the jugular vein it generally reached the organs supplied by the systemic circulation in 8–12 s and about 5 s sooner when injected into the left ventricle.

**Statistical evaluation of the results**

Statistical evaluation was made by ‘Analysis of Variance’ (Moroney, 1951), with reference being made to Variance Ratio Tables (Fisher & Yates, 1948).

**RESULTS**

**Dose–response relationship**

The results in the twelve sheep are shown in Fig. 1 and indicate that intravenous injections of angiotensin I are more effective than left ventricular ones in raising the blood pressure over a
wide dose range. Angiotensin II was shown to be equally effective by either route. The results were similar with the dog and pig.

The average pressor responses of 9–24 mmHg with injections of angiotensin I into the left ventricle were associated with delays in the onset of the pressor response of 9.9–7.6 s in the experiments in which both factors were determined. The corresponding figures with intravenous injections were 12–28 mmHg and 14.2–13.2 s. The pressor responses of 13–31 mmHg with angiotensin II by either route started 6.3–5.7 s and 14.9–12.6 s after the left-ventricular and jugular vein injections respectively. The development of the pressor response was similar with both hormones with doses giving the same rise in blood pressure.

![Graph showing pressor responses with various doses of angiotensin I and II](image)

**Pressor responses with the same dose of angiotensin I by both routes**

Both angiotensin I preparations gave similar pressor responses when they were given to the same animal. There was also usually good agreement between the ratio of the pressor response after intravenous injections to that after intraventricular injections (JV/LV ratio) with both hormones. These averaged 1.15±0.09 (SD) and 1.18±0.10 (SD) for the Ciba and Schwarz material respectively [average difference 0.04±0.03 (SD)]. Similar results were obtained in the dog and pig. The response to intravenous injections was always significantly greater than that to ventricular injections. This was true with either preparation of angiotensin I.

**Blood-pressure responses with equipressor doses of the hormones**

The average rise in blood pressure was the same [23±6 mmHg (SD)] with injections of angiotensin I into the left ventricle and jugular vein; there was the same average response with a similar scatter with injections of angiotensin II by both routes. The average JV/LV ratio of
0.99 for angiotensin I and of 1.01 for angiotensin II each had SD±0.09. These are probably greater than can be explained by purely experimental errors; the studies on replication suggest that in most of the experiments the average JV/LV ratio was estimated with an accuracy of ±0.05 or less.

The onset of the rise in blood pressure was determined in twenty-five of these experiments. The pressure started to rise 7.6±1.2 s (SD) and 12.0±2.5 s (SD) after injections of angiotensin I into the left ventricle and jugular vein respectively; the corresponding results for angiotensin II were 6.0±1.1 s (SD) and 11.6±2.1 s (SD). There was a delay of 1.6±0.8 s (SD) with left-ventricular injections of angiotensin I compared with angiotensin II and of 0.4±0.5 s (SD) with the intravenous ones for the individual experiments. The lag in the response with angiotensin I was always statistically significant (P mostly <0.01) with injections into the left ventricle, but the significance was usually much less with the intravenous route in a particular experiment.

The relative sensitivity to the hormones varied appreciably; the average ratio with the left-ventricular injections was 0.99±0.10 (SD) and with the intravenous ones it was 0.98±0.07 (SD). The maximum response was reached 14±3 s (SD) and 12±2 s (SD) after the onset of the pressor response with the left-ventricular injections of angiotensin I and angiotensin II respectively; the corresponding times for the intravenous injections were 13±3 s (SD) and 13±2 s (SD).

The response was better sustained with the angiotensin I especially with the left-ventricular injections (Fig. 2); 1 min after the peak of the response the blood pressure had fallen by 10 and 12 mmHg on average with injections of angiotensin I into the left ventricle and jugular vein respectively and by 14 mmHg with injections of angiotensin II by both routes.

The area under the pressor-response curve was increased by 12±15% (SD) and by 20±16% (SD) with injections over 10 and 30 s respectively compared with those over 3 s.

Pressor responses to angiotensin II with injection and infusion over different times

The average rise in blood pressure was the same (25 mmHg) with injections over 3 and 10 s; the response was lower (23 mmHg) with injections over 30 s; each had SD ±5 mmHg. The peak of the response was reached 13±2 s (SD), 18±3 s (SD) and 25±5 s (SD) respectively after the start of the response. The area under the pressor–response curve was increased by 12±15% (SD) and by 20±16% (SD) with injections over 10 and 30 s respectively compared with those over 3 s.

In vitro activation of angiotensin I by sheep blood

It is known that angiotensin I can be activated by enzymes present in blood (Helmer, 1955, 1957; Skeggs et al., 1956; Andersen, 1967; Ng & Vane, 1967, 1968; Oparil, Sanders & Haber, 1970; Osborn et al., 1970). The in vitro activation of angiotensin I was studied with blood from five sheep. Blood (50 ml) was collected from the right atrium and lower abdominal aorta with heparin as anticoagulant. Angiotensin I (0.36 and 1.08 μg of both preparations) was mixed with either 50 ml of Tyrode
solution or 50 ml of blood in the organ-bath. Angiotensin II (0.03, 0.09 and 0.27 µg) was similarly used. These doses were chosen to give acceptable contractions of the colon and to approximate the in vivo blood concentrations initially achieved with injections into the circulation.

The contractions produced by each of the five doses of the angiotensins in Tyrode solution were determined at the beginning of each experiment, the doses being chosen at random. The solution was then removed from the organ-bath and replaced with blood immediately after it had been taken from the sheep. The colon was exposed to the blood until a steady baseline was achieved (usually within 1 min) and the contractions of the colon after the addition of both doses of angiotensin I and of 0.09 µg of angiotensin II were then measured in duplicate. The response to the same dose of angiotensin II in Tyrode solution was also estimated at regular intervals.

The results are shown in Fig. 3; mixing of angiotensin I with blood considerably enhanced its potency whereas the effect of angiotensin II was considerably less when mixed with blood instead of Tyrode solution.

**In vitro destruction of the angiotensins by blood**

Experiments were made in eight sheep to study the in vitro rate of inactivation of both preparations of angiotensin I and of angiotensin II by arterial and venous blood. The hormones
(1-2–12 µg of angiotensin I and 0.5–5-0 µg of angiotensin II in 5 ml of 0.15 m-NaCl) were mixed with 45 ml of blood for 45 s–5 min at 38°C in a water-bath. The mixtures were then infused into the jugular vein over 10 s and the pressor responses compared with those after similar infusions of 45 ml of blood diluted to 50 ml with 0.15 m-NaCl and of various doses of the hormones.

The destruction of the angiotensins was similar in arterial and venous blood and for the various concentrations; about 25% of the activity was lost within 45 s and about 60% after 5 min.

![Graph](a) and (b)

**FIG. 3.** Contraction of rat colon after addition of (a) angiotensin I (Ang. I) and (b) angiotensin II (Ang. II) to the organ-bath containing either Tyrode solution or blood. Similar results were obtained with arterial and venous blood and these were considered together.

**DISCUSSION**

The experiments described in the present paper show that in the sheep, dog and pig both preparations of angiotensin I have similar activity despite differences in them. Also the compounds were prepared and isolated by widely dissimilar techniques.

Ng & Vane (1967, 1968) suggested that in the dog the conversion of angiotensin I into angiotensin II is probably restricted to the pulmonary circulation. Our results, however, show that when angiotensin I is injected into the left ventricle it exerts a pressor effect before it passes through the pulmonary circulation; study of circulation times in sheep shows that if conversion occurred only in the lungs the response to an intraventricular injection of angiotensin I would occur 16–20 s later than that to angiotensin II. We observed no delay of that order either in the onset of the pressor response or in the time required for the maximum response to develop over a wide range of doses. These findings suggest that angiotensin I has either an immediate direct effect on the vasculature in the systemic circulation or that it is rapidly converted into angiotensin II.
Studies on angiotensin I conversion

Our observations that the onset and development of the pressor response to angiotensin I, whether after left-ventricular or intravenous injection, is only marginally slower than that to angiotensin II suggest that both hormones are about equally rapidly attached to receptors.

There was an average delay of 1.6 s with angiotensin I compared with angiotensin II after injections into the left ventricle, and of 0.4 s after injections into the jugular vein with equipressor doses of the hormones. This shortened delay with the intravenous route suggests that the lungs may play a role in the response to angiotensin I.

Angiotensin I was appreciably more effective in increasing the blood pressure when injected intravenously rather than into the left ventricle, whereas angiotensin II gave similar responses with both routes. These findings indicated that mixtures of the angiotensins could probably be analysed by comparing the pressor responses and the JV/LV ratio, obtained with an unknown mixture, with those observed with the hormones used singly and in various proportions. Studies in a sheep showed that the total dose and relative proportions of a mixture of 5 µg of angiotensin I and 4 µg of angiotensin II could be determined with considerable accuracy.

Equipressor doses gave an appreciable prolongation of the average blood-pressure response, especially with injections into the left ventricle, of angiotensin I compared with angiotensin II. The ability of angiotensin I to produce a more prolonged response, especially with the left ventricular route, may result from several factors. These could include; (1) an inherent ability of its own to increase vascular resistance for longer periods than angiotensin II; (2) a steady release of angiotensin II from angiotensin I after its removal from the blood (this is probably unimportant because slowing the rate of infusion of angiotensin II also appreciably slows the development of the response; however, left-ventricular injections of the angiotensins give similar rates of development); and (3) a boost to the pressor response starting about one circulation time after its onset, resulting from the conversion of the angiotensin I, which escapes immediate removal by the organs in the systemic circulation, into angiotensin II in the lungs.

Studies in the rat, cat, dog and rabbit by Biron & Huggins (1968) have indicated that the rises in blood pressure after intravenous injection of angiotensin I are greater than those after injection of the same doses into the ascending aorta. Their findings are similar to our own in different animals. Stanley & Biron (1968) concluded from experiments in the dog on cardio-pulmonary bypass that the lungs are probably the major site of conversion. Oparil et al. (1970) studied the in vivo and in vitro activity of radioactive angiotensin I in the dog and reached a similar conclusion. The relative importance of the systemic and pulmonary circulations in the activation of angiotensin I is clearly related to the way in which renin liberates angiotensin I from renin substrate. Thus the situation would be markedly different if angiotensin I were liberated only on, or near, the wall of arterioles in the systemic circulation as Page (1960) has suggested, or in venous blood alone, or generally throughout the circulation as Ng & Vane (1968) have suggested.

The relatively slow rate of activation and destruction by arterial and venous blood demonstrated in our in vitro studies make it unlikely that either conversion or destruction of the angiotensins in the circulating blood are important in determining the observed pressor effects. Previous studies have shown that the in vitro conversion by blood or plasma occurs at a similar rate to that we have observed in sheep blood (Andersen, 1967; Ng & Vane, 1967; Oparil et al., 1970; Osborn et al., 1970).

Arakawa, Smeby & Bumpus (1962) have shown that it is not necessary for angiotensin II to penetrate the cell for activity. It is possible that angiotensin I is positioned on the cell surface
where it may exert an action of its own or be rapidly converted by circulating converting enzyme into an active form, possibly angiotensin II, after becoming more susceptible to conversion following removal from the blood stream.

Investigations reported by Carlini, Picarelli & Prado (1958), Halvorsen, Fasciolo & Calvo (1959), Gross & Turrian (1960), Barac (1962a, b), Osborn, Tildesley, Leach & Mahler (1971), Tildesley, Osborn, Willicombe & Mahler (1971) and E. C. Osborn & G. Tildesley (unpublished work) have shown that angiotensin I immediately reduced blood flow when injected into the kidney, hind-quarters and hind-limb of the dog and other species. These studies are therefore at variance with those of Ng & Vane (1968) who concluded that, while angiotensin I is substantially removed during a single passage through the kidney and hind-limb in the dog, it has no immediate vasoconstrictor effect.

ACKNOWLEDGMENTS

The financial support of the joint Clinical Research Sub-Committee of the Welsh Hospital Board and the University Hospital of Wales (Cardiff) Hospital Management is gratefully acknowledged. We are also greatly indebted to Professor F. Gross for the Ciba angiotensin I preparation and to Dr K. G. Leach, Mrs A. T. Parkes and Mr P. R. Willicombe for occasional technical help. The late Dr L. R. West gave useful advice and Professor R. F. Mahler and Dr J. Williams assisted with the manuscript. The studies were carried out in the Dr Leonard West Research Laboratory, Sully Hospital, and were made possible by the unfailing services of Mr J. Wilson, Mr O. F. Mason and Mr P. Stock.

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Studies on angiotensin I conversion


