The effect of captopril on blood pressure and angiotensins I, II and III in sodium-depleted dogs: problems associated with the measurement of angiotensin II after inhibition of converting enzyme

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Summary

1. Changes in arterial blood pressure, blood angiotensin I, plasma angiotensin II and plasma angiotensin III were measured in conscious sodium-depleted dogs after infusion of captopril, an orally active inhibitor of converting enzyme.

2. Angiotensins II and III were measured after chromatography to remove angiotensin I, which increased in concentration after inhibition of converting enzyme and which interfered in the direct assay for angiotensin II.

3. Infusion of captopril at 20, 200, 2000 and 6000 μg h⁻¹ kg⁻¹, each for 3 h, produced a rapid fall in blood pressure and in concentration of angiotensin II. Angiotensin II was undetectable at 6000 μg h⁻¹ kg⁻¹ (mean pre-infusion value for all samples was 39 ± sd 15 pmol/l, n = 14).

4. The percentage fall in blood pressure correlated with the percentage fall in plasma angiotensin II (r = 0.65, P < 0.001).

5. These results suggest that the initial fall in blood pressure may be mediated in part by the suppression of angiotensin II.

6. Blood angiotensin I concentration rose with each rate of infusion of drug to a maximum 16-fold increase at 6000 μg h⁻¹ kg⁻¹ (26–416 pmol/l). The rise in angiotensin I was inversely related to the fall in angiotensin II (r = −0.68, P < 0.001).

Key words: angiotensin, captopril, converting-enzyme inhibitor, paper chromatography.

Abbreviation: ANG, angiotensin.

Introduction

Captopril (SQ 14225) (Cushman, Cheung, Sabo & Ondetti, 1977) has been shown to be an effective inhibitor of the angiotensin-converting enzyme (peptidyldepeptide carboxyhydrolase) both in animals (Ondetti, Rubin & Cushman, 1977) and man (Fergusson, Turini, Brunner & Gavras, 1977). Its potential as an antihypertensive agent is currently being investigated and from initial studies it appears to be capable of lowering blood pressure (Bengis, Coleman, Young & McCaa, 1978; Brunner, Waeber, Wauters, Turini, McKinstry & Gavras, 1978; Muirhead, Prewitt, Brooks & Brosius, 1978). However, its mechanism of action and also that of the nonapeptide inhibitor, teprotide (SQ 20881), is unclear. Hypotension may be a consequence of suppression of angiotensin II (ANG II), potentiation of bradykinin or a combination of both (Ferreira, 1965; Engel, Schaeffer, Gold & Rubin, 1972), or it may also result from some other as yet undefined mechanism. Most investigators have used indirect methods, including renin activity or aldosterone concentration (Atlas, Case, Sealey, Laragh & McKinstry, 1979), to investigate the role of ANG II in the hypotensive response to captopril, and in the few studies which
claim to measure ANG I and/or ANG II (Williams & Hollenberg, 1977; Johnston, Millar, McGrath & Matthews, 1979; Swartz, Williams, Hollenberg, Moore & Dluhy, 1979) a number of anomalies exist, which suggest that there may still be unsolved technical problems in the measurement of these peptides after inhibition of conversion.

This report highlights one of the problems: the interference of ANG I in the immunoassay of ANG II. Elimination of this interference allowed an accurate investigation to be made into the fall in blood pressure and the fall in ANG II concentration after the infusion of captopril at different rates. A preliminary account of part of this work has been given (Morton, Brown, Casals-Stenzel, Lever, Robertson & Tree, 1979a).

Methods

Five male conscious beagle dogs, weighing between 12 and 17 kg, were infused with captopril (SQ 14225, Squibb). The right carotid artery had previously been exteriorized within a narrow pedicle of skin to facilitate arterial cannulation. Four days before each infusion the dogs were given an intravenous injection of frusemide (5 mg/kg) and were then maintained on a diet low in sodium (mean 0.7 mmol/day) but with a normal potassium content (mean 27.7 mmol/day). On the morning of day 5, the dogs were placed on the experimental table and allowed to stand or sit only. The exteriorized carotid artery was cannulated (19 gauge butterfly needle), under local anaesthesia for blood sampling and blood pressure recording. A fine polyethylene catheter was inserted into a forelimb vein for the infusion.

The dogs were first infused for 1 h with 5% glucose solution. This was followed by an infusion of the inhibitor in glucose solution for 3 h. Four dogs were each infused at three different rates (20, 200 and 2000 µg h⁻¹ kg⁻¹). Each rate was used on a different day and a rest period of at least 2 weeks was allowed for each dog between successive infusion rates. One of the four dogs was also infused at 6000 µg h⁻¹ kg⁻¹ and a fifth dog was infused at 6000 µg h⁻¹ kg⁻¹ only. During each experiment blood was removed for hormone and electrolyte determination, at the end of the control infusion and at 30 min, 2 and 3 h after starting the captopril infusion. Blood pressure was measured and recorded continuously via the carotid arterial loop, a mercury manometer and kymograph drum being used.

ANG I was extracted from blood after ethanol precipitation and measured by a radioimmunoassay method (Waite, 1973) as applied to dog blood (Morton, Semple, Ledingham, Stuart, Tehrani, Garcia & McGarrity, 1977). Blood ANG I concentrations have been corrected for a recovery of 67% (Morton et al., 1977) and are quoted as pmol/l of blood.

Total plasma ANG II immunoactive material was measured by radioimmunoassay (Düsterdieck & McElwee, 1971) as applied to dog blood (Morton et al., 1977). These have been corrected for a recovery of 85% (Morton et al., 1977) and are quoted as pmol/l of plasma.

The individual quantities of ANG II and ANG III ([des-Asp¹]ANG II) in the ANG II immunoactive extracts and also the extent to which ANG I interfered in the ANG II immunoassay were determined after chromatographic separation of the ANG II extracts as previously described (Morton, Casals-Stenzel, Lever, Millar, Riegger & Tree, 1979b). The recovery of a mixture of 2 ng of [Ile⁵]ANG II and 2 ng of [Ile⁴]ANG III, added to dog blood (10 ml) after extraction and chromatography, was determined on six separate occasions. The recovery of ANG II was 47 ± 6 SD% and of ANG III was 44 ± 5%. ANG II and ANG III concentrations have both been corrected for recovery losses.

Plasma electrolytes were measured by flame photometry.

Results

Cumulative electrolyte balance, plasma electrolytes and packed cell volume

All dogs were in negative sodium balance before the study (mean −83 ± 23 mmol). Regardless of the infusion rate, there were no consistent changes in packed cell volume, plasma sodium or plasma potassium after captopril infusion.

ANG I interference in the ANG II assay

The effect of captopril infused at four different rates on the total ANG II immunoactive material and on circulating ANG I concentration is shown in Fig. 1. When the unchromatographed extracts were assayed it was not possible to show the complete removal of ANG II immunoactive material from blood. Interference by ANG I in the assay was shown after chromatography of the ANG II extracts (Fig. 2a). After captopril infusion at 6000 µg h⁻¹ kg⁻¹ there was a large peak of ANG
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**Captopril**

**FIG. 1.** Effect of captopril infused at different doses on total plasma ANG I1 immunoactivity (●) and blood ANG I concentration (△). Samples were taken 3 h after starting the infusion. Bars are ± SEM. Each value for the 6000 pg h⁻¹ kg⁻¹ infusion is given as the mean of two results.

II immunoactivity running in the ANG I position. In contrast, there was no detectable ANG I cross-reaction either before or after captopril infused at the lowest rate (20 μg h⁻¹ kg⁻¹) (Fig. 2b).

Apart from data in Fig. 1, all plasma ANG II values quoted in this study have been determined by the second method, that is, after chromatography and removal of any ANG I interference. ANG III concentrations were similarly determined.

**Blood pressure and plasma ANG II and III concentrations after captopril**

These results are shown in Fig. 3. Blood pressure fell with all four captopril infusions, the maximum fall being reached within the first 30 min of starting each infusion rate. Pressure remained suppressed (falling further slightly at the two lower doses) for the remaining period of the infusion. The maximum fall in pressure was 32 mmHg (30% of the basal value) at the end of the 6000 μg h⁻¹ kg⁻¹ infusion; this was similar to that achieved at the end of the 2000 μg h⁻¹ kg⁻¹ infusion (31 mmHg, 32% of the basal value), indicating that maximal suppression had been achieved at a dose of 2000 μg h⁻¹ kg⁻¹ or less.

Plasma ANG II concentration showed a somewhat similar pattern of change to that for blood pressure in that it also fell in a dose-related manner. The maximum fall was reached within the first 30 min and thereafter it remained suppressed. ANG II was suppressed by more than 92% at 2000 μg h⁻¹ kg⁻¹ and was undetectable at 6000 μg h⁻¹ kg⁻¹.

The percentage fall in plasma ANG II correlated with the percentage fall in blood pressure for values at 30 min, 2 h and 3 h after starting the captopril infusion (r = 0.61, P < 0.05; r = 0.85, P < 0.001; r = 0.64, P < 0.02 respectively). There was a highly significant correlation between the percentage fall in ANG II and the percentage fall in blood pressure for all samples taken (r = 0.65, P < 0.001) (Fig. 4).

It is noteworthy that for the basal samples, where there was no ANG I cross-reaction, the combined mean plasma ANG II and III concentration obtained after chromatography was similar to the mean plasma ANG II immunoactive concentration obtained without chromatography.
FIG. 3. Effect, with time, of captopril infused at different doses (●, 20; △, 200; ○, 2000; ×, 6000 μg h⁻¹ kg⁻¹) on mean blood pressure and plasma ANG II and III concentrations (after chromatography). Bars are ± SEM. At 6000 μg h⁻¹ kg⁻¹ samples for plasma ANG II and III estimation were taken at the end of the pre-infusion and at 3 h after captopril only. Each value for the 6000 μg h⁻¹ kg⁻¹ infusion is given as the mean of two results.

FIG. 4. Relationship between the percentage fall in blood pressure and the percentage fall in plasma ANG II concentration for all samples taken after captopril infusion. $r = 0.65, P < 0.001$.

FIG. 5. Effect with time of captopril infused at different doses (●, 20; △, 200; ○, 2000; ×, 6000 μg h⁻¹ kg⁻¹) on circulating ANG I concentration. Each value is given as the mean ± SEM except for the 6000 μg h⁻¹ kg⁻¹ infusion, where each value is given as the mean of two results.

Circulating ANG I

Circulating ANG I increased with each infusion rate, the extent of the rise being dose-related (Fig. 5). The rise in ANG I reached a maximum 30 min after starting the infusion. Thereafter the response flattened, remaining elevated until the end of the infusion. There was a highly significant inverse relationship between the fall in ANG II and the rise (47 ± 19 and 56 ± 25 pmol/l respectively). Smaller ANG II immunoactive fragments such as the N-terminal hexapeptide probably account for some of the difference. There was also a significant correlation for individual basal samples between the combined chromatographed ANG II and III concentrations and the unchromatographed ANG II immunoactive concentrations ($r = 0.77, P < 0.01, n = 12$). These findings confirm the reproducibility of the chromatographic procedure, as was also shown by the recovery of exogenous ANG II and III.
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in ANG I ($r = -0.68$, $P < 0.001$). Also there was a significant, though weaker, inverse relationship between the fall in blood pressure and the rise in ANG I ($r = -0.43$, $P < 0.01$).

Discussion

Atlas et al. (1979) conclude that the inability to measure low concentrations of ANG II after inhibition of conversion excludes its measurement as a means of studying the involvement of ANG II in the hypotensive response after captopril treatment. As an alternative, they suggest that changes in plasma aldosterone should be used as an index of ANG II suppression. One of the problems in the measurement of ANG II after inhibition of converting enzyme is increased concentrations of ANG I, which cross-react in the ANG II immunoadsay and lead to falsely high values for ANG II. This has previously been highlighted (Morton et al., 1977, 1979a) and is confirmed in the present study. However, rather than use some secondary factor as an index of ANG suppression, we have attempted to overcome this problem by removing the ANG I interference before immunoassay for ANG II. Although there are other reports claiming to measure plasma ANG II after treatment with captopril or teprotide, none of these mentions or takes into account this problem (Williams & Hollenberg, 1977; Johnston et al., 1979; Swartz et al., 1979). Although these studies have generally shown a partial fall in plasma ANG II concentration, none has demonstrated complete suppression. In particular, not only were Swartz et al. (1979) unable to suppress ANG II by more than approximately 30% with an initial dose of teprotide, but further administration of increasing doses caused an apparent return of ANG II concentration to a value similar to that for the pre-teprotide concentration. Both this anomaly and the inability to show complete suppression of ANG II could be explained if ANG I was interfering in the assays used.

The rise in ANG I at all four infusion rates is also of interest in that it does not accord with the result of the only other group claiming to measure circulating ANG I concentration after captopril treatment (Johnston et al., 1979). These workers were unable to demonstrate in man a rise in circulating ANG I after captopril; this result was at variance with their own renin activity measurements, which did show an increase. The explanation for this lack of agreement is uncertain, but technical differences cannot be excluded.

The inverse relationship between the fall in ANG II and the rise in ANG I is indirect evidence in favour of the rise of ANG I being, in part, a consequence of increased renin secretion after removal of the negative feedback effect of ANG II (Vander & Geelhood, 1965). The fall in blood pressure also probably has a part to play in the increase in renin secretion leading to increased ANG I concentration. That the rise in ANG I is not due to the blockage of its breakdown to ANG II is suggested by the observation that there was no accumulation of ANG I with time. ANG I must therefore be rapidly metabolized by other hydrolases to peptides other than ANG II or ANG III.

The correlation between the fall in blood pressure and the fall in ANG II suggests that one of the factors which may be involved in the acute hypotensive response in these dogs after captopril treatment is suppression of ANG II. This would agree with the conclusions of a somewhat similar study by McCaa, Hall & McCaa (1978), but in which ANG II was not measured. However, our results do not suggest that suppression of ANG II is the sole cause of the fall in blood pressure, nor do they allow us to quantify the magnitude of its involvement, and it is certainly possible that mechanisms other than the suppression of ANG II may also be important. This is supported from the results of a study in which infused ANG II failed to restore blood pressure completely to normal in sodium-depleted dogs given captopril (Tree & Morton, 1979).

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References


