Angiotensin II and Its Heptapeptide (2-8), Hexapeptide (3-8), and Pentapeptide (4-8) Metabolites in Arterial and Venous Blood of Man

PETER F. SEMPLE, M.B., Alistair S. Boyd, B.Sc., Paul M. Dawes, Ph.D., and James J. Morton, Ph.D.

SUMMARY We made separate measurements of angiotensin II (A II) and of its immunoreactive metabolites (2-8 heptapeptide, 3-8 hexapeptide, and 4-8 pentapeptide) in arterial and venous plasma of subjects with widely different plasma levels of the peptides. A II and its three metabolites were extracted from blood, separated by paper chromatography, and measured by radioimmunoassay using an A II antiserum which had a 100% cross-reaction with each metabolite. In contrast to results of previous studies, A II was found to predominate in both arterial (60-100%) and venous (55-100%) blood. The biologically active 2-8 heptapeptide metabolite accounted for only 10% of the activity in arterial plasma. Radioimmunoassay of venous plasma extracts using an A II antiserum which had a low cross-reaction with the 3-8 hexapeptide and the 4-8 pentapeptide confirmed the results obtained using the antiserum which had a 100% cross-reaction with the metabolites. We conclude that radioimmunoassay methods for measuring A II in venous blood may be more accurate and relevant than has previously been recognized. The small difference between A II concentrations in arterial and venous plasma suggests further that there may be significant conversion of angiotensin I (A I) to A II in the limb vasculature.

Methods

SYNTHETIC PEPTIDES

The following peptides were used: 1-Asp-5-Ile-angiotensin I (A I), (Schwarz/Mann); 1-Asp-5-Ile-angiotensin II (A II) (National Institute for Biological Standards and Control, London); Arg-Val-Tyr-Ile-His-Pro-Phe, Val-Tyr-Ile-His-Pro-Phe, and Tyr-Ile-His-Pro-Phe (Schwarz/Mann); and 1-Asp(NH₂)-5-Val-angiotensin II (CIBA).

EXTRACTION OF PEPTIDES FROM BLOOD

Method of Düsseldiek and McElwee

The method of extracting A II and its immunoreactive metabolites has been described in detail by Düsseldiek and McElwee. Blood was drawn in samples of 20 ml from the arm and, within 30 seconds after its first appearance, was added in a plastic container to 1 ml of an aqueous solution of 0.1 M ethylenediaminetetraacetic acid (EDTA) (disodium salt) with 0.05 M o-phenanthroline, a mixture that effectively inhibits blood converting enzyme and angiotensinas. After chilling in ice, the mixture was extracted at +5°C and 2,000 g for 20 minutes. The peptides were extracted from a measured volume of plasma by mixing with Dowex AG 50W-X2, 50-100 mesh (H⁺ form) and twice eluted from the resin with ammonia-methanol (9:1, vol/vol). In recovery experiments, venous or arterial blood was collected into syringes containing known quantities of peptides.

Method of Cain et al.

Comparison was made with the extraction method of Cain et al., in which converting enzyme and angiotensin-
nases were destroyed by adding whole blood directly to ethanol, the peptides being extracted by Sephadex column chromatography.

**PAPER CHROMATOGRAPHY**

Residues from Dowex eluates were redissolved in 100 µl of a mixture of ammonia and methanol (9:1, vol/vol), and 60 µl of this were applied as a 1-cm line to the origin of 2.5-cm-wide lanes of a paper chromatogram (Whatman No. 3MM). Marker standards (20 µg) of 1-Asp-5-Ile-angiotensin II or 1-Asp(NH₂)-5-Val-angiotensin II and the immunoreactive metabolites were applied to adjacent lanes. Residues from eluates of Sephadex were treated similarly but dissolved in a mixture of ethanol and 0.1 m acetic acid (7:3, vol/vol). After equilibration in the solvent system for 4 hours, the chromatograms were developed in either solvent A (n-butanol-acetic acid-water-pyridine, 15:3:12:10, vol/vol, pH 5.2) or solvent B (upper phase, n-butanol-acetic acid-water-ethyl acetate, 10:15:10, vol/vol, pH 3.1) for 15-16 hours. After the chromatograms were dried in air, the marker lanes were stained by heating at 60°C for 20 minutes after saturation with a mixture of 1% ninhydrin in acetone and 1% cadmium acetate in 50% aqueous acetic acid (85:15, vol/vol).

Chromatogram lanes bearing the samples were transversely cut at 1-cm intervals and each segment was eluted with 2 ml of ammonia-methanol mixture (see above) in 2-ml syringes for 4 hours. The eluates were then ejected from the syringe into tubes containing 50 µl of aqueous human serum albumin solution (1 g/100 ml), the remaining drops in the syringe being recovered by centrifugation (2,000 × g, 5 minutes). The solution was evaporated to dryness at 37°C under a stream of cold air.

**RADIOIMMUNOASSAY**

Radioimmunoassay of A II was performed by the method of Düsterdieck and McElwee. To corroborate the chromatographic findings, A II immunoreactive material (A II IM) in Dowex extracts of normal venous plasma (80 ml) was measured with two antisera that had different affinities for A II metabolites (see Results).

A I was measured by the radioimmunoassay method of Waite.

**BIOASSAY**

Bioassay of A II in the pentolinium-treated rat was performed by the method of Peart.

**CONTRIBUTION OF A I TO IMMUNOREACTIVE A II CONCENTRATIONS**

A I was not separated from A II by paper chromatography, but the A II antiserum used in the radioimmunoassay had a small (0.6%) cross-reaction with A I (see Results). Because chemical inhibitors do not inhibit the renin-renin substrate reaction, A I may be generated in plasma during Dowex extraction. To measure the contribution to apparent A II concentrations made by A I, the following experiments were conducted: (1) Samples (20 ml) of normal venous blood drawn into inhibitor solution were kept at room temperature (20°C) for times varying from 0 to 300 minutes before cooling in an ice bath. Residues from Dowex extraction were divided into two equal portions, one of which was assayed for A I and the other for A II. (2) A I and A II were measured in four extracts of normal venous plasma (40 ml) developed in system A.

**CATABOLISM OF A II AND ITS METABOLITES IN BLOOD**

Eight picomoles of A II of the hepta-, hexa-, or pentapeptide in 100 µl of tromethamine (Tris) buffer, pH 7.5, were added to samples (4 × 50 ml) of venous blood containing heparin (10 U/ml) but not inhibitor, and freshly drawn from a normal subject. A further sample (control) received only Tris buffer. The samples were incubated at 37°C and 10-ml portions, removed at 0, 2, 5, 15, and 30 minutes, were mixed immediately with 0.5 ml of inhibitor solution and cooled in ice. After Dowex extraction and chromatography in system A, the sample values were corrected for the control values.

**EFFECT OF DELAY IN ADDING INHIBITOR SOLUTION TO BLOOD**

Samples of venous blood (20 ml) were incubated at 20°C for up to 10 minutes without anticoagulant and for up to 60 minutes with heparin (10 U/ml) before adding inhibitor and chilling in ice. One sample was totally hemolyzed with saponin (Zaponin, Coulter Electronics) before incubation for 60 minutes. Samples containing inhibitor from the same subject were incubated as controls. The Dowex extracts were developed in system A.

**SUBJECTS**

Informed consent was obtained from all subjects, and the protocol of the study was agreed to by the Ethical Supervisory Committee of the Western Infirmary, Glasgow. To obtain information on the distribution of A II and its metabolites over a wide range of circulating renin levels, the following groups were studied. Sample volumes of blood varied from 20 to 40 ml.

**Normal Subjects**

Forearm venous blood was taken from 22 normal men and women (25-59 years old) who had been recumbent for 5 minutes. The range of plasma concentrations of A II IM measured without paper chromatography by Düsterdieck and McElwee in normal subjects studied in the same way was 5-35 pmol/liter. Comparison was also made of simultaneous forearm venous and arterial blood samples taken after a 1-hour period of recumbency from each of five normal subjects between 20 and 73 years in age on an unrestricted diet. In a previous study of eight normal subjects who had been on a fixed normal daily intake of sodium and potassium for 3 days and recumbent overnight, we found a mean concentration of A II IM in brachial arterial plasma of 10.0 pmol/liter (range, 6-17) compared to a mean of 10.4 pmol/liter (range, 6-19) in brachial venous plasma drawn concurrently.

**Subjects with Raised Plasma Renin Concentration (PRC)**

Forearm venous blood was drawn from: (1) five women between the 30th and 40th weeks of normal pregnancy who...
had been supine for 1 hour; plasma concentrations of renin and A II 1M are generally high at this stage of pregnancy; a 20-year-old man with untreated malignant phase hypertension and a peripheral venous PRC of 179 µU/ml (normal range, 20–100 µU/ml, international standard renin\textsuperscript{15, 16}); (3) a 25-year-old man with Bartter's syndrome and a PRC of 1,141 µU/ml; and (4) a 54-year-old woman with rheumatic heart disease, being treated with 160 mg of furosemide daily, whose PRC was 110 µU/ml.

\textbf{Subjects with Low Plasma Renin Concentration}

Arterial blood was drawn from one male (aged 31) and one female (aged 27), from whom both kidneys had been removed 5 weeks and 9 months previously. PRC was 21 and 32 µU/ml, respectively. Forearm arterial and venous blood was also obtained from an asthmatic patient, on 80 mg of prednisolone a day, whose PRC was 21 µU/ml.

\textbf{INFUSION OF 1-Asp(NH$_2$)-5-Val-ANGIOTENSIN II}

The pattern of metabolites was examined in venous blood from three normal subjects (ages, 20–30 years) and the patient with Bartter's syndrome during intravenous infusion of 1-Asp(NH$_2$)-5-Val-angiotensin II. Samples were taken after 1 hour of an infusion of 5% glucose and after 1 hour of an infusion of 1-Asp(NH$_2$)-5-Val-angiotensin II in 5% glucose at a rate of 8 pmol/kg per min, the subjects being recumbent throughout. In the patient with Bartter's syndrome, simultaneous venous and arterial samples were obtained.

\textbf{Results}

\textbf{SEPARATION OF A II FROM IMMUNOREACTIVE METABOLITES BY PAPER CHROMATOGRAPHY}

Solvent A separated A II, the 2-8 heptapeptide, and 3-8 hexapeptide (R$_v$ values = 0.50, 0.67, and 0.77) but did not separate the 4-8 pentapeptide (R$_v$=0.73) from the 3-8 hexapeptide. Solvent B separated A II/2-8 heptapeptide (R methyl orange 0.1) from 3-8 hexapeptide/4-8 pentapeptide (R methyl orange 0.6). Neither solvent separated A I from A II.

\textbf{CROSS-REACTING PROPERTIES OF THE PEPTIDE ANTISERA}

The A II antiserum (final dilution, 1:20,000) used for the paper chromatographic studies (antisera 1) had an equal affinity for A II and 1-Asp(NH$_2$)-5-Val-angiotensin II and, in molar terms, a cross-reaction of 0.6% with A I\textsuperscript{19} and 100% with the three A II metabolites (Fig. 1). The other A II antiserum (antisera 2), used at a final dilution of 1:20,000, had a slightly higher (2.3%) cross-reaction with A I, but a much lower cross-reaction with both the hexapeptide (10%) and pentapeptide (0.6%) (Fig. 2). The A I antiserum had a cross-reaction with A II of less than 1 x 10\textsuperscript{-4}\%.

\textbf{ACCURACY: RECOVERY OF PEPTIDES FROM BLOOD}

A II recoveries were corrected for simultaneously estimated endogenous levels of peptide.

Through the Dowex extraction procedure\textsuperscript{12}, recoveries of 2 pmol of the heptapeptide, hexapeptide, and pentapeptide added to venous blood were 86 ± 3% (SD) (n = 4), 80 ± 5% (n = 4), and 82 ± 4% (n = 4), respectively. The recovery of 1-Asp-5-Ile-angiotensin II was 83 ± 11% (n = 44), measured by immunoassay, with similar results on bioassay.\textsuperscript{17} Through the alcoholic precipitation and Sephadex extraction method,\textsuperscript{14} the recovery of 2 pmol of 1-Asp-5-Ile-angiotensin II was 72 ± 12% (n = 6).

Through the entire extraction and chromatographic procedures, there were no differences in recovery of the individual peptides or between the different methods (Table 1), although recoveries were generally higher when large amounts of peptide were used. The results of blood analysis have therefore not been corrected for recovery. Figure 3 compares the recoveries in two single experiments in which 2 ng\textsuperscript{12} each of A II, heptapeptide, and hexapeptide were added to blood and 50 µl of the redissolved Dowex extracts developed in system A or system B.

When an experiment was performed in which 200 ng of each peptide were added to blood and the Dowex extract developed in system A the major peak coincided with the A II region. A minor peak coincided with the heptapeptide region and no pressor activity was found running with the hexa- or pentapeptides.

\textbf{COMPARISON OF A II AND IMMUNOREACTIVE FRAGMENTS IN ARTERIAL AND VENOUS BLOOD (TABLE 2)}

A II was the predominant peptide in Dowex extracts of normal venous plasma (78%) and Sephadex extracts of venous blood (96%) developed in system A. Examples are shown in Figure 4. The concentration of A II was slightly but significantly higher in arterial plasma than in simultaneously drawn venous plasma (paired t-test, P < 0.02). A II was also the predominant peptide in venous samples from pregnant subjects, in subjects infused with 1-Asp(NH$_2$)-5-Val-angiotensin II, and in subjects with abnormally high or low PRC. Plasma from both the anephric subjects studied contained immunoreactive material which chromat-
graphed as authentic A II. It was possible to test the pressor activity of immunoreactive A II recovered from a chromatograph developed in solvent system A by analyzing a Dowex extract of a 40-ml sample of venous plasma from a subject with a high PRC. Bioactive angiotensin (10-21 pmol) and 15 pmol of immunoreactive A II (15 pmol) were recovered from the A II position. No other pressor peaks were seen but vasodepressor material chromatographed with an $R_f$ value of 0.78.

Results obtained with system B were similar to those as with system A, the majority of the A II IM being identified in the A II/heptapeptide position (Table 3, Fig. 5).

**COMPARISON OF IMMUNOREACTIVE A II CONCENTRATIONS OBTAINED USING ANTIKERUM 1 AND ANTISERUM 2**

Dowex extracts of normal venous blood were assayed for A II concentration using both antisera 1 and 2. The mean concentration of A II IM using antiserum 2 was $18.4 \pm 4.4$ (SD) pmol/liter ($n = 8$) and using antiserum 1 it was $18.9 \pm 4.8$, ($n = 8$). The mean percentage difference between individual extracts was 0.8%. Since antiserum 1 cross-reacted 100% with the metabolites these data suggest that the concentrations of the hexa- and pentapeptide in normal venous blood were small.

**CONTRIBUTION OF A I TO APPARENT A II**

The plasma concentrations of A I and A II IM in samples of normal venous blood containing inhibitor solution incu-

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**Table 1 Recovery of Angiotensin II (A II) and Metabolites from Blood after Paper Chromatography**

<table>
<thead>
<tr>
<th>Extraction method</th>
<th>Solvent</th>
<th>Arterial (A) or venous (V)</th>
<th>Amount of peptide added (ng)</th>
<th>Percentage recoveries</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A II</td>
</tr>
<tr>
<td><strong>Plasma</strong></td>
<td>A</td>
<td>V</td>
<td>0.2</td>
<td>39-46 (3)</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>V</td>
<td>2</td>
<td>53 ± 4 (8)</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>A</td>
<td>2</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>V</td>
<td>5</td>
<td>49-58 (3)</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>V</td>
<td>100</td>
<td>61 ± 3 (4)</td>
</tr>
<tr>
<td><strong>Blood</strong></td>
<td>A</td>
<td>V</td>
<td>0.2</td>
<td>44-50 (3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th>A II/heptapeptide</th>
<th>Hexapeptide/pentapeptide</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma</strong></td>
<td>A</td>
<td>V</td>
<td>0.2</td>
<td>40-47 (2)</td>
<td>42-45 (2)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>V</td>
<td>2</td>
<td>51-56 (2)</td>
<td>48-52 (2)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>V</td>
<td>100</td>
<td>71</td>
<td>66</td>
</tr>
<tr>
<td><strong>Blood</strong></td>
<td>B</td>
<td>V</td>
<td>0.2</td>
<td>45-50 (2)</td>
<td>46-53 (2)</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD where four or more experiments (numbers in parentheses) were performed. One peptide was added to each 20-ml blood sample.
IMMUNOREACTIVE METABOLITES OF ANGIOTENSIN II/Semple et al.

Table 2  Angiotensin II (A II) and Metabolites in Plasma and Blood (Solvent A)

<table>
<thead>
<tr>
<th>Extraction method</th>
<th>Clinical problem</th>
<th>Arterial (A) or venous (V)</th>
<th>No. of samples</th>
<th>A II</th>
<th>Heptapeptide</th>
<th>Hexapeptide/ pentapeptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma*</td>
<td>Normal</td>
<td>V</td>
<td>12</td>
<td>12.6 ± 2.5 (78%)</td>
<td>1.6 ± 0.4 (10%)</td>
<td>2.0 ± 0.6 (12%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>6</td>
<td>9.0 ± 2.2 (72%)</td>
<td>1.3 ± 0.3 (10%)</td>
<td>2.2 ± 0.8 (18%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>V*</td>
<td>6</td>
<td>8.7 ± 2.0 (65%)</td>
<td>1.7 ± 0.6 (14%)</td>
<td>2.5 ± 1.0 (21%)</td>
</tr>
<tr>
<td>Pregnancy</td>
<td></td>
<td>V</td>
<td>5</td>
<td>30.0 ± 10.1 (70%)</td>
<td>4.8 ± 1.3 (9%)</td>
<td>8.0 ± 1.1 (15%)</td>
</tr>
<tr>
<td>Malignant hypertension</td>
<td></td>
<td>V</td>
<td>1</td>
<td>40 (69%)</td>
<td>12 (21%)</td>
<td>6 (16%)</td>
</tr>
<tr>
<td>Barter's syndrome</td>
<td></td>
<td>V</td>
<td>1</td>
<td>118 (98%)</td>
<td>1 (1%)</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Status asthmaticus on high dose steroids</td>
<td></td>
<td>V*</td>
<td>1</td>
<td>3 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Anephric</td>
<td></td>
<td>A</td>
<td>2</td>
<td>3 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td>Basal</td>
<td>V</td>
<td>118 (72%)</td>
<td>39 (24%)</td>
<td>9 (43%)</td>
</tr>
<tr>
<td>Blood*</td>
<td></td>
<td>A II amide infusion</td>
<td>V</td>
<td>239 (81%)</td>
<td>47 (16%)</td>
<td>9 (3%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Barter's syndrome</td>
<td>Basal</td>
<td>118 (98%)</td>
<td>1 (1%)</td>
<td>1 (1%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A II amide infusion</td>
<td>V</td>
<td>239 (81%)</td>
<td>47 (16%)</td>
<td>9 (3%)</td>
</tr>
</tbody>
</table>

Results are expressed as pmol/liter of plasma or pmol/liter of blood; ± represents 1 standard error.

* Samples drawn simultaneously with arterial blood.

bated at room temperature for up to 5 hours are shown in Table 4. Plasma levels of A II IM remained constant despite a progressive increase in the concentration of A I. Interference from A I would therefore appear to be negligible. In the remaining experiments, A I and A II were assayed in four normal venous plasma extracts after chromatography in solvent system A. The mean ratio of A I to A II was 13:1 (range, 9:1–17:1), indicating that only about 8% of the A II could be accounted for by A I, taking into account the cross-reaction of the A II antiserum with A I.

CATABOLISM OF A II AND ITS METABOLITES IN BLOOD

Half-lives for A II, the heptapeptide, hexapeptide, and pentapeptide were 4.4, 2.0, 1.9, and 2.4 minutes, respectively (Fig. 6). The value for the hexapeptide may be an underestimate because conversion of hexa- to pentapeptide will not be accounted for. The delay between withdrawing blood from a subject and its mixing with inhibitor was never more than 30 seconds, and thus the peptide pattern was not likely to alter significantly during this period.

This was corroborated by comparing the pattern of A II and its metabolites in three samples of normal venous blood drawn into syringes containing inhibitor solution with the pattern obtained when blood was mixed with inhibitor in a container after sampling. No differences could be determined. A mean of 76% of the A II IM chromatographed with the mobility of A II in both groups.

EFFECT OF DELAY IN ADDING INHIBITOR SOLUTION

These results are summarized in Table 5. Incubation of blood without anticoagulant and without inhibitor solution for 10 minutes resulted in an 80% increase in the concentration of A II but no significant change in the proportions of the various peptide metabolites. In the heparinized samples a progressive rise in A II concentrations was also apparent but again there was no change in the proportion of metabolites. The rise in A II concentration was smaller in the hemolyzed sample, probably because of liberation of red cell angiotensinases.

Figure 4  Diagrams showing the pattern of immunoreactive peptides recovered from chromatographs of extracts of normal venous blood. Top panel: Dowex plasma extract. Bottom panel: Sephadex blood extract.
TABLE 3  Angiotensin II (A II) and Metabolites in Plasma and Blood (Solvent B)

<table>
<thead>
<tr>
<th>Extraction method</th>
<th>Clinical problem</th>
<th>Arterial (A) or venous (V)</th>
<th>No. of samples</th>
<th>A II/heptapeptide</th>
<th>Hexapeptide/pentapeptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>Normal</td>
<td>V</td>
<td>4</td>
<td>6.5 ± 1.0 (87%)</td>
<td>1.0 ± 0.7 (13%)</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>A</td>
<td>4</td>
<td>1.0 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td>Status asthmaticus on steroids</td>
<td>A*</td>
<td>1</td>
<td>5 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td></td>
<td>1</td>
<td>3 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Blood</td>
<td>Normal</td>
<td>V</td>
<td>3</td>
<td>3.4 ± 0.6 (94%)</td>
<td>0.2 ± 0.03 (6%)</td>
</tr>
</tbody>
</table>

Results are expressed as pmol/liter of plasma or pmol/liter of blood; ± represents 1 se.

* Sample drawn simultaneously with arterial blood.

Discussion

The results of the present study differ substantially from those reported in preliminary forms by Cain et al. 11 These workers found that only some 28% of A II immunoreactive material in venous blood is A II, the bulk of such material being in their view the 3-8 hexapeptide metabolite. We have found, by contrast, that 55-100% of such immunoreactive material is A II in venous, as in arterial, plasma and have confirmed our observations on repeating the extraction and chromatographic procedures used by Cain et al. 11 and Catt et al. 21

Several possible explanations for the discrepancy between our findings and the earlier work have been considered. A contribution of A I to the apparent A II content was excluded by specific measurements of both peptides and the relationship of these to the cross-reactivity of the A II antiserum with A I.

When the effect of deliberate delay in inhibiting blood converting enzyme and angiotensinases was studied, it was found that there was an increase in the concentration of A II but that the relative proportion of metabolites were little changed. Thus it seems unlikely that changes occurring after blood sampling could explain the differences between the studies, particularly when we routinely accomplished angiotensinase inhibition rapidly in relation to the rates of

Results are corrected for recovery.

1M = immunoreactive material.

FIGURE 5  Diagram showing the pattern of immunoreactive peptides recovered from a chromatograph of a Dowex extract of normal venous plasma developed in system B.

FIGURE 6  The rates of breakdown of A II and its three immunoreactive metabolites by normal blood incubated at 37°C for up to 30 minutes. Results are expressed as percentage of 0 time values. O---O = A II; Δ--Δ = 2-8 heptapeptide; ●●●● = 3-8 hexapeptide; ▲▲▲▲ = 4-8 pentapeptide.
breakdown of the peptides studied. The concentrations which we found are probably close to those prevailing in vivo.

It may be that the A II antiserum we used had different affinities for the peptide metabolites from that of the previous workers. Cain et al.6,11 and Catt et al.11 did not provide details of the affinity of their antiserum for the three metabolites. In a separate study the same group referred to an A II antiserum which had a slightly higher affinity for the 3-8 hexapeptide than for A II at low concentrations of peptide, although the cross-reaction of this particular antiserum with the 4-8 pentapeptide was not documented. A particularly high affinity of an A II antiserum for peptide metabolites, such as the 4-8 pentapeptide, would result in a falsely high estimate of its concentration in blood. Emanuel et al.13 have described an A II antiserum with a disproportionately high affinity (15- to 40-fold) for the 3-8 hexapeptide. Using this antiserum, these workers found similar venous and arterial concentrations of A II immunoreactive materials, a finding which led them to query the results of Cain et al.6. Our observations with an antiserum having a low affinity for the hexa- and pentapeptide complement those of Emanuel et al. and corroborate our chromatographic findings.

The arteriovenous difference in endogenous plasma A II concentrations across the limb was small (14%) (Table 2). This contrasts with observations that exogenous A II, in amounts above the physiological range, loses 50-60% of its biological activity in one circulation through the limb vasculature of animals.8,11,17 Studies on the fate of A I in the arterial bypass have appreciable circulating levels of A II.10 During intravenous infusion of A II the arteriovenous gradient in plasma levels of A II IM increases with the infusion rate.14 These observations suggest that A II is both generated and destroyed in the limb vessels and this may explain our finding of a modest difference between arterial and venous plasma levels of the hormone.

Since the bulk of a A II immunoreactive material in arterial and venous blood is authentic A II, it seems unnecessary to include a procedure which separates A II from its immunoreactive metabolites in routine immunoreactive methods for measuring the hormone in human blood.

**References**

Changes in Extracellular Potassium Activity in Response to Decreased pH in Rabbit Atrial Muscle

ROBERT B. SKINNER, JR., AND DIANA L. KUNZE, PH.D.

SUMMARY. The extracellular and intracellular potassium (K⁺) activities of isolated superfused rabbit atrial muscle were measured using K⁺-sensitive liquid ion exchanger microelectrodes. When the pH of the bathing medium was decreased from 7.5 to 6.8, intracellular K⁺ activity fell and extracellular K⁺ activity rose from a mean control level of 3.6 mM to a new steady state level of 3.9 mM after 1 hour. When the pH was further decreased to 6.1, extracellular K⁺ activity increased to a mean of 4.9 mM. Following the change in pH, the increase in extracellular K⁺ activity occurred over a period of 30-40 minutes at which time a stable value was reached and maintained for the next hour. On return to normal pH the extracellular K⁺ activity returned to control with a time constant of 20 minutes or less. Measurements of intracellular K⁺ activity over 1 hour showed a mean loss of 3 mM at pH 6.8, and a mean loss of 8 mM at pH 6.1. The loss was reversible within 20 minutes of return to control pH. The increase in extracellular K⁺ activity was accompanied by a decrease in resting membrane potential as well as decreases in maximum dV/dt and overshoot of the action potential. The action potential contour underwent complex changes consisting of decrease in the plateau and a prolongation of the time to full repolarization.

POTASSIUM LOSS from cardiac tissues following experimental coronary occlusion first was reported by Harris et al. It since has been substantiated in a variety of cardiac preparations (for review, see Gettes). A small potassium loss might be expected to increase the extracellular concentration next to the myocardial cell membrane, thereby altering the potassium equilibrium potential and, consequently, because of the relatively high potassium permeability, the resting membrane potential. This, in turn, would alter voltage-dependent conductances such as the sodium system. This has led to the postulation that an increase in extracellular potassium is involved in the genesis of cardiac arrhythmias immediately following myocardial infarction.

The cause or causes of potassium loss following coronary occlusion have not been established. Immediately following coronary occlusion a rise in lactate concentration of coronary sinus blood occurs as a result of anaerobic metabolism. Simultaneously, coronary sinus potassium rises. Coronary sinus lactate and potassium rise simultaneously in man in angina pectoris. This has led to speculation that increases in either hydrogen or lactate ion concentrations are at least partially responsible for potassium loss. Direct measurements have been made of pH in ischemic heart tissue. Gebert et al. have demonstrated that pH decreases 0.65 pH unit in the ischemic area after 30 minutes of occlusion and 1.2 pH units following fibrillation. Couch and Middleton reported a surface pH of 5.99 in heart after 1 hour of ischemia at 38°C and Cohn et al., a mean pH of 7.08 in ischemic areas during fibrillation. The effect of extracellular acidity at constant PCO₂ on cardiac potassium has been investigated by Page et al., who showed that total potassium decreases in cat papillary muscle bathed in Ringer's solution at pH 5.2, and by Grassi et al., who showed potassium loss from isolated perfused rat heart at pH 6.8. Cameron and Poole-Wilson, however, reported intracellular cardiac po-

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P F Semple, A S Boyd, P M Dawes and J J Morton

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