Angiotensin I, II, and III in Sheep
A Model of Angiotensin Production and Metabolism

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SUMMARY The arterial and central venous concentrations of angiotensin I (AI), Val'-angiotensin II ([Val']AII), and Val'-angiotensin III ([Val']AIII(2-8)) were quantitatively determined in conscious sheep before and after sodium depletion. All three angiotensins were elevated in blood with progressive sodium loss. During sodium deficiency the arteriovenous concentration ratios (A:V) of AI, [Val']AII, and [Val']AIII(2-8) were found to be 0.48 ± 0.03 (n = 9), 1.30 ± 0.05 (n = 16), and 1.52 ± 0.05 (n = 11) respectively. Intravenous infusion of [Val']AII or [Val']AIII(2-8) significantly elevated the A:V of respective angiotensins, being 2.09 ± 0.28 (n = 5) for [Val']AII and 2.2 ± 0.37 (n = 6) for [Val']AIII(2-8). The blood clearance rates of exogenous [Val']AII and [Val']AIII(2-8) in sodium-depleted sheep were calculated to be 135 ± 15 liter/hr (n = 10) and 140 ± 13 liter/hr (n = 10) respectively. Based on these experimental data, a steady-state model of angiotensin metabolism was constructed.

If it is assumed that endogenous arterial blood [Val']AII and [Val']AIII(2-8) cleared metabolically at a similar rate as exogenous arterial blood angiotensins, it can be calculated that at steady-state 55% of the arterial [Val']AII concentration was derived from the peripheral vascular bed. For [Val']AIII(2-8), 63% of the arterial concentration was derived from the pulmonary circulation. The concentration of [Val']AIII(2-8) in arterial blood was 42% of [Val']AII. (Hypertension 3: 730-737, 1981)

KEY WORDS • angiotensin I • angiotensin II • angiotensin III • plasma renin concentration • sodium depletion • sodium repletion • sheep

RECENT findings in sodium-replete sheep have shown that, following exogenous infusion of either angiotensin III(2-8) heptapeptide (AIII(2-8)) or angiotensin III (3-8) hexapeptide (AIII(3-8)), both peptides could be found in arterial blood.1 The calculated blood clearance rates of these two smaller fragments of angiotensin were similar to that of angiotensin II(1-8) octapeptide (AII). Also, it has been reported that in moderately sodium-depleted conscious sheep the arteriovenous ratios of angiotensin I(1-10) decapeptide (A1) and immunoreactive AII were 0.78 and 0.92 respectively.2

These findings pose several puzzles. An arteriovenous ratio of approximately 1 is not consistent with the high clearance rate and short half-life of AII. Also the fact that AII(2-8) and AIII(3-8) have clearances similar to AII implies that if these peptides occur in venous blood they must also occur in arterial blood. The present study was therefore undertaken to measure the concentration of A1, [Val']AII, and [Val']AIII(2-8) in both arterial and central venous blood of sheep using sodium depletion as a physiological stimulus. It was planned to use the data obtained in this way to set up a model of the production and metabolism of AII in the sodium-depleted sheep.
Methods

Experiments were performed on 10 conscious Merino sheep: six ewes (weighing 35–42 kg) and four wethers (40.5–46 kg). These animals were housed in separate metabolism cages, and fed daily with 0.8 kg of a mixture of lucerne and oaten chaff (1:3) with free access to water; this diet contained 80–100 mEq sodium and 250–350 mEq potassium. All animals had bilateral carotid loops prepared at least 6 weeks prior to experimentation. Animals were sodium-depleted by uncompensated loss of saliva for 48 hours through a parotid duct cannula.8

Blood Sampling

Arterial and central venous blood samples (in triplicate) were taken simultaneously into 3 volumes of ethanol at room temperature, mixed, and stored at −20°C until assayed. The volume of blood taken for each sample ranged from 15 ml in sodium-replete sheep to 10 ml in sodium-depleted sheep and for those taken during angiotensin infusion. Arterial blood was obtained from the carotid artery by direct needle puncture, and central venous blood was drawn rapidly through a 35 mm cannula positioned in the right atrium into a plastic syringe and transferred immediately into ethanol.

Infusions

In the first series of experiments, [Val5]AII was continuously infused into the pulmonary artery of sodium-depleted wethers at a rate of 42 nmoles/hr for 60 minutes. The infusate was made up to 2 nmoles/ml with 0.9% saline solution containing 0.05% ovine γ-globulin. Samples were taken simultaneously from the carotid and the right atrium before and 60 minutes after the commencement of infusion. When the infusion was completed, the animal was allowed to rest until its blood pressure and heart rate returned to preinfusion values. A second infusion of [Val5]AIII(2–8) at a rate of 42 nmoles/hr followed. Both arterial and central venous samples were again taken before and just prior to the end of the 60-minute infusion.

Extraction of Angiotensin

Extraction of angiotensin from blood was performed within 2 weeks of collection. Previous studies have indicated that the ethanolic blood mixture can be handled individually. On the day of extraction, approximately 1000 cpm of either [131]AI (~ 1 fmole), or 4H-AII (~ 50 fmole), or 4H-AIII (2–8) (~ 30 fmole) in 0.5 ml diluent solution (0.1% human serum albumin, 0.01% merthiolate, and 0.002% neomycin sulphate in 0.1 M phosphate buffer, pH = 7.4) were added to sample. The tracer was allowed to mix and equilibrate with the ethanolic blood mixture for 20 minutes before the start of extraction. The method of extraction of angiotensin from whole blood was similar to the procedure described previously.4 The ethanolic blood mixture was centrifuged at 1000 × g for 10 minutes, and the supernatent collected into siliconized glass containers. The residues were resuspended in 70% ethanol and centrifuged. The supernatents were pooled, and an equal volume of diethyl ether added. With occasional shaking, the mixture was left standing at room temperature for 2 hours before filtration through Whatman No. 1 filter paper into a separating funnel. The filtrate was acidified to pH 2.5, and an equal volume of distilled water was added. With vigorous shaking, the mixture solution was separated into two phases. The colored ether layer was discarded. The aqueous layer was reabsorbed with an equal volume of diethyl ether. The pH of the aqueous phase was then adjusted to pH 3 with 1 M NaOH solution and transferred onto a 10 × 30 mm column of SP-Sephadex C50 (Pharmacia, Sweden), which was equilibrated in 0.05 M acetic acid (pH 3). When the sample was absorbed completely, the column was eluted with 2.7 ml of diluent, discarding the first 0.7 ml. The eluate containing AI was counted to correct for losses that occurred during the extraction procedure.

For [Val5]AII and [Val5]AIII(2–8) analyses, the sample was eluted from the SP-Sephadex column with 3 ml of 0.05 M triethylamine (pH 8.3), and the dried eluates transferred by means of 0.05 M acetic acid and 70% ethanol onto Whatman No. 1 chromatography paper. Descending chromatography was carried out for 16 hours with the upper phase (the mobile phase) of the solvent system n-butanol/acetic acid/water/pyridine = 5:1:4:1 (pH 4.6). The Rf values for various peptides in this solvent system were as following: [Val5]AII, 0.30; 4H-AII, 0.31; [Val5]AIII(2–8), 0.46; [H-AIII(2–8)], 0.48; [Val5]AIII(3–8), 0.64; [Val5]AIII(4–8), 0.62; [Val5]AIII(5–8), 0.55. Strips were dried and sections of 2 × 2 cm were then eluted for 3 hours in 2 ml of diluent solution. The eluate was divided into two halves and two to three replicates were counted to correct for losses during extraction and the other to determine mass by radioimmunoassay. After subtracting the mass of tracer and correcting for losses during extraction, we then determined the concentration of either [Val5]AII or [Val5]AIII(2–8).

Tracer and Standards

Labelling with 131I was performed by a modification of the chloramine-T method4 as described previously. The tritium-labelled AII and AIII(2–8), i.e., Asp4, Ile6 (Tyr-3,5-3H(N))-angiotensin II (24 Ci/mmole) and Ile6(Tyr-3,5-3H(N))AIII (41.2 Ci/mmole), were obtained from New England Nuclear Corporation, Boston, Massachusetts. Their purity was checked prior to use and, if necessary, chromatography on paper using the solvent system mentioned above was carried out. AI was a gift from the Division of Biological Standards, National Institute for Medical Research, Holly Hill, London. [Val5]AI and [Val5]AIII(2–8) were purchased from the Peninsula...
Laboratories, San Carlos, California. The purity of these peptides was established by amino acid analysis and paper chromatography. \([\text{Val}^8]\text{AIII}(3-8), [\text{Val}^8]\text{AIII}(4-8)\) and \([\text{Val}^8]\text{AIII}(5-8)\) were obtained from Ciba-Geigy, Basle, Switzerland. \([\text{Des-Asp}']\text{AI}\) was a gift from Dr. F. M. Bumpus, Cleveland Clinic.

**Antibodies**

Antibodies against AI or AII were raised in rabbits by coupling the peptide to hemocyanin by the carbodiimide method, as previously described.4 AI antibody (No. B2) did not cross-react with either \([\text{Val}^8]\text{AI}I\) or \([\text{Val}^8]\text{AIII}(2-8)\) (< 0.1% at 1 pmole concentration). It had a cross-reactivity with \([\text{des-Asp}']\text{AI}\) of 32%. It had a sensitivity of detecting 5 fmoles per tube. The AII antibody (No. 825) cross-reacts with AII (100%), \([\text{Val}^8]\text{AII}I\) (100%), \([\text{Val}^8]\text{AIII}(2-8)\) (102%), \([\text{Val}^8]\text{AIII}(3-8)\) (98%), \([\text{Val}^8]\text{AIII}(4-8)\) (65%), and \([\text{Val}^8]\text{AIII}(5-8)\) (42%) but minimally with AI (< 2% at 1 pmole concentration). It had a sensitivity of detecting 2 fmoles \([\text{Val}^8]\text{AI}I\) and 5 fmoles \([\text{Val}^8]\text{AIII}(2-8)\) per tube. From immunological evidence, it has been shown previously that in sheep the amino acid in position 5 of AII is valine.19

**Plasma Renin Concentration**

Plasma renin concentration (PRC) was determined by radioimmunoassay of AI production, using an antibody capture technique* developed by Poulsen and Jorgensen.1

**Statistical Analysis**

Unless otherwise stated, all results were expressed as means ± standard error of the mean. Student's paired \(t\) test was performed when required. For the analysis of ratios, the Wilcoxon rank sum test was used.

**Results**

**Reliability of Angiotensin Assays**

To determine the accuracy of the method for assaying AI, known amounts of AI (ranging from 500 to 2 pmoles) were added to arterial blood samples taken from sodium-loaded sheep. With \(^{125}\text{I}-\text{AI}\) as an internal marker, the samples were extracted, concentrated, and assayed. Figure 1 shows the measured level of AI in the blood, after correction for losses during extraction, plotted against the added AI to the blood samples. There was a linear relationship, with a correlation coefficient of 0.99 (\(p < 0.001\)).

For the chromatographic method used to determine \([\text{Val}^8]\text{AI}I\) and \([\text{Val}^8]\text{AIII}(2-8)\), the recovery of 2 pmoles of each peptide in the blood was 96% ± 2% \((n = 6)\) and 108% ± 5% \((n = 5)\) respectively.

**Chromatographic Profile of Arterial and Central Venous Blood Extract**

Figure 2 shows the result of radioimmunoassay of eluates following paper chromatography of arterial and central venous blood extracts. These blood samples were taken from two sheep (ALJA and FRYA) before and after sodium depletion. The presence of immunoreactive fragments of \([\text{Val}^8]\text{AII}\) in both arterial and central venous blood was apparent. Both arterial and central venous profiles were similar.

**Effect of Sodium Depletion on Blood Angiotensin Concentration**

Table 1 shows the concentration of AI, \([\text{Val}^8]\text{AI}I\), and \([\text{Val}^8]\text{AIII}(2-8)\) in arterial and central venous blood taken before and after sodium depletion. These blood concentrations, corrected for losses during extraction and chromatography, are expressed as fmoles per ml of blood. All three angiotensins were significantly \((p < 0.001)\) elevated after 24 hours sodium depletion by uncompensated salivary loss. With the exception of AI in the sodium-replete sheep, there was a significant concentration gradient across the pulmonary circulation for all three peptides in both the sodium-replete and sodium-depleted state.

Table 2 summarizes the arteriovenous ratios of AI, \([\text{Val}^8]\text{AII}\) and \([\text{Val}^8]\text{AIII}(2-8)\) obtained before and after sodium depletion. The mean arteriovenous ratio \((A:V)\) for AI was significantly different \((p < 0.001)\) between the sodium-replete \((0.80 ± 0.03, n = 7)\) and sodium-depleted state \((0.48 ± 0.03, n = 9)\). There was no significant difference of arteriovenous ratios of \([\text{Val}^8]\text{AII}\) before and after sodium depletion \((1.28 ± 0.06, n = 9\) vs 1.30 ± 0.05, \(n = 16)\). Similarly, the arteriovenous ratio for \([\text{Val}^8]\text{AIII}(2-8)\) obtained in the sodium-replete state was not significantly different from that in the sodium-depleted state, being 1.5 ± 0.09 \((n = 7)\) and 1.52 ± 0.05 \((n = 11)\) respectively.

Because we determined the levels of all three angiotensins from the same animal under the same physio-
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Figure 2. Chromatogram of arterial (black column) and central venous blood (open column) taken from two sheep before and after sodium depletion. The position of [Val⁵]AlI, [Val⁵]AlI(2-8), and [Val⁵]AlI(3-8) standards in this solvent system (n-butanol: acetic acid: H₂O: pyridine = 5:1:4:1, pH = 4.6) is shown in the lower panel. In these samples, no recovery tracer were added.

Table 1. Concentration of Al, [Val⁵]AlI, and [Val⁵]AlI(2-8) in Arterial (A) and Central Venous (V) Blood Before and After Sodium Depletion

<table>
<thead>
<tr>
<th>Na status</th>
<th>Blood</th>
<th>Al</th>
<th>[Val⁵]AlI</th>
<th>[Val⁵]AlI(2-8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replete</td>
<td>A</td>
<td>14.1 ± 1.4 (6)</td>
<td>16.2 ± 3.4 (9)</td>
<td>12.4 ± 1.7 (7)</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>16.4 ± 2.4 (6)</td>
<td>12.9 ± 2.4 (9)</td>
<td>8.2 ± 1.3 (7)</td>
</tr>
<tr>
<td>Deplete</td>
<td>A</td>
<td>27.1 ± 3.0 (5)</td>
<td>59.5 ± 10.6 (5)</td>
<td>26.7 ± 1.9 (6)</td>
</tr>
<tr>
<td>(24 hr)</td>
<td>V</td>
<td>59.1 ± 6.2 (5)</td>
<td>50.4 ± 4.7 (5)</td>
<td>16.6 ± 1.7 (5)</td>
</tr>
<tr>
<td>Deplete</td>
<td>A</td>
<td>50.3 ± 11.2 (5)</td>
<td>114.0 ± 18.8 (5)</td>
<td>37.6 ± 2.5 (6)</td>
</tr>
<tr>
<td>(48 hr)</td>
<td>V</td>
<td>98.8 ± 11.0 (5)</td>
<td>88.3 ± 16.0 (5)</td>
<td>28.5 ± 2.4 (6)</td>
</tr>
</tbody>
</table>

All values were expressed as fmole/ml ± standard error of the mean. Figures in parentheses were the number of determinations. NS = paired t test not significant.

*Paired t test p < 0.05.
**Paired t test p < 0.025.

Logical conditions, we were able to establish the relationships between Al, [Val⁵]AlI, and [Val⁵]AlI(2-8) concentrations in arterial and central venous blood. Under sodium-deplete conditions, the concentration ratio of AI to [Val⁵]AlI in arterial blood was 0.34 ± 0.05 (n = 8), and in the central venous blood, 0.95 ± 0.06 (n = 8). The concentration ratio of [Val⁵]AlI(2-8) to [Val⁵]AlI in arterial blood was 0.42 ± 0.06 (n = 8), and in central venous blood, 0.32 ± 0.04 (n = 8).
Arteriovenous Ratio during Exogenous Angiotensin Infusions

Of 10 sodium-depleted sheep, six had both arterial and central venous blood taken simultaneously before and during angiotensin infusion. Intravenous infusion of [Val8]AII resulted in an elevation of the arteriovenous concentration ratio from preinfusion values (table 3). The mean arteriovenous concentration ratio of [Val8]AII before infusion was 1.24 ± 0.12 (n = 6). It was significantly elevated to 2.09 ± 0.28 (n = 5) just prior to the end of 1 hour of infusion of [Val8]AII at 42 nmoles/hr.

Table 3. Arterial (A) and Central Venous (V) Blood Concentration of [Val8]AII and Plasma Renin Concentration (PRC) during [Val8]AII Infusions in Na-Deficient Sheep

<table>
<thead>
<tr>
<th>Sheep</th>
<th>Infusion rate (n mole/hr)</th>
<th>[Val8]AII concentration (f mole/ml)</th>
<th>A:V</th>
<th>PRC (f mole/hr/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KATO</td>
<td>0</td>
<td>22.1</td>
<td>1.50</td>
<td>19.8</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>304.0</td>
<td>2.40</td>
<td>26.7</td>
</tr>
<tr>
<td>RASMY</td>
<td>0</td>
<td>89.9</td>
<td>1.20</td>
<td>13.2</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>428.0</td>
<td>4.30</td>
<td>10.4</td>
</tr>
<tr>
<td>DARREN</td>
<td>0</td>
<td>67.1</td>
<td>2.40</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>242.0</td>
<td>17.0</td>
<td>13.1</td>
</tr>
<tr>
<td>MAMDO</td>
<td>0</td>
<td>48.2</td>
<td>0.97</td>
<td>27.0</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>497.0</td>
<td>7.20</td>
<td>26.6</td>
</tr>
<tr>
<td>CLEEVE</td>
<td>0</td>
<td>86.4</td>
<td>1.60</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>260.0</td>
<td>4.50</td>
<td>12.0</td>
</tr>
<tr>
<td>BERNICE</td>
<td>0</td>
<td>85.1</td>
<td>1.10</td>
<td>3.18</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>300.4</td>
<td>2.40</td>
<td>2.5</td>
</tr>
<tr>
<td>Mean A:V</td>
<td>1.24 ± 0.12 (n = 6)</td>
<td>p &lt; 0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean of two determinations.

The blood clearance rate of [Val8]AII was calculated after correction for the fall in endogenous level of [Val8]AII due to suppression of plasma renin concentration using the formula of Johnston and colleagues:

$$BCR = \frac{IR}{C_o - C_i + \frac{PRC_i}{PRC_o}}$$

where IR = the infusion rate (pmole/hr); C0 = the concentration of blood [Val8]AII (pmole/liter) at the commencement of [Val8]AII infusion; C_i = the equilibrium concentration of blood [Val8]AII (pmole/liter) just prior to the end of [Val8]AII infusion; and PRC0 and PRC_i = the plasma renin concentration (pmole/hr/ml) at 0 and 60 minutes after commencement of the [Val8]AII infusion. The blood clearance rate of exogenous [Val8]AII in the sodium-depleted sheep was 135 ± 15 liter/hr (n = 10).

During [Val8]AIII(2-8) infusion, a similar elevation of the arteriovenous concentration ratio of [Val8]AIII(2-8) was observed. The mean arteriovenous concentration ratio was 1.39 ± 0.1 (n = 6) before infusion and 2.2 ± 0.37 (n = 6) just prior to the end of infusion (table 4). The blood clearance rate of [Val8]AIII(2-8) in the sodium-depleted sheep was 140 ± 13 liter/hr (n = 10).

Plasma renin concentration decreased by 59% ± 8% (n = 10) during [Val8]AII infusion, and by 57% ± 9% (n = 10) during [Val8]AIII(2-8) infusion.

Model of Steady-State Angiotensin Metabolism

The experimental results (tables 1 and 2) obtained above allowed construction of a steady-state model of the production and fate of the various angiotensins as they moved through the circulation in sodium-deficient sheep. It was assumed that the cardiac output remained constant. The central venous [Val8]AII concentrations were arbitrarily selected as 100 concentration units. In one passage across the pulmonary circulation, 30 units of [Val8]AII were produced, because the measured arteriovenous concentration ratio for [Val8]AII was 1.30; thus, the arterial [Val8]AII concentration was assigned as 130 concentration units.

Because the measured concentration ratios for A1/[Val8]AII and [Val8]AIII(2-8)/[Val8]AII in central venous blood were 0.95 and 0.32 respectively, this allowed the designation of central venous AI concentration as 95 units and [Val8]AIII(2-8) as 32 units, shown on the left of the model. Using the known arteriovenous concentration ratios of AI (0.48) and [Val8]AIII(2-8) (1.52), we calculated the arterial concentration of AI and [Val8]AIII(2-8) as 46 units and 49 units respectively.

How do these steady-state concentrations arise? The AI that disappeared across the pulmonary bed was most likely converted to [Val8]AII (30 units) and [Val8]AIII(2-8) (17 units), as shown. This is internally consistent with the ratio values. About half the AI

### Table 4. Arterial (A) and Central Venous (V) Blood Concentration of [Val8]AII (2-8) and Plasma Renin Concentration (PRC) during [Val8]AIII(2-8) Infusions in Na-Deficient Sheep

<table>
<thead>
<tr>
<th>Sheep</th>
<th>Infusion rate (n mole/hr)</th>
<th>[Val8]AIII(2-8) concentration (f mole/ml)</th>
<th>A:V</th>
<th>PRC (f mole/hr/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KATO</td>
<td>0</td>
<td>48.1</td>
<td>1.3</td>
<td>22.8</td>
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<td></td>
<td>42</td>
<td>226.0</td>
<td>1.94</td>
<td>3.1</td>
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<td>RASMY</td>
<td>0</td>
<td>15.5</td>
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<td>12.2</td>
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<td></td>
<td>42</td>
<td>312.0</td>
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<td>4.3</td>
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<td>DARREN</td>
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<td>1.5</td>
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<td></td>
<td>42</td>
<td>235.0</td>
<td>1.1</td>
<td>14.0</td>
</tr>
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<td>1.33</td>
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<td></td>
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<td>12.8</td>
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<tr>
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<td></td>
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<td>275.3</td>
<td>2.4</td>
<td>10.3</td>
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<td>Mean A:V</td>
<td>1.39 ± 0.10 (n = 6)</td>
<td>p &lt; 0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean of two determinations.
Figure 3. Diagram of a model of angiotensin production and metabolism in the sodium-deficient sheep. The number inside each column represents the total value (in arbitrary units) of the column. The number by the side of the column represents only a portion of the total column. For example, 130 is the value of [Val*]AII in arterial blood, out of which 30 was derived from the pulmonary circulation. A/V means the arteriovenous ratio obtained before infusion; A/V (INF) means the arteriovenous ratio obtained during exogenous infusion. Cross-hatched bar represents peripheral production; light-dotted bar represents pulmonary production; darker dotted bar represents irreversibly metabolized production.

present in central venous blood arises during the passage from the arterial side of the circulation to the central venous sampling site — either in the blood or from the peripheral vascular beds.

[Val*]AII from in vivo studies on its effect on blood pressure or by direct measurement following exogenous injection is known to have a very short biological half-life, which is consistent with its high metabolic clearance rate. The arteriovenous ratio of 1.30 is not consistent with this, as only 30% of the [Val*]AII is being metabolized in each passage through the vascular beds. The most compelling explanation of this discrepancy was the local production of [Val*]AII and [Val*]AIII(2-8). During infusion of exogenous [Val*]AII, the arteriovenous ratio was 2.1, indicating that 50% of [Val*]AII arising in the arterial blood was removed irreversibly, so that, as shown in the right hand column (figure 3), approximately half the [Val*]AII from arterial blood finished up in the central venous side (62 units). If the model is to be internally consistent, it implies that 38 units of [Val*]AII was generated on passage through the capillary beds or in the blood. Similarly, 22 units of [Val*]AIII(2-8) transferred to the central venous side while 10 units were added from a new source, most likely from metabolism of [Val*]AII.

It can be calculated that, in a single passage through the peripheral vascular bed, 38% of the [Val*]AII in central venous blood is locally produced (shown by the hatched column in fig. 3). In the steady-state, it can be calculated that 73% of the [Val*]AII in central venous blood has arisen in the peripheral vascular bed and that this local production represents 55% of the steady-state arterial concentration of [Val*]AII. Similar calculations show that 37% of the arterial [Val*]AIII(2-8) has arisen in the peripheral circulation, as shown in the diagram in figure 4.

Figure 4. Diagram of peripheral and pulmonary production of [Val*]AII and [Val*]AIII(2-8) at steady-state in arterial and central venous blood.
Discussion

The idea that AII can be generated locally at sites additional to the pulmonary vascular bed has been suggested by various investigators. However, no previous in vivo studies have estimated the actual dimension of the contribution from the systemic vascular bed. Our study reports, for the first time, additional to the pulmonary vascular bed has been the systemic circulation, for sodium deficient sheep.

From these data, we have derived a model describing the in vivo production of AI, [Val]AII, and [Val]AIII(2-8) across both the pulmonary bed and the systemic circulation, for sodium deficient sheep. The model is easy to construct because of the high concentrations of angiotensin during sodium deficiency; moreover, preliminary results indicate that it also is applicable to sodium-replete sheep.

It is of interest that there is a significant decrease of the arteriovenous ratio of AI with progressive sodium depletion (from 0.80 sodium-replete to 0.48 sodium-deplete). We have reported previously that the in vivo conversion of AI to AII by the lung in sheep may vary from 54% to 107%. Although the cause of this variability in conversion was not known, the present study suggests that the rate of conversion of AI to AII is influenced by the sodium status of the animal.

At steady-state, production of [Val]AII and [Val]AIII(2-8) in the peripheral vascular bed accounts for a highly significant component of the production of these peptides. Although AI in arterial blood could serve as substrate for converting enzyme, it does not rule out the possibility that AI and subsequently [Val]AII and [Val]AIII(2-8) could be formed by local renin acting on blood renin substrate.

Elevation of the arteriovenous concentration ratio of [Val]AII during [Val]AII infusion is in agreement with earlier reports in sheep and man. This latter study showed that a delay of up to 4 minutes in the collection of venous blood may result in an increase of 50% of total venous immunoreactivity prior to infusion, while during infusions, total venous immunoreactive material may decrease by 10%. In our present study, however, particular care was taken in the collection of central venous blood; it was drawn into a plastic syringe and immediately transferred into ethanol. The whole procedure took less than 20 seconds to collect 10 ml of central venous blood. Other experiments incubating blood at 37°C and measuring AI, [Val]AII, and [Val]AIII(2-8) production with time would indicate that increased time in the venous circulation would not give rise to the results above, except for some production of AI.

From qualitative studies in sheep reported earlier, it was suggested that the concentration of AII in venous blood was small. Using more sophisticated methodology and especially recovery tracers, we have shown here that the arterial and central venous profiles are very similar. The chromatographic separation used has better resolution than that used previously, and the AI assay is also more sensitive. In man, it also has been shown that the immunoreactive profile of arterial and venous blood are very similar.

The level of AIII(2-8) in arterial blood relative to AI has been reported to be high in rats and low in dog and man. The present studies have shown that, in sheep, arterial [Val]AIII(2-8) is 42% of the arterial [Val]AII concentration. Also, the existence of an arteriovenous gradient (tables 1 and 4) indicates that substantial [Val]AII(2-8) is produced across the pulmonary bed. In the sheep, the blood clearance rates of [Val]AIII(2-8) and [Val]AI are similar. This is in contrast to studies in the dog in which it has been shown, both in vitro and in vivo, that the metabolism of the AIII(2-8) is more rapid than that of AI. However, in both the dog and sheep, sodium restriction or depletion does not alter the clearance rate. Although [Val]AIII(2-8) has a similar clearance rate to [Val]AII in sodium-depleted sheep, the arteriovenous concentration ratio found in the present study is significantly different from that for [Val]AII (1.52 ± 0.05, n = 11, vs 1.30 ± 0.05, n = 16, p < 0.05). This suggests that [Val]AII and [Val]AIII(2-8) may have different production rates in vivo, and that [Val]AIII(2-8) may derive in part from an alternative pathway other than from [Val]AII, as was first proposed by Goodfriend and Peach.

There is both in vitro and in vivo evidence to support the hypothesis that the production of AIII(2-8) may occur via a pathway involving the production of [des-Asp]AI from AI, and its subsequent conversion by converting enzyme to form AIII(2-8). It has recently been reported that [des-Asp]AI can be produced from AI in the isolated pulmonary vascular bed of the rat. This paper suggests that angiotensinase activity in rat lung may degrade specifically the amino-terminal of AI. In our present model, the pulmonary production of [Val]AIII(2-8) also suggests that either the angiotensinase activity of AI may occur in the pulmonary circulation of sheep, or that [des-Asp]AI in central venous blood is the source for producing the [Val]AIII(2-8) found in the arterial circulation. Because the AI antibody has some cross-reactivity with [des-Asp]AI (32% at 1 pmol concentration), it is possible that some contribution to [Val]AIII(2-8) production may occur via the latter route. Full evaluation of the role of the pathway involving [des-Asp]AI in the metabolism of AI in sheep will require the measurement in blood of [des-Asp]AI and its smaller degradation products.

In conclusion, the present model of angiotensin production and metabolism based on the quantitative measurement of AI, [Val]AII, and [Val]AIII(2-8) in central venous and arterial blood shows that 55% of [Val]AII production and 37% of [Val]AIII(2-8) occurs outside the pulmonary circulation in the sheep. The quantitative evaluation of this peripheral production of [Val]AII and [Val]AIII(2-8) has not been described previously in any in vivo experiments.
References

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