Mechanism of Angiotensin I Converting Enzyme Inhibition by SQ20,881 (<Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro) in Vivo
Further Evidence for Extrapulmonary Conversion

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SUMMARY The mechanism by which the angiotensin I (AI) converting enzyme inhibitor SQ20,881 (<Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro) blocks the pressor response to exogenous AI was studied in vivo in the intact anesthetized dog. When administered as a single dose 250 times that of injected AI (250 nmoles/kg) into either the pulmonary or systemic circulation, SQ20,881 produced inhibition of pulmonary conversion of exogenous AI to All that lasted for more than 6 hours as judged by the absence of immunoreactive or labeled All in the pulmonary venous effluent. In contrast, the pressor response to exogenous AI began to reappear within 1 hour of SQ20,881 administration. Six hours following SQ20,881, the pressor response to AI had nearly returned to normal, still in the absence of demonstrable intrapulmonary conversion and without release of detectable amounts of All into the pulmonary venous effluent. These experiments demonstrated that AI has a pressor effect in the presence of SQ20,881 that is independent of pulmonary conversion. Studies with (Des-Asp^1, Ile*) All showed that the delayed pressor response to AI following SQ20,881 administration could not be accounted for by circulating peptide metabolites of AI or All. A competitive inhibitor of All, (D-Asp^1, Ile*) All completely blocked the returning pressor response, suggesting that extrapulmonary generation of All was responsible. The data strongly suggest that the systemic vascular bed taken as a whole contains large amounts of AI converting enzyme that is capable of rapid generation of All without releasing the peptide into circulation. The extrapulmonary enzyme is more resistant to long-lasting blockade by SQ20,881 than pulmonary converting enzyme. The physiological role of extrapulmonary conversion in systemic and local circulatory homeostasis remains to be assessed. (Hypertension 1: 13-22, 1979)

KEY WORDS • pulmonary circulation • pressor response • converting enzyme inhibition • SQ20,881 • angiotensin

THE pressor effects of angiotensin I (AI) have been attributed mainly to its rapid conversion to angiotensin II (All) by converting enzyme (EN 3.4.15.1) in the pulmonary circulation. Previous studies from our laboratory have shown that SQ20,881 inhibits both the acute pressor response and the generation of All seen immediately after the injection of AI into the pulmonary circulation of the dog. Previous studies from our laboratory have shown that SQ20,881 inhibits both the acute pressor response and the generation of All seen immediately after the injection of AI into the pulmonary circulation of the dog. Because of the importance of converting enzyme inhibitors such as SQ20,881 as tools for elucidating the role of the renin-angiotensin system in normal circulatory homeostasis and in the pathophysiology of hypertension, further studies of the mechanism of antihypertensive action of SQ20,881 were undertaken. The time course of converting enzyme inhibition by a single dose of SQ20,881 was evaluated in the pulmonary circulation of the intact dog. The pressor response to injected AI returned before demonstrable intrapulmonary conver-
sion of All as judged by the appearance of All in the pulmonary venous effluent. The returning pressor response was completely blocked by (D-Asp₁, Ile⁶) angiotensin II (D-Asp₁, Ile⁶) All, suggesting that extrapulmonary generation of All was responsible.

Methods

Peptide Synthesis

Angiotensin I, All, (D-Asp₁, Ile⁶) All, (Des-Asp¹) All, (Des-Asp¹) All, (Des-Asp¹) All and (Des-Asp¹, Arg²) All were synthesized manually by the solid-phase technique.¹⁰,¹¹ The t-butyloxycarbonyl derivative of d-asparatic acid was prepared as a custom synthesis by Bachem, Inc.; the t-butyloxycarbonyl aspartic acid, t-amyloxycarbonyl tosyl arginine and t-butyloxycarbonyl tosyl histidine, were obtained from Beckman. We used t-butyloxycarbonyl tosyl histidine to minimize racemization.¹² The subsequent steps in synthesis and purification of the peptides were the same as those described previously.¹ Final yields of the purified peptide were 35-40%. The converting enzyme blocker SQ20,881 was a gift from the Squibb Institute for Medical Research.

Bioassay

The blood pressure responses to (D-Asp₁, Ile⁶) All, (Des-Asp¹) All, (Des-Asp¹, Arg²) All and SQ20,881 were determined in dogs anesthetized with sodium pentobarbital (20 mg/kg, I.V.). Test samples were paired with standards of known doses of AI and All. Peptides were injected intravenously, and pressure was measured with a Statham strain gauge attached to a large-bore catheter in the abdominal aorta.

Iodination Procedure

Angiotensin I, All, (Des-Asp¹) All and (Des-Asp¹, Arg²) All were labeled with ¹²⁵I by the chloramine-T method of Greenwood et al.¹⁴ as modified by Nielsen et al.¹⁵ The details of the iodination procedure and the purification of the monoiodinated peptides on columns of DEAE Sephadex A-25 have been described previously.¹⁶ Over 95% of the labeled material eluted from the columns was monoiodinated peptide with an estimated specific activity of 700 mc/µmole. The monoiodinated peptides were either used immediately or frozen with human serum albumin (1 mg/ml) and stored at -20°C.

Radioimmunoassay

Angiotensin I, All, (D-Asp₁, Ile⁶) All, (Des-Asp¹) All and (Des-Asp¹, Arg²) All were subjected to radioimmunoassay with antibodies to AI¹⁷ and All.¹⁸ The amount of peptide required to produce 50% displacement of ¹²⁵I-AI from the antibody was determined and compared with the amount of All required to do so. The ratio represents percent cross-reactivity.

Preparation of Peptide Standards

To develop a system for distinguishing generated All from AI, (Des-Asp¹) All, (Des-Asp¹, Arg²) All and the products of circulating angiotensinases, peptide standards were prepared as described previously.¹⁸ Monoiodinated AI and All were incubated with trypsin, chymotrypsin or leucine aminopeptidase and each reaction mixture was subjected to high-voltage paper electrophoresis or column chromatography on DEAE Sephadex A-25. The (Des-Asp¹) All and (Des-Asp¹, Arg²) All were subjected to the same analytical procedures for comparison. Figure 1 summarizes the separation of the peptide fragments of ¹²⁵I-AI and ¹²⁵I-All from one another, from intact ¹²⁵I-AI and ¹²⁵I-All, and from ¹²⁵I-(Des-Asp¹) All and ¹²⁵I-(Des-Asp¹, Arg²) All. Trypsin digestion of AI yielded a single labeled product that traveled in the same position as the Val¹-Leu¹⁰ peptide from AI synthesized by the solid-phase technique; trypsin digestion of All yielded a product which traveled in the same position as (Des-Asp¹, Arg²) All. The product of leucine aminopeptidase digestion traveled in the same position as monoiodinated tyrosine. Chymotrypsin yielded a single peptide that presumably consisted of the 1-4 sequence. Since all of the conversion experiments were analyzed by both techniques, it was possible to distinguish All from most of the probable peptide metabolites of AI and All, including (Des-Asp¹) All and (Des-Asp¹, Arg²) All. All of the peptides generated in the in vivo studies could be identified by comparison with these standards.

In Vivo Studies

Twenty-two mongrel dogs of both sexes weighing 20-30 kg were anesthetized with sodium pentobarbital (20 mg/kg, I.V.), received a tracheostomy, and were placed on a Harvard respirator. Each animal was given heparin (1000 U/kg, I.V.). A Swan-Ganz catheter was advanced into the pulmonary artery, as verified by the contour of the pressure tracing, and then withdrawn until a typical right ventricular pressure tracing was obtained. The catheter was left in the right ventricle for the remainder of the experiments. Large-bore retrograde catheters were placed in the ascending aorta and the abdominal aorta. Blood pressure was continuously monitored from the catheter in the abdominal aorta by a Statham pressure transducer and recorded on an Electronics for Medicine photographic recorder.

Then SQ20,881 (5 µmole total dose, or 0.25 mg/kg) was injected as a single bolus into the right ventricle of 16 dogs followed in 30 seconds and at 30, 60 and 120 minutes by a second bolus of either unlabeled AI (20 nmoles) or ¹²⁵I-AI (100 pmole or 1 x 10⁶ counts/min). This dose of ¹²⁵I-AI gave a concentration of iodinated peptide in aortic plasma that approached physiological levels (1 x 10⁶ counts/min ml⁻¹ or 0.1 pmole/ml). Five minutes after each inject-
tion of unlabeled AI, a 5-nmole bolus of unlabeled All was injected into the right ventricle to evaluate pressor responsiveness to All. In four animals, additional injections of unlabeled AI or \(^{125}\text{I}-\text{AI}\) were made at 3, 4 and 6 hours following SQ20,881. In six experiments, SQ20,881 (5 \(\mu\text{mole}\) total dose, or 0.25 \(\mu\text{g/kg}\)) was injected into the ascending aorta at time 0, and unlabeled AI or \(^{125}\text{I}-\text{AI}\) was injected into the right ventricle at intervals as previously described. In this way, the effects of systemic and pulmonary administration of the inhibitor were compared.

Bolus injections of either 20 or 100 nmole of unlabeled (Des-Asp\(^1\)) All or (Des-Asp\(^1\), Arg\(^2\)) All, or 100 pmole of \(^{125}\text{I}-(\text{Des-Asp}^1)\) All or \(^{125}\text{I}-(\text{Des-Asp}^1, \text{Arg}^2)\) All were administered into the right ventricle of four animals to evaluate pressor activity, immunologic activity and metabolic products of the two peptides in vivo.

After injection of each peptide into the right ventricle, blood was collected in five sequential 7-ml samples from the ascending aorta at 5-second intervals into syringes containing dimercaprol, 8-OH quinoline and EDTA and the plasma fraction separated and stored as previously described. Plasma samples containing unlabeled peptides were subjected to radioimmunoassay for AI\(^1\) and All\(^1\). Radioactive samples were subjected to high-voltage paper electrophoresis and column chromatography on DEAE Sephadex A-25 for identification and quantification of labeled peptides. The second and third sequential samples obtained 5 to 15 seconds after injection represented one circulation through the lung as determined by the appearance time of radioactive material. These were subjected to the most detailed chemical and immunological analysis. At least 30 minutes were allowed to lapse between injections to avoid interference with residual radioactive and immunoreactive material.
TABLE 1. Amino Acid Analysis of AI, AII, (D-Asp<sup>1</sup>, Ile<sup>6</sup>) AII, (Des-Asp<sup>1</sup>) AII, (Des-Asp<sup>1</sup>, Arg<sup>2</sup>) AII

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>AI</th>
<th>AII</th>
<th>(D-Asp&lt;sup&gt;1&lt;/sup&gt;, Ile&lt;sup&gt;6&lt;/sup&gt;) AII</th>
<th>(Des-Asp&lt;sup&gt;1&lt;/sup&gt;) AII</th>
<th>(Des-Asp&lt;sup&gt;1&lt;/sup&gt;, Arg&lt;sup&gt;2&lt;/sup&gt;) AII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.08</td>
<td>1.08</td>
</tr>
<tr>
<td>Arg</td>
<td>1.08</td>
<td>1.08</td>
<td>1.06</td>
<td>1.06</td>
<td>1.06</td>
</tr>
<tr>
<td>Val</td>
<td>1.14</td>
<td>1.14</td>
<td>1.10</td>
<td>1.14</td>
<td>1.14</td>
</tr>
<tr>
<td>Tyr</td>
<td>1.06</td>
<td>1.06</td>
<td>1.03</td>
<td>1.06</td>
<td>1.06</td>
</tr>
<tr>
<td>Ile</td>
<td>1.04</td>
<td>1.04</td>
<td>2.10</td>
<td>1.04</td>
<td>1.04</td>
</tr>
<tr>
<td>His</td>
<td>2.25</td>
<td>1.10</td>
<td>1.05</td>
<td>1.23</td>
<td>1.23</td>
</tr>
<tr>
<td>Pro</td>
<td>1.25</td>
<td>1.25</td>
<td>1.20</td>
<td>1.23</td>
<td>1.23</td>
</tr>
<tr>
<td>Phe</td>
<td>1.10</td>
<td>1.10</td>
<td>1.10</td>
<td>1.10</td>
<td>1.10</td>
</tr>
<tr>
<td>Leu</td>
<td>1.19</td>
<td>1.19</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Asp = aspartic acid; Arg = arginine; Val = valine; Tyr = tyrosine; Ile = isoleucine; His = histidinyl; Pro = proline; Phe = phenylalanine; Leu = leucine; AI = angiotensin I; AII = angiotensin II.

Results

Peptide Characterization

Amino acid analyses were performed on a Durrum analyzer after hydrolysis with 5.7 N HCl for 24 hours at 110° C. Acid hydrolysis yielded the amino acids in the expected ratios (table 1). Each unlabeled peptide (10 µg) or each iodinated peptide was subjected to descending paper chromatography followed by electrophoresis in a second dimension as previously described. Each peptide produced a single spot with ninhydrin-collidine stain or a single labeled spot. The (D-Asp<sup>1</sup>, His<sup>8</sup>) AII was evaluated for racemization of D-Asp during synthesis by incubation with leucine aminopeptidase and for susceptibility to cleavage at the carboxyl terminus by incubation with carboxypeptidase A, as previously described. There was no measurable L-aspartic acid at the amino terminus, and the molecule was susceptible to enzymatic cleavage at the carboxyl terminus.

Bioassay

In dogs anesthetized with sodium pentobarbital, a 5-µmole dose of (D-Asp<sup>1</sup>, Ile<sup>6</sup>) AII caused a rise in mean blood pressure of 9 ± 2 (se) mm Hg; a 10-nmole dose of AII caused a rise in mean blood pressure of 44 ± 6 mm Hg. Thus, the Ile<sup>6</sup> substituted peptide had approximately 1/2500th the pressor activity of AII. The heptapeptide (Des-Asp<sup>1</sup>) AII had 25% and the hexapeptide (Des-Asp<sup>1</sup>, Arg<sup>2</sup>) AII had 1% of the pressor potency of an equimolar amount of AII. In doses up to 1 mg/kg, SQ20,881 had no effect on resting blood pressure or on the pressor response to exogenous AII.

Radioimmunoassay

The concentration of each peptide at 50% displacement and the percent cross-reactivity are summarized in table 2. The cross-reactivity of hepta- and hexapeptides with anti-AII was 20% and 25%, respectively. This was sufficient to allow detection of pressor doses of both peptides in aortic blood by radioimmunoassay using anti-AII and <sup>125</sup>I-AII. In a concentration 1000 times that of AI or AII, SQ20,881 did not cross react with either anti-AI or anti-AII.

In Vivo Studies

Figure 2 represents a typical response of blood pressure to injection of SQ20,881 followed by AI.
Angiotensin I alone produced a mean increase of 52 ± 5 (SE) mm Hg in systolic pressure and 39 ± 3 mm Hg in diastolic pressure. SQ20,881 acutely abolished the pressor response to AI, but it did not lower baseline blood pressure. Responsiveness to the pressor effects of AI gradually returned toward normal. Mean pressor response to a 20-nmole dose of AI at 60 minutes was 25 ± 3 mm Hg systolic and 23 ± 4 mm Hg diastolic; at 120 minutes, 29 ± 4 mm Hg systolic and 23 ± 4 mm Hg diastolic. The pressor response to 5 nmoles of administered All was 27 ± 3 mm Hg systolic and 21 ± 4 mm Hg diastolic. Prior administration of SQ20,881 did not alter the pressor response to All. Intraaortic injection of SQ20,881 produced the same complete inhibition of the acute pressor response to AI as intrapulmonary injection. Recovery of the pressor response took place over a similar time course. In the four experiments that were carried out up to 6 hours after SQ20,881 administration, the pressor response to 20 nmoles of AI reached a peak of 48 ± 3 mm Hg systolic and 44 ± 2 mm Hg diastolic (92% and 113% of baseline, respectively) at 6 hours. Neither the systolic nor the diastolic pressor response to AI was significantly different from baseline. The pressor response to All did not change over time.

Injection of 5 μmoles of (D-Asp^1, Ile^8) All blocked both the immediate pressor response to AI and All and the delayed pressor response to AI following injection of SQ20,881, but it did not alter the conversion of AI to All (table 3). This result supports the All dependence of all of the observed pressor effects.

Table 4 summarizes the pressor responses and concentrations of immunoreactive AI and All in aortic blood 5 to 15 seconds following injection of AI before and at intervals after administration of SQ20,881. Pulmonary conversion of AI as judged by appearance of immunoreactive All in the pulmonary venous effluent was blocked by SQ20,881 and did not return by 6 hours following administration of the nonapep-
tide inhibitor, even after the pressor response had returned. Similar results were obtained following intraaortic injection of SQ20,881.

Figure 3 illustrates the results of a representative experiment with $^{125}$I-AI. Within 5 to 15 seconds after intrapulmonary injection of $^{125}$I-AI alone, 70% of the labeled material appearing in aortic blood after a single circulation time was All; 10% was intact AI, and 20% was accounted for by peptide metabolites. These included Tyr, the (3–10) peptide and the (1–4) peptide. Because of the small molar quantity injected, no pressor response was seen. After injection of SQ20,881 into either the right ventricle or the aorta, no $^{125}$I-AI was converted to $^{125}$I-II in the pulmonary circulation. Furthermore, no labeled AI appeared at 30, 60 or 120 minutes after SQ20,881 injection. The (Des-Asp$^1$) All and (Des-Asp$^1$, Arg$^2$) All peptides did not appear in the pulmonary effluent following administration of $^{125}$I-AI alone or at any time interval after SQ20,881. Table 5 summarizes the results of all experiments with $^{125}$I-AI.

Injection of (Des-Asp$^1$) All or (Des-Asp$^1$, Arg$^2$) All into the pulmonary circulation yielded results that are summarized in table 6. The pressor responses to (Des-Asp$^1$) All and (Des-Asp$^1$, Arg$^2$) All were approximately 25% and 1% of the responses to equimolar amounts of All, respectively. Apparent concentrations of All in aortic blood were estimated by radioimmunoassay with anti-All antiserum. Within 5 to 15 seconds following injection of (Des-Asp$^1$) All, the apparent All concentration in the pulmonary effluent was 20% of that predicted from an equimolar dose of injected All; following (Des-Asp$^1$, Arg$^2$) All, 25% of the predicted concentration. These data are consistent with our bioassay data and with our in vitro cross-reactivity studies using anti-All antiserum (table 1). The experiments gave evidence that the returning pressor response to AI following injection of SQ20,881 is not due to the generation of (Des-Asp$^1$) All or (Des-Asp$^1$, Arg$^2$) All, the only fragments of All that have been shown to have pressor activity.

### Table 3. Conversion and Pressor Effect of Angiotensin I ($20$ nmoles)

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Time</th>
<th>No.</th>
<th>Blood pressure increase (mm Hg)</th>
<th>Peptide concentration (pmole/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Systolic</td>
<td>Diastolic</td>
</tr>
<tr>
<td>AI control (20 nmoles)</td>
<td>6</td>
<td>48 ± 7</td>
<td>40 ± 3</td>
<td>16 ± 2</td>
</tr>
<tr>
<td>AI control (10 nmoles)</td>
<td>6</td>
<td>45 ± 5</td>
<td>39 ± 3</td>
<td>0.2</td>
</tr>
<tr>
<td>Post (D-Asp$^1$, Ile$^4$) All</td>
<td>30 secs</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Post SQ20,881</td>
<td>2 hrs</td>
<td>6</td>
<td>30 ± 6</td>
<td>24 ± 5</td>
</tr>
<tr>
<td>Post SQ20,881 +</td>
<td>30 secs</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(D-Asp$^1$, Ile$^4$) All</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: AI = angiotensin I; All = angiotensin II.

### Table 4. Conversion of AI ($80$ nmoles) Injected into Right Ventricle Alone (Control) and at Intervals After SQ20,881 (5 μmoles)

<table>
<thead>
<tr>
<th>Time interval</th>
<th>No.</th>
<th>Blood pressure increase (mm Hg)</th>
<th>Peptide concentration (pmole/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Systolic</td>
<td>Diastolic</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>52 ± 5</td>
<td>39 ± 3</td>
</tr>
<tr>
<td>Post SQ20,881</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 secs</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30 mins</td>
<td>6</td>
<td>19 ± 6</td>
<td>18 ± 7</td>
</tr>
<tr>
<td>1 hr</td>
<td>8</td>
<td>25 ± 3</td>
<td>23 ± 4</td>
</tr>
<tr>
<td>2 hrs</td>
<td>6</td>
<td>29 ± 4</td>
<td>23 ± 4</td>
</tr>
<tr>
<td>3 hrs</td>
<td>3</td>
<td>32</td>
<td>26</td>
</tr>
<tr>
<td>4 hrs</td>
<td>2</td>
<td>34</td>
<td>21</td>
</tr>
<tr>
<td>6 hrs</td>
<td>4</td>
<td>48 ± 3</td>
<td>44 ± 2</td>
</tr>
</tbody>
</table>

Abbreviations: AI = angiotensin I; All = angiotensin II.
**Figure 3.** Pulmonary conversion in vivo. Paper electrophoretic separation and column chromatography of \(^{125}\text{I}-\text{AI}\) administered before and at intervals after SQ20,881 collected in aortic blood 15 seconds after a bolus injection into the right ventricle. Peaks are labeled as in figure 1.

**Discussion**

This study has delineated the time course of AI converting enzyme inhibition and of blockade of the pressor response to pharmacologic doses of injected AI by a single dose of SQ20,881 administered into either the pulmonary circulation or the systemic circulation of the intact anesthetized dog. The pressor response to injected AI returned before demonstrable intrapulmonary conversion of AI as judged by the appearance of immunoreactive AI in aortic blood. The response to SQ20,881 was the same whether the blocker was introduced into the pulmonary or systemic circulation, suggesting that local destruction of SQ20,881 in a single passage through peripheral capillary beds was insufficient to decrease the extent or duration of pulmonary converting enzyme blockade.

The pressor response to AI injected into the pulmonary circulation gradually returned to 50% of control within 1 hour of SQ20,881 administration. This is consistent with previous reports of a 60- to 90-minute blockade of the pressor response to exogenous
AI in anesthetized⁶ and conscious¹⁹ dogs following administration of SQ20,881 in doses comparable to those used in our study. The degree and duration of inhibition by SQ20,881 of the AI-induced pressor response have been shown to be dose-related.¹⁹ In our study, further increases in pressor responsiveness to AI occurred between 1 and 6 hours after SQ20,881, approaching pre-SQ20,881 levels by 6 hours. Hypovolemia did not appear to be responsible, since care was taken to fully replace blood loss with normal saline. Plasma renin activity and endogenous circulating AII did not increase with time following SQ20,881 administration in these animals. Furthermore, the pressor response to injections of AII administered 1 to 6 hours following SQ20,881 did not differ from the response to control injections, indicating that the failure of the AI pressor response to return to control levels was not due to refractoriness to AII or nonspecific unresponsiveness of the preparation to pressor substances. The long-lasting diminution in the pressor response to injected AI following SQ20,881 administration therefore appeared to be due to impaired production of AII.

Minute amounts (< 1 pmole/ml above control) of material that reacted with anti-AII antiserum appeared in aortic blood following AI injection within 6 hours of SQ20,881 administration. A portion of this increase in apparent AII concentration represents cross-reactivity of anti-AII antiserum with circulating AI and its metabolites. The remainder may result from a small amount of residual intrapulmonary conversion of AI. It should be noted that the apparent AII concentration in the pulmonary effluent did not increase with time and did not parallel the return of the pressor response to AI. Furthermore, the amount of AII generated was far too small to account for the observed pressor response.

When ¹²⁵I-AI was administered in near-physiologic doses into the pulmonary circulation, no ¹²⁵I-AII appeared in aortic blood. This confirms the results of studies with pharmacologic doses of unlabeled AI and suggests that the duration of pulmonary converting enzyme inhibition by SQ20,881 is greater than the duration of its major antihypertensive action. The data indicate that AI in pharmacologic doses has a pressor effect in the presence of SQ20,881 that is independent of demonstrable intrapulmonary conversion. Possible explanations include: 1) pressor effects of circulating peptide metabolites of AI or AII; 2) extrapulmonary conversion of AI to AII; and 3) an independent pressor effect of AI, either direct or mediated by the central nervous system or adrenal medullary catecholamines.

### Table 5. Conversion of ¹²⁵I-AI

<table>
<thead>
<tr>
<th>Time interval</th>
<th>Control</th>
<th>SQ20,881</th>
<th>Metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>AI</td>
<td>AII</td>
<td>No.</td>
</tr>
<tr>
<td>30 secs</td>
<td>12</td>
<td>10 ± 6</td>
<td>70 ± 12</td>
</tr>
<tr>
<td>30 mins</td>
<td>6</td>
<td>71 ± 8</td>
<td>0</td>
</tr>
<tr>
<td>1 hr</td>
<td>8</td>
<td>58 ± 9</td>
<td>0</td>
</tr>
<tr>
<td>2 hrs</td>
<td>3</td>
<td>63</td>
<td>0</td>
</tr>
<tr>
<td>6 hrs</td>
<td>4</td>
<td>61 ± 5</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: AI = angiotensin I; AII = angiotensin II.

### Table 6. Injection of (DES-ASP¹) AII or (DES-ASP¹, ARG²) AII

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Blood pressure increase (mm Hg)</th>
<th>Apparent peptide concentration (pmole/ml)</th>
<th>Percent of total counts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Systolic</td>
<td>Diastolic</td>
<td>AI</td>
</tr>
<tr>
<td>(DES-ASP¹) AII</td>
<td>20 nmoles</td>
<td>4</td>
<td>25 ± 8</td>
</tr>
<tr>
<td></td>
<td>100 nmoles</td>
<td>4</td>
<td>92 ± 11</td>
</tr>
<tr>
<td>(DES-ASP¹, Arg²) AII</td>
<td>20 nmoles</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>100 nmoles</td>
<td>4</td>
<td>5 ± 3</td>
</tr>
<tr>
<td>Standard AI</td>
<td>20 nmoles</td>
<td>12</td>
<td>52 ± 5</td>
</tr>
<tr>
<td>Standard AII</td>
<td>5 nmoles</td>
<td>10</td>
<td>24 ± 6</td>
</tr>
<tr>
<td>¹¹¹I-(DES-ASP¹) AII</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>¹¹¹I-(DES-ASP¹, Arg²) AII</td>
<td>2</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

Abbreviations: AI = angiotensin I; AII = angiotensin II.
To investigate the first possibility, (Des-Asp<sup>1</sup>) AII and (Des-Asp<sup>1</sup>, Arg<sup>2</sup>) AII, the only peptide fragments of AII or AI that have pressor activity<sup>20-22</sup> were synthesized, labeled with <sup>125</sup>I and evaluated for immunologic activity with anti-AII antisemur and for pressor activity in the closed chest anesthetized dog. Results indicated that both the pressor and immunologic activities of (Des-Asp<sup>1</sup>) AII and the immunologic activity of (Des-Asp<sup>1</sup>, Arg<sup>2</sup>) AII were reduced to 20-25% of an equimolar quantity of AII; the pressor activity of (Des-Asp<sup>1</sup>, Arg<sup>2</sup>) AII was only 1% of AII. Thus a substantial pressor response could not have been produced by either of these AI derivatives in the absence of appreciable immunoreactive peptide in aortic blood. In addition, the heptapeptide and hexapeptide derivatives of AII were not found in aortic blood following the intrapulmonary administration of AII. For all of these reasons, circulating metabolites of AI and AII did not appear to account for the pressor response to AI observed in the presence of SQ20,881.

To evaluate the AII dependence of the pressor response to AI before and after administration of SQ20,881, (D-Asp<sup>1</sup>, Ile<sup>6</sup>) AII, a potent, specific and long-lasting inhibitor of the pressor effect of AII,<sup>23</sup> was administered in 250-fold molar excess of AI. The pressor responses to exogenous AI and AII completely blocked the pressor response to exogenous AII and AII alone and the pressor response to exogenous AI administered 1-6 hours following SQ20,881. Since (D-Asp<sup>1</sup>, Ile<sup>6</sup>) AII is thought to be an antagonist of AI,<sup>25</sup> these results suggest that it tends to release pressor effect of pharmacologic doses of AI following administration of SQ20,881 is due to AI generation in extrapulmonary sites. These results make an independent pressor effect of AI an unlikely explanation for this phenomenon.

Previous studies in which AI was administered into various peripheral capillary beds of the dog, including kidney,<sup>24, 25</sup> mesenteric circulation,<sup>24</sup> hindlimb<sup>27</sup> and liver,<sup>28</sup> have given evidence that some AI conversion occurs in extrapulmonary sites. Also, the presence of all components of the renin-angiotensin system has been reported in isolated tissues not perfused with blood.<sup>29-31</sup> Experiments in the rat have shown that the pressor response to exogenous AI,<sup>32</sup> renin<sup>33</sup> or AII<sup>34</sup> was blocked more completely by SQ20,881 or the AI antagonist (Sar<sup>1</sup>, Ala<sup>6</sup>) AII than by anti-AI antiserum. These data have been interpreted as support for the hypothesis that AI is generated at a site in blood vessel walls that is accessible to the readily diffusible converting enzyme or AI antagonist but not to the large molecules of anti-AI antibody. Similarly, SQ20,881 has been shown to lessen the vasoconstrictor effect of AI but not of AII on human forearm vessels, suggesting that converting enzyme resides in the peripheral vessels of man.<sup>35</sup>

The current study provides strong evidence that a single bolus of exogenous AI administered to the dog can be extensively and rapidly converted to AII under conditions in which pulmonary conversion appears to be blocked by SQ20,881 without releasing detectable amounts of AII into the circulation. The magnitude of the pressor response seen under these circumstances suggests that the systemic vascular bed as a whole may contain nearly as much converting enzyme as the lung. This had not been apparent in previous studies conducted in the absence of SQ20,881 because peripheral organ beds were studied individually and because the greater concentration of pulmonary converting enzyme and higher pulmonary blood flow caused pulmonary conversion to predominate.<sup>5, 24</sup> In the current study, a single injection of SQ20,881 totally blocked both pulmonary and peripheral conversion of exogenous AI for a brief time, but peripheral converting enzyme recovered more rapidly than the pulmonary enzyme. This may be explained in part by the greater accessibility of the blocker to pulmonary converting enzyme, which has been demonstrated on the luminal surface of pulmonary endothelial cells,<sup>26</sup> than to peripheral converting enzyme. Additional possibilities are that SQ20,881 may be broken down more rapidly in the systemic than in the pulmonary circulation, or that there may be qualitative differences between pulmonary and peripheral converting enzyme(s). Further investigation will be needed to evaluate this problem and to identify the predominant site of conversion under physiological conditions.

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