Effects of Angiotensin I and Angiotensin II on Canine Hepatic Vascular Resistance

By Joseph Di Salvo, Steven Britton, Patrick Galvas, and Thomas W. Sanders

ABSTRACT

The effects of angiotensin I (0.2–3.2 µg) and angiotensin II (0.1–1.6 µg) injections into the pump-perfused arterial supply of the liver were studied in dogs anesthetized with sodium pentobarbital. Marked increases in hepatic artery perfusion pressure (10–50%), reflecting directionally similar changes in resistance to blood flow, were caused by either angiotensin I or angiotensin II. Resistance increases produced by angiotensin I were significantly attenuated by the synthetic nonapeptide SQ 20881 (Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro, 50 µg/kg, iv) that inhibits enzymatic conversion of angiotensin I to angiotensin II. In contrast, responses caused by angiotensin II were unaltered by SQ 20881. However, resistance increases caused by either angiotensin I or angiotensin II were blocked by 1-Sar-8-Ala-angiotensin II (100 µg/kg min⁻¹, ia), a specific angiotensin II antagonist. These findings parallel the finding that responses to angiotensin I in the vasculature supplied by the hepatic artery are largely caused by local enzymatic conversion of angiotensin I to angiotensin II. Such conversion appears to occur to the extent of about 46%.

KEY WORDS angiotensin antagonist angiotensin-converting enzyme hepatic arterial perfusion hepatic vasoconstriction local blood flow peptides perfusion pressure

The decapeptide angiotensin I produces marked vasoconstriction in canine renal (1, 2), superior mesenteric (3), and hind-limb vasculatures (2). These constrictor responses are abolished by a synthetic pentapeptide (SQ 20475, Pyr-Lys-Trp-Ala-Pro) that inhibits enzymatic conversion of angiotensin I to the vasoactive octapeptide, angiotensin II (1–7). The inhibitory agent SQ 20475, originally isolated from Bothrops jararaca venom (BFT5a), does not inhibit responses to angiotensin II or norepinephrine. Therefore, renal, mesenteric, and hind-limb vasoconstriction caused by angiotensin I appears to be largely attributable to its local conversion to angiotensin II. The reported findings bear directly on the hypothesis that local formation of angiotensin II from angiotensin I could be importantly involved in local regulation of blood flow. We examined the effects of angiotensin I and angiotensin II on vascular resistance to blood flow in the vasculature supplied by the hepatic artery with and without a new inhibitor of the angiotensin-converting enzyme and with and without an angiotensin II antagonist.

Methods

Fourteen mongrel dogs of either sex (14–17 kg), anesthetized with sodium pentobarbital (30 mg/kg, iv), were intubated with a cuffed endotracheal tube and permitted to breathe spontaneously. Systemic arterial blood pressure was measured with a catheter inserted into the right femoral artery and advanced into the thoracic cavity until its tip was close to the heart. We examined the effects of angiotensin I and angiotensin II on vascular resistance to blood flow in the vasculature supplied by the hepatic artery with and without a new inhibitor of the angiotensin-converting enzyme and with and without an angiotensin II antagonist.

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showed that perfusion pressure was stable at such levels for 3-4 hours. Changes in perfusion pressure during constant flow conditions reflect directionally similar active changes in resistance to blood flow (8, 9).

Therefore, increases in hepatic artery perfusion pressure represent increases in resistance to blood flow in the vasculature supplied by the hepatic artery.

Dosed doses of angiotensin I (Schwarz Bio-Research Inc. [1-Arp-5-De-At]-Al, 0.05 μmol peptide/mg) or angiotensin II (Ciba [1-Aam-S-Val-All], 0.78 μmol peptide/mg) were rapidly injected (0.2 ml) into the inflow side of the perfusion pump. The maximal increase in hepatic artery perfusion pressure was taken as the principal response. Results were expressed as a percent of the control perfusion pressure measured immediately before agonist injection. The doses of angiotensin I tested were 0.2, 0.4, 0.8, and 1.6 μg, and the doses of angiotensin II were 0.1, 0.2, 0.4, 0.8, and 1.6 μg. In each experiment, control responses to saline (0.2 ml) were obtained and the injection schedule was randomized with respect to agonist and dose. Sufficient time (5-10 minutes) was allowed between injections to prevent tachyphylaxis to angiotensin I or angiotensin II.

Calculation of the percent conversion of angiotensin I to angiotensin II has been described previously (1, 3). The ratio of equipotent doses of angiotensin II to angiotensin I, with respect to increasing hepatic artery perfusion pressure, was multiplied by 1.25 to calculate the percent conversion of angiotensin I to angiotensin II, because 1.25 corrects the difference in molecular weights between angiotensin I and angiotensin II and converts the dose ratio into a molar ratio (1, 3). Since dose-response curves follow a sigmoidal relationship, comparisons of potency between two or more agonists and estimates of the percent conversion must be performed at doses that elicit the same response (10, 11).

In seven dogs responses to angiotensin I and angiotensin II were studied before and after administration of the synthetic nonapeptide SQ 208811 (Pyr-Trp-Pro-Arg-Pro-Glu-Be-Pro-Pro, 50 μg/kg, iv) that inhibits angiotensin-converting enzyme (12-14). In these experiments, responses to angiotensin II were also assessed at a dose of 3.2 μg. The significance of differences between responses with and without SQ 20881 was assessed with Student’s paired t-test (15). Data collected were plotted by the double-reciprocal method of Lineweaver and Burk (16). Specifically, the percent increase in hepatic artery perfusion pressure was plotted on the ordinate and the reciprocal of agonist dose (μg) was plotted on the abscissa. The straight line generated conforms to the equation: 1/V = 1/Vmax + Ke/Vmax)1/S, where V is response (percent increase in perfusion pressure), 1/Vmax (the ordinate intercept) is the reciprocal of the maximal increase in perfusion pressure, Ks is the dissociation constant of the agonist-receptor complex or the enzyme-substrate complex (17-19), and S is the agonist dose (μg). Ks was calculated from the slope of the line (1, 3, 17, 19). We recognize that applicability of Lineweaver-Burk plots to in vitro or in vivo, do provide insight into mechanisms of antagonist action (17-19).

In three dogs responses to angiotensin I (0.8, 1.6, 3.2 μg) and angiotensin II (0.4, 0.8, and 1.6 μg) were studied before and during infusion of 1-Sar-8-Ala-angiotensin II (100 μg/kg min⁻¹) into the inflow side of the hepatic artery perfusion pump. 1-Sar-8-Ala-angiotensin II was recently described as a specific potent angiotensin II antagonist in rats (20); however, its effects on angiotensin II response in dogs have not been reported. The significance of differences between increases in hepatic artery perfusion pressure caused by angiotensin I and angiotensin II before and after administration of 1-Sar-8-Ala-angiotensin II was evaluated with Student’s paired t-test (11).

All drugs were dissolved in saline, adjusted to pH 7.3, and frozen in plastic vials. In each experiment the dog was challenged with angiotensin I (1.3 μg/kg, iv) and angiotensin II (1.3 μg/kg, iv) to ensure that agonist potency did not deteriorate during storage.

Results are expressed as means ± SE.

**Results**

**EFFECTS OF ANGIO TENSIN I AND ANGIO TENSIN II**

Mean systemic arterial blood pressure for all dogs was 132 ± 9 mm Hg at the beginning of the experiments and averaged 140 ± 11 mm Hg when the experiments were terminated. Corresponding values for hepatic artery perfusion pressure were 105 ± 10 mm Hg and 108 ± 12 mm Hg, respectively. Mean blood flow through the pump-perfused hepatic artery was 82 ± 9 ml/min.

Angiotensin I and angiotensin II caused dose-dependent increases in hepatic artery perfusion pressure (Fig. 1). Although dose-response curves for angiotensin I and angiotensin II were similar in shape, the response to angiotensin II was always greater than the response to an equal weight of angiotensin I. At low agonist doses (0.1-0.4 μg) angiotensin II was about four times as potent as angiotensin I and over the remaining portions of the response curves (0.4-3.2 μg) angiotensin II was only about two to three times as potent as angiotensin I. The dose-response curves for angiotensin I and II were parallel at doses above 0.4 μg.

For each dose of angiotensin I tested, the corresponding dose of angiotensin II required to produce an equivalent increase in hepatic artery...
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Percent Conversion of Angiotensin I to Angiotensin II in the Vasculature Supplied by the Pump-Perfused Hepatic Artery

<table>
<thead>
<tr>
<th>Angiotensin I</th>
<th>Angiotensin II</th>
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<td>0.34</td>
<td>53.1</td>
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<tr>
<td>1.6</td>
<td>0.60</td>
<td>41.6</td>
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<tr>
<td>3.2</td>
<td>1.15</td>
<td>44.0</td>
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<tr>
<td>MEAN</td>
<td></td>
<td>46.4</td>
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</table>

*Estimated equivalent dose of angiotensin II from the dose-response curves in Figure 1.

\[ \text{ANGIOTENSIN II/ANGIOTENSIN I} \times 1.25 \times 100. \]

### FIGURE 1

Dose-response curves for the increase in hepatic artery perfusion pressure (percent of control) caused by angiotensin I (open circles) and angiotensin II (solid circles). Each point represents the mean response from ten dogs; small vertical bars represent ± SE. Agonist dose is shown on a log scale (abscissa). Angiotensin II was more potent than angiotensin I in causing hepatic vascular resistance to increase.

### TABLE 1

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Influenz of SQ 20881 on Angiotensin Responses

SQ 20881 is a synthetic nonapeptide originally derived from Bothrops jararaca venom. It inhibits angiotensin-converting enzyme in vitro and in vivo (5, 12-14).

Systemic pressor responses produced by intravenous injection of angiotensin I (1.3 μg/kg) and angiotensin II (1.3 μg/kg) were examined in each of six dogs before and after administration of SQ 20881 (50 μg/kg, iv). Before administration of SQ 20881, the pressor response produced by angiotensin I was more than 90% of the pressor response produced by angiotensin II (Table 2). However, after administration of SQ 20881, the systemic response caused by the same dose of angiotensin I was abolished, but the response to angiotensin II was unaltered. Usually, SQ 20881 caused a small transient decrease in systemic arterial blood pressure.

In the liver, increases in hepatic artery perfusion pressure caused by angiotensin I were abolished or attenuated with SQ 20881, and the increases produced by angiotensin II were unaltered (Figs. 2 and 3). In a representative experiment, injection of angiotensin II (1.6 μg) into the pump-perfused arterial supply of the liver increased perfusion pressure from an initial value of 100 mm Hg to about 135 mm Hg, and injection of angiotensin I (3.2 μg) increased perfusion pressure to 125 mm Hg (Fig. 2). After administration of SQ 20881,
Effects of angiotensin I (A-I) and angiotensin II (A-II) on hepatic arterial perfusion pressure (HAPP) and systemic arterial pressure (SAP). Responses in the absence of SQ 20881 are shown on the left, whereas responses in the presence of SQ 20881 (50 μg/kg, iv) are shown on the right. SQ 20881 significantly attenuated the increase in hepatic arterial perfusion pressure and systemic arterial blood pressure caused by angiotensin I but did not alter markedly the responses to angiotensin II.

However, angiotensin I increased perfusion pressure to only 105 mm Hg, and angiotensin II increased perfusion pressure to about 140 mm Hg. SQ 20881 also inhibited the slight systemic pressor response caused by angiotensin I injected into the hepatic arterial supply. Presumably, these small pressor responses were due to angiotensin I which had circulated from the site of injection, through the hepatic vasculature, and into the systemic circulation.

In all dogs studied, SQ 20881 consistently reduced the increase in hepatic artery perfusion pressure produced by each dose of angiotensin I tested (Fig. 3, left). In seven dogs, angiotensin I (0.8 μg) increased perfusion pressure to 130 ± 2% of control, and the same dose of angiotensin I increased perfusion pressure to only 109 ± 1% of control (P < 0.001) after administration of SQ 20881 (50 μg/kg, iv). However, SQ 20881 did not alter hepatic responses to graded doses of angiotensin II in any of the dogs (Fig. 3, right). The mean increase in perfusion pressure produced by 0.4 μg angiotensin II in the same seven dogs was 126 ± 1.5% of control before administration of SQ 20881 and 122 ± 1.3% of control after administration of SQ 20881. SQ 20881 did not produce any change in hepatic artery perfusion pressure.

Double-reciprocal plot analysis of responses obtained from graded doses of angiotensin I and angiotensin II for all dogs shows that 1/Vmax (ordinate intercept) was 0.0032 for angiotensin II, 0.0145 for angiotensin I without SQ 20881, and 0.0032 for angiotensin I with SQ 20881 (Fig. 4). Corresponding values for the estimated Ks (dissociation constant of the agonist-receptor complex or the enzyme-substrate complex) were 0.25 μg, 0.70 μg, and 4.0 μg, respectively. Clearly, SQ 20881 had
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Table 3

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Hepatic artery perfusion pressure (mm Hg)</th>
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<tbody>
<tr>
<td>Before antagonist</td>
<td>After antagonist</td>
</tr>
<tr>
<td>Angiotensin I (μg)</td>
<td>117 ± 4</td>
</tr>
<tr>
<td>1.0</td>
<td>104 ± 7*</td>
</tr>
<tr>
<td>3.2</td>
<td>101 ± 8*</td>
</tr>
<tr>
<td>Angiotensin II (μg)</td>
<td>129 ± 4</td>
</tr>
<tr>
<td>1.6</td>
<td>106 ± 4*</td>
</tr>
<tr>
<td>Norepinephrine (μg)</td>
<td>118 ± 4</td>
</tr>
<tr>
<td>1.0</td>
<td>144 ± 5</td>
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*P < 0.01 for response before and after administration of the antagonist 1-Sar-8-Ala-angiotensin II.

Discussion

Using the pump-perfused arterial supply of the canine liver we demonstrated that angiotensin I and angiotensin II produce dose-dependent increases in hepatic artery perfusion pressure. Responses to angiotensin I are abolished by SQ 20881, a nonapeptide that inhibits enzymatic conversion of angiotensin I to angiotensin II, but responses to angiotensin II are not altered by SQ 20881. Responses to either angiotensin I or angiotensin II are abolished by 1-Sar-8-Ala-angiotensin II, a specific angiotensin II antagonist. These findings are consistent with the view that increases in hepatic artery perfusion pressure caused by angiotensin I are largely attributable to its intrahepatic enzymatic conversion to angiotensin II. Also the evidence suggests that antagonism of responses to angiotensin I with SQ 20881 involves a competitive mechanism.

Since control levels of hepatic artery perfusion pressure were not altered significantly when the experiments were terminated and tachyphylaxis to injected agonists did not develop, the vasculature supplied by the hepatic artery maintained adequate responsiveness to injected agonists throughout the experimental period.

Increases in perfusion pressure which occur during constant flow conditions reflect corresponding increases in resistance to blood flow (8, 9). Presumably, these resistance increases are largely due to decreases in arteriolar radius. Our finding that angiotensin II produces marked vasoconstriction in the vasculature supplied by the hepatic artery is supported by results from other laboratories (21-25). However, the demonstration that angiotensin I causes vasoconstriction in the liver is a new observation. Increases in hepatic vascular resistance produced by angiotensin I and angiotensin II seem to be mediated through a similar receptor system, since dose-response curves generated for angiotensin I and angiotensin II (Fig. 1) are very similar in shape (18, 19).
Possibly, increases in hepatic artery perfusion pressure caused by angiotensin I are due to its enzymatic conversion to angiotensin II in blood during the time required for transit from the site of injection to the responsive hepatic vasculature. However, this phenomenon is not likely since such conversion would have to occur to the extent of 42-53% within 15 seconds (Table 1, Figs. 1 and 2). The converting enzyme activity in dog plasma is simply too low to permit significant conversion of angiotensin I to angiotensin II in the time elapsed between the injection of angiotensin I and the onset of the hepatic response (13, 20-28).

Accordingly, our results suggest that angiotensin I constricts the vasculature supplied by the hepatic artery by acting directly on hepatic vascular smooth muscle or as a result of intrahepatic conversion to angiotensin II, or both. Inhibition of hepatic constrictor responses caused by angiotensin I and angiotensin II with 1-Sar-8-Ala-angiotensin II is consonant with the view that angiotensin I is locally converted to angiotensin II (Table 3). Since 1-Sar-8-Ala-angiotensin II did not alter hepatic responses produced by norepinephrine, it appears that inhibition of angiotensin responses is specific (20). However, the possibility that 1-Sar-8-Ala-angiotensin II blocks angiotensin I receptors as well as angiotensin II receptors should be considered. Similarly, SQ 20881 could inhibit hepatic and systemic pressor responses to angiotensin I itself or it could inhibit angiotensin-converting enzyme. Either view is consistent with the finding that SQ 20881 did not alter responses to angiotensin II (Figs. 2 and 3, Table 2). Data bearing on interaction between SQ 20881 and angiotensin I receptors are lacking. Nevertheless ample evidence exists showing that SQ 20881 inhibits angiotensin converting enzyme in vitro and in vivo (12-14). Therefore, the effects of SQ 20881 that we observed are probably due to inhibition of enzymatic conversion of angiotensin I to angiotensin II. Vasocostrictor responses to angiotensin I which occur after administration of SQ 20881 could result partly from incomplete inhibition of the converting enzyme and partly from the small degree of vasoactivity of angiotensin I (4). However, Peach et al. (30) recently reported that angiotensin I releases catecholamines from cat adrenal medullae. Therefore, a portion of the hepatic vasocostrictor activity of angiotensin I that persists after inhibition of angiotensin-converting enzyme possibly releases catecholamine from neural or chromaffin cell storage sites in the liver. Our data do not permit accurate assessment of the role of angiotensin I-induced release of catecholamine in mediating hepatic vasocostrictor responses resulting from angiotensin I. Nevertheless, such a role appears insignificant since responses to angiotensin I obtained after administration of SQ 20881 were small.

Double-reciprocal plots of increases in hepatic artery perfusion pressure obtained with angiotensin I with and without SQ 20881 permit examination of the mechanism of SQ 20881 inhibition (17-19). Ks values derived from such data are related directly to the dissociation constant of the converting enzyme–angiotensin I complex. The analysis (Fig. 4) suggests a predominantly competitive type of enzyme inhibition since calculated Ks values in the presence (4.9 µg) and the absence (0.70 µg) of SQ 20881 were very different, but ordinate intercepts (1/Vmax) were similar (1-3, 17-19).

Presumably, angiotensin II formed from angiotensin I in the vasculature supplied by the hepatic artery interacts with the same vascular receptors which interact with exogenous angiotensin II. Our estimated Ks for angiotensin I (0.25 µg) differ from the Ks for angiotensin II (0.25 µg). This difference could be related to probable direct interaction of exogenous angiotensin II with its receptor, although the increases in hepatic artery perfusion pressure produced by angiotensin I require prior conversion to angiotensin II.

Our conclusion that the hepatic vasocostrictor responses elicited by angiotensin I are caused by local enzymatic formation of angiotensin II fundamentally rests on two findings: (1) angiotensin I responses are abolished by 1-Sar-8-Ala-angiotensin II, an angiotensin II antagonist, and (2) angiotensin I responses are abolished by inhibition of angiotensin-converting enzyme with SQ 20881.

Oparil et al. (29), reporting that conversion could not be demonstrated in the canine liver, used a radioimmunoassay technique for detecting newly formed angiotensin II; but we based our finding that angiotensin II was formed from angiotensin I (1-3, 17-19) on hepatic vasoconstrictor responses. Studies by Cain et al. (31) and Goodfriend et al. (35) indicate that interaction between angiotensin II and angiotensin II antibodies is often incomplete and subject to considerable variability. Both et al. (33), utilizing a synthetic substrate (Z-phenylalanylhistidylleucine) for the converting enzyme, found low levels of converting
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activity in rat liver homogenates. In contrast, Huggins and Thampi (34) found that rat liver homogenates contained almost as much converting enzyme activity as did lung homogenates. The reasons for these disparate results are unclear but may be partly due to species differences and partly to differences in methods of measuring converting enzyme activity.

Recent findings that significant conversion of angiotensin I to angiotensin II also occurs in the renal (4, 2), mesenteric (5), and hind-limb vasculatures (2) are consistent with the view that local formation of angiotensin II from angiotensin I may be importantly involved in local regulation of peripheral blood flow. Clearly, the influence of local formation of angiotensin II on hepatic function and hemodynamics merits further study.

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References


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