Reversal of Low Dose Angiotensin Hypertension by Angiotensin Receptor Antagonists

Glenn J. Smits, John P. Koepke, and Edward H. Blaine

During acute angiotensin II (Ang II) infusion (200 ng/kg/min i.v.) into anesthetized rats, mean arterial pressure rose from 124±1 to 154±2 mm Hg. The peptidic Ang II antagonist saralasin lowered arterial pressure in a dose-dependent manner. The maximal decrease in pressure was similar to that observed after the Ang II infusion was discontinued. The nonpeptide Ang II antagonist, 4'-{[2-butyl-4-chloro-5-(hydroxymethyl)-1H-imidazole-1-yl]methyl}[1,1'-biphenyl]-2-carboxylic acid (SC-48742), lowered acutely elevated arterial pressure to a level similar to that on discontinuation of the angiotensin infusion. Chronic (8 days) infusion of Ang II (20 ng/kg/min i.v.) increased mean arterial pressure from 116±3 to 164±7 mm Hg, which then decreased to 121±6 mm Hg on termination of the infusion. Saralasin (10 µg/kg/min, a maximally effective dose during acute angiotensin infusion) decreased mean arterial pressure from 168±7 to 141±3 mm Hg, a pressure significantly higher (p<0.05) than the pressure observed after the angiotensin infusion was discontinued. SC-48742 decreased mean arterial pressure from 167±7 to 127±3 mm Hg, a pressure not statistically different from the minimum pressure observed after the angiotensin infusion was terminated. The mechanism of blood pressure elevation during acute high dose or chronic low dose Ang II infusion is different, the latter having a significant neural component as measured by the response to trimethaphan. The peptidic antagonist saralasin was fully effective in lowering acute angiotensin hypertension but only partially effective during chronic hypertension. The nonpeptide SC-48742 was fully effective during acute and chronic angiotensin hypertension. It is likely that SC-48742 and other nonpeptide Ang II antagonists will be effective antihypertensive agents where low levels of angiotensin operate through a central nervous system mechanism. (Hypertension 1991;18:17-21)

Saralasin is a potent angiotensin II (Ang II) receptor antagonist but has partial agonist activity. In animal models of hypertension conflicting results with respect to antihypertensive activity have been obtained, presumably because of this partial agonist activity.

An important model of hypertension results from the chronic infusion of low levels of Ang II — levels that are not acutely pressor. This form of hypertension has an important central nervous system component.

Whereas saralasin lowers pressure in other forms of angiotensin-dependent hypertension (e.g., one- and two-kidney Goldblatt hypertension) no data have been reported showing that saralasin is effective in the low dose Ang II infusion model. We hypothesize that saralasin is not effective in this model because only low levels of Ang II are required to sustain the hypertension, and the agonist action of saralasin offsets or even dominates its antagonist actions.

The discovery of specific nonpeptidic Ang II antagonists devoid of agonist activity provides an opportunity to test this hypothesis. We have, therefore, compared the blood pressure-lowering action of saralasin with that of the nonpeptide Ang II antagonist SC-48742 (4'-{[2-butyl-4-chloro-5-(hydroxymethyl)-1H-imidazole-1-yl]methyl}[1,1'-biphenyl]-2-carboxylic acid), in rats infused acutely with high levels of Ang II, where we expect saralasin to be effective, or infused chronically with low levels of Ang II, where we expect saralasin to be ineffective. Furthermore, we compared the mean arterial pressure (MAP) responses to saralasin and SC-48742 after the Ang II infusion was discontinued.

Methods

Acute Angiotensin II-Induced Hypertension

Male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, Ind.) weighing 300–400 g were
anesthetized with Inactin (100 mg/kg i.p., BYK-Gulden, Constance, FRG), and the trachea was cannulated to facilitate airway patency. A polyethylene catheter (PE-50) was inserted into a femoral artery to measure MAP and two PE-50 catheters were inserted into a femoral vein for the administration of compounds. Arterial pressure was measured throughout the experiment by connecting the femoral arterial catheter to a Gould P23dB pressure transducer (Statham Division, Gould Inc., Oxnard, Calif.) and chart recorder (Gould Inc., Cleveland, Ohio). Rectal temperature was maintained at 37±1°C by placing the animal on a servocontrolled heating pad (Harvard Apparatus, South Natick, Mass.).

After a 30-minute stabilization period, Ang II (Sigma Chemical Co., St. Louis, Mo.) was infused intravenously at 200 ng/kg/min in 0.9% NaCl to provide a stable elevation in MAP. After acute Ang II–mediated hypertension, the angiotensin infusion was discontinued in one group of rats (n=4) to determine the time course of the return of MAP to normotensive levels. In other groups of rats, the peptidic Ang II antagonist saralasin was infused intravenously at 1, 3, or 10 µg/kg/min (n=6, 5, and 7, respectively). For the nonpeptide SC-48742, a bolus injection of 10 µg/kg preceded a continuous infusion of 1 mg/kg/min to assure steady-state plasma levels (n=5). Infusions of Ang II receptor antagonists were maintained for 25 minutes and stable blood pressure was confirmed.

**Chronic Angiotensin II–Induced Hypertension**

Male Sprague-Dawley rats were anesthetized with ketamine/acepromazine. Sterile Tygon microbore catheters were implanted in the femoral artery and vein and tunneled subcutaneously to the back of the neck. The catheters were exteriorized, protected within a spring and swivel apparatus (Alice King Chatham Medical Arts, Los Angeles), and filled with a heparin-saline (100 units/ml) solution. The rats were individually housed and given standard rat chow and water ad libitum.

After a 3-day recovery period, Ang II was infused at 20 ng/kg/min in a total volume of 20 ml/day isotonic NaCl, which increased by threefold the normal sodium intake. This was provided to enhance blood pressure elevation. Solutions were prepared fresh daily. After 7 days of Ang II infusion, the six rats were randomly assigned to receive the same three treatments used in the acute study (i.e., discontinuing the Ang II infusion, intravenous infusion of SC-48742, and intravenous infusion of saralasin). SC-48742 was infused at the same dose used in the acute study, and saralasin was infused at 10 µg/kg/min, the maximally effective dose as determined in the acute dose–response study. The treatments lasted 120 minutes and the Ang II infusions were reestablished in those rats in which it had been discontinued. Each rat received all three treatments in random order over a 3-day period.

The neurogenic component of chronic Ang II hypertension was assessed by intravenous bolus injection of the ganglion blocker trimethaphan (5 mg/rat i.v.) on the day before Ang II infusion began and on the eighth day of Ang II infusion. The difference in the blood pressure response to ganglion blockade was interpreted as the neurogenic component of blood pressure (e.g., neural tone).

**Statistical Analysis**

In the acute study, analysis of variance for repeated measures followed by Bonferroni multiple comparison t test was used to determine significant differences within any one treatment group with respect to time. Comparisons between groups were analyzed by analysis of variance and Bonferroni t test. Because all rats received each of the three treatments in the chronic Ang II infusion experiments, both intragroup and intergroup comparisons of these data were performed with the repeated measures analysis of variance and subsequent Bonferroni multiple comparison t test. Data were considered to be significantly different when p<0.05, and values are expressed as mean±SEM.

**Results**

**Acute Angiotensin II–Induced Hypertension**

Figure 1A illustrates the acute pressor response to 200 ng/kg/min Ang II infusion and the subsequent blood pressure-lowering action of 1, 3, and 10 µg/kg/min saralasin in anesthetized rats. The fall in MAP resulting from discontinuation of the Ang II infusion is also shown.

For all rats studied (n=27), MAP averaged 124±1 mm Hg before and 154±2 mm Hg during the infusion. The rise in MAP was prompt and reached its steady-state level 4.2±0.2 minutes after the Ang II infusion was begun. Discontinuation of the Ang II infusion resulted in a similarly prompt fall in pressure to control level within 4 minutes (Figure 1).

Saralasin dose-dependently decreased MAP (Figure 1A). Saralasin (1 µg/kg/min) was not fully effective at lowering MAP to the level observed after the Ang II infusion was discontinued, even after 25 minutes of infusion. Three and 10 µg/kg/min saralasin, on the other hand, matched the hypotensive effect observed after the Ang II infusion was discontinued, although the effect of the 3 µg/kg/min infusion was delayed compared with the 10 µg/kg/min infusion. The blood pressure–lowering response to the highest dose of saralasin was similar both in time course and magnitude to that observed when Ang II was discontinued.

SC-48742, the nonpeptidic receptor antagonist, was similarly fully effective in blocking the acute pressor effects of Ang II (Figure 1B). The time course and magnitude were identical to that observed when the Ang II infusion was discontinued.
**Chronic Angiotensin II-Induced Hypertension**

To determine the nervous system component of Ang II hypertension, the ganglion blocker trimethaphan was administered intravenously to eight rats before and after 8 days of infusion with 20 ng/kg/min Ang II. Before Ang II, MAP averaged 108±2 mm Hg, and after ganglion blockade, pressure fell to 60±2 mm Hg. This difference of 48±2 mm Hg was considered to be the basal neural tone supporting blood pressure during normotension before Ang II infusion. After 8 days of Ang II infusion, MAP increased to 162±7 mm Hg. Now, treatment with trimethaphan reduced MAP to 58±3 mm Hg, a value similar to that observed after ganglion blockade before Ang II infusion (i.e., 60±2 mm Hg). Thus, neural tone increased from 48±2 mm Hg to 105±10 mm Hg during Ang II infusion.

Before chronic Ang II infusion, MAP was 116±3 mm Hg. After 7 days of Ang II infusion, MAP was 164±7 mm Hg (Figure 2A). Termination of the Ang II infusion decreased MAP within 45 minutes to 121±6 mm Hg, a level not different from preinfusion pressure (Figure 2B). SC-48742 decreased MAP to 127±3 mm Hg (Figure 2B). The time course and magnitude of the fall in MAP after SC-48742 was similar to that observed after discontinuation of the Ang II infusion (i.e., MAP returned to a level not significantly different from preinfusion levels within 45 minutes). However, saralasin at 10 μg/kg/min, the dose that restored pressure to normal during acute Ang II infusion, decreased MAP from 168±7 to only 141±3 mm Hg, a level significantly higher than preinfusion levels.

**Discussion**

The hypertensive action of chronically elevated circulating Ang II may have a predominant central nervous system component. In 1961, Bickerton and Buckley demonstrated in dogs that delivery of Ang II to the brain elevated arterial pressure independent of a direct action on peripheral vascular smooth
Figure 2. Line graphs show mean arterial pressure (MAP) of six conscious rats infused chronically with 20 ng/kg/min angiotensin II (Ang II) in 20 ml/day of 0.9% NaCl solution for 7 days (panel A). Panel B: MAP response to saralasin (10 μg/kg/min), SC-48742 (10 mg/kg bolus followed by 1 mg/kg/min), or discontinuation of the 20 ng/kg/min Ang II infusion. The six rats were randomly assigned to receive the three treatments during days 8, 9, and 10 of the Ang II infusion such that each rat received each treatment. For those rats in which Ang II infusion was discontinued for 120 minutes, the infusion was restarted at the end of that time interval. All, angiotensin II. 

Increased sympathetic nervous system outflow with long-term Ang II infusion, as measured by recording of splanchnic nerve activity, indicates that Ang II can increase sympathetic outflow. The observation that surgical ablation of the area postrema blocked the chronic hypertension resulting from Ang II infusion identified a critical central site for Ang II-induced hypertension. The degree of arterial pressure elevation after chronic Ang II infusion was similar to the elevation in arterial pressure that was sensitive to ganglion blockade (i.e., the neurogenic tone) further supporting the contention that chronic elevation of circulating Ang II increases arterial pressure via increased sympathetic nervous system outflow.

Acute, high level infusion of Ang II increases MAP within 4 minutes, and MAP is normalized within 4 minutes after termination of the Ang II infusion. These responses are consistent with the short half-life of Ang II in the systemic circulation and the direct effect of Ang II on vascular smooth muscle. Both SC-48742 and 10 μg/kg/min saralasin restored MAP to pretreatment levels, and the response was...
not distinguishable from that observed when the Ang II infusion was discontinued.

When Ang II was infused chronically at 20 ng/kg/min, the rise in blood pressure was more protracted. The full expression of the hypertension did not occur until 3 days after the infusion was begun. This dose of Ang II produces an acute pressor response in rats due to a direct action on vascular smooth muscle, but as shown by Bruner and Fink and confirmed in the present study, elevated blood pressure is maintained through activation of central nervous system receptors accessible to blood-borne Ang II. It is not known why there is a delay in the activation of the brain centers, but similar findings have been reported in rabbits, dogs, sheep, and pigs (unpublished observations from our laboratory).

Discontinuation of chronic Ang II infusion decreased blood pressure with a time course distinct from that observed after the acute infusion. MAP returned to control levels within 45 minutes after the chronic infusion was discontinued but was back to normal within 4 minutes after the acute infusion was discontinued. These data suggest different mechanisms for the chronic and acute blood pressure elevations. These findings suggest that under chronic conditions, only a very small amount of Ang II is necessary to drive the arterial pressure regulatory system and that this drive is exerted primarily through the central nervous system. Thus, in conditions where Ang II may be chronically elevated (e.g., renal hypertension or salt deficiency) only a minimal amount of Ang II is required to activate the central nervous system pathway, and the elevated circulating levels may be superfluous.

Saralasin has agonistic activity that may be adequate to activate the central nervous system pathway. Our data with saralasin reveals only a partial blood pressure-lowering action in the chronically Ang II-infused animal. The same dose was fully effective during acute Ang II infusion.

SC-48742, on the other hand, was completely effective in restoring blood pressure to normal at the same dose that was effective acutely. This is consistent with the lack of agonist activity of this nonpeptide Ang II receptor antagonist.

Another possibility is that SC-48742 reaches Ang II-sensitive areas in the central nervous system not accessible to saralasin. However, it is difficult to rationalize that saralasin, a close structural analogue of Ang II, would not have access to Ang II–sensitive sites and SC-48742, which is structurally distinct from Ang II, would.

Overall, the data show that the nonpeptide Ang II antagonist SC-48742 is fully effective in restoring normotension in animals infused chronically with low doses of Ang II and saralasin is not. SC-48742 and similar nonpeptide Ang II antagonists appear to offer unique possibilities for the treatment of hypertension.

References


**Key Words** • angiotensin hypertension • saralasin • angiotensin antagonists
Reversal of low dose angiotension hypertension by angiotensin receptor antagonists.
G J Smits, J P Koepke and E H Blaine

*Hypertension*. 1991;18:17-21
doi: 10.1161/01.HYP.18.1.17

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/18/1/17

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Hypertension* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Hypertension* is online at:
http://hyper.ahajournals.org/subscriptions/