Comparison of Renin and Converting Enzyme Inhibition in Sodium-Deficient Dogs

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SUMMARY While renin is a highly specific protease, converting enzyme has at least two principal substrates, angiotensin I and bradykinin. Changes in the rate of formation of angiotensin II or degradation of bradykinin can influence the hypotensive action of angiotensin converting enzyme inhibitors. The present study was designed to determine if there were differences in the maximal blood pressure reduction in Na-deficient dogs after angiotensin converting enzyme or renin inhibitor treatment. Five conscious dogs received 0.1, 0.5, and 1.0 mg/kg of i.v. enalaprilat, a potent angiotensin converting enzyme inhibitor, which reduced blood pressure to 75 ± 4, 71 ± 5, and 71 ± 5 mm Hg. Plasma immunoreactive angiotensin II levels were reduced in a dose-related fashion to 35% of control level at the highest dose. Infusion of a maximally effective dose of a statine-containing renin inhibitor (SCRP) with the high dose of enalaprilat produced no further fall in blood pressure (68 ± 7 mm Hg), but immunoreactive angiotensin II levels fell to essentially zero in four of five dogs. The order of drug administration was reversed in another experiment in a group of nine dogs in which SCRP reduced plasma immunoreactive angiotensin II to 25% of control at 0.04 mg/kg/minute (n = 5), with reduction to near zero levels at higher doses. Maximal blood pressure reduction was achieved at 0.32 to 0.64 mg/kg/minute (76 ± 4 mm Hg); 1 mg/kg of enalaprilat lowered blood pressure an additional 11 ± 2 mm Hg (p < 0.01) while not further decreasing immunoreactive angiotensin II levels. A modest tachycardia was observed during enalaprilat treatment, alone or with SCRP, but not during treatment with SCRP alone. The data indicate that enalaprilat is a more effective hypotensive agent in Na-deficient dogs than SCRP and suggest actions in addition to inhibition of the formation of circulating angiotensin II. Both the renin inhibitor and the angiotensin converting enzyme inhibitor may exert their major effects outside the plasma renin-angiotensin system.

Key Words • blood pressure • heart rate • plasma renin activity • angiotensin II • enalaprilat • statine-containing renin inhibitor
Studies to elucidate the antihypertensive mechanism of ACE inhibitors using angiotensin antagonists\textsuperscript{5, 8, 11-14} have yielded conflicting results, presumably because of the inherent agonist activity of these compounds.\textsuperscript{15, 16} Conflicting data have also been obtained in the few studies with renin inhibitors. Two studies found no additional decrease in blood pressure when a converting enzyme inhibitor was combined with a peptide renin inhibitor,\textsuperscript{17, 18} while another study showed an additional blood pressure decrement attributable to ACE inhibition.\textsuperscript{19} The present study used Na-deficient dogs to test this hypothesis because their MAP is highly renin dependent and these animals respond well to all interventions that block the renin-angiotensin system.

Materials and Methods

Adult female mongrel dogs (15.2–19.6 kg) were trained to stand quietly in a modified Pavlov sling. Before experimentation, vascular catheters were placed in one iliac artery and vein with sterile surgical procedures. Those catheters were tunneled subcutaneously to exit between the scapulae. If a venous catheter became nonfunctional during the study period, another sterile catheter was inserted percutaneously into a cephalic vein for the duration of the experiment.

The dogs were rendered Na deficient by feeding them dog food containing less than 12.5 mEq of Na per day (Hills' HD Prescription Diet, Topeka, KS). During the first week of maintenance on the low NA diet, each animal received 5.0 mg/kg of oral furosemide on alternate days (3 doses) to maximize the Na-deficient state. During the afternoon before an experiment, another sterile catheter was inserted percutaneously into a cephalic vein for the duration of the experiment.

On the day of the experiment, the dogs were placed in a sling and MAP and heart rate were recorded with a MP-15D pressure transducer (Micron Instruments, Los Angeles, CA). The blood pressure was monitored at 1-minute intervals with a datalogger (Digitrec Corporation, Dayton, OH) and traced on a strip chart recorder (Gould Instruments, Cleveland, OH). The blood pressure reported for any period was the average of at least five consecutive 1-minute readings during steady state (see below).

In one group of dogs ($n = 9$), control observations were followed by an intravenous infusion of 0.04, 0.08, 0.16, 0.32, and 0.64 mg/kg/minute of the statine-containing renin inhibitory peptide (SCRIP)\textsuperscript{20, 21} To hasten the attainment of the steady state (as indicated by a constant MAP for at least 5 minutes), a bolus injection of four times the per minute infusion rate was given at the beginning of each infusion level (e.g., 0.16 mg/kg was injected as a bolus followed by infusion of 0.04 mg/kg/minute), except at the highest dose of 0.64 mg/kg/minute, when no bolus was given. The infusions of the increasing doses were continuous, and the bolus injections were administered immediately before increasing the infusion rate. Previous experiments\textsuperscript{20} indicated the ED$_{50}$ (effective dose, 50%) for blood pressure lowering was approximately 0.05 mg/kg/minute when SCRIP was infused into conscious Na-deficient dogs. A dose-response relationship was determined for each dog in this study, and the maximally effective dose of SCRIP for blood pressure lowering was found to be 0.32 mg/kg/minute. Twice the maximally effective dose was administered to ensure that complete renin inhibition had been achieved. In this group of dogs, 1.0 mg/kg of enalaprilat, a potent non-sulfhydryl-containing ACE inhibitor,\textsuperscript{1} was injected during the steady state infusion of 0.64 mg/kg/minute of SCRIP, and MAP was monitored for an additional 15 minutes. The SCRIP infusion subsequently was terminated, and MAP was observed for at least 30 minutes.

Nine dogs were studied in this group because plasma ANG II levels were not determined in two dogs and were not satisfactory for technical reasons in a third. In order to have an adequate sample size for this important variable, the additional dogs were included in this group. In addition, one animal was removed from the study due to an adverse reaction of unknown cause when the highest dose of SCRIP (0.64 mg/kg/min) was infused. The data collected from that dog up to that point were satisfactory and have been included in the average values. One plasma sample from the control plasma renin activity (PRA) was lost before analysis, and one PRA value at the 0.04 mg/kg/minute was greater than two SD from the mean and, as an outlier, was excluded from the average.

A similar protocol was followed in another group ($n = 5$), except the animals were treated first with 0.1, 0.5, and 1.0 mg/kg of i.v. enalaprilat. Because enalaprilat has a long duration of action, it was administered as bolus injections rather than by intravenous infusions. Mean arterial blood pressure was monitored for at least 30 minutes before injection of the next higher dose, and MAP had achieved steady state during this period. After a clear steady state MAP had been achieved at the highest dose of enalaprilat, SCRIP was infused at 0.32 mg/kg/minute (preceded by a fourfold bolus injection) and MAP was recorded for at least 15 minutes. Subsequently, the dose was increased to 0.64 mg/kg/minute and MAP was monitored for an additional 15 minutes. The SCRIP infusion was then terminated, and MAP observed for 30 minutes.

Blood samples for measurement of PRA and plasma ANG II concentration were obtained from the arterial catheter at the end of each observation period. Blood for PRA determination was collected in prechilled tubes containing EDTA and centrifuged at 4°C, and the plasma was stored frozen until assayed with standard PRA methodology (1-hour incubation at 37°C and pH 6) and radioimmunoassay components from Clinical Assays (Cambridge, OH) The samples to be used for ANG II determination were collected in prechilled tubes containing EDTA (4 $\times$ 10$^{-3}$ M final concentration) and diisopropyl fluorophosphate (DFP) in isopropanol (1.25 $\times$ 10$^{-3}$ final concentration) and assayed according to the method of Nussberger et al.\textsuperscript{22}
Recent data by Nussberger et al. suggest that this assay method may give a slightly higher value for ANG II concentration than is observed after separation of the various angiotensin metabolites by high-performance liquid chromatography. For this reason we refer to our measurements as immunoreactive ANG II (irANG II) concentrations.

Results

Administration of 0.1 mg/kg of i.v. enalaprilat resulted in a prompt decrease in MAP of 28 ± 3 mm Hg (mean ± SEM; Figure 1A). A second injection of enalaprilat 30 minutes later resulted in an additional decrease of only 5 ± 3 mm Hg. No additional decrease in MAP was observed after the injection of the highest dose, 1.0 mg/kg, and MAP was constant at 71 ± 5 mm Hg. Infusion of 0.32 or 0.64 mg/kg/minute of SCRIP produced no additional fall in MAP (MAP was 68 ± 5 and 68 ± 7 mm Hg respectively during the two periods of SCRIP infusion), nor did discontinuing the SCRIP infusion (see Figure 1A).

Heart rate averaged 105 ± 3 beats/minute during the control period and rose to 127 ± 5 beats/minute (p < 0.01, paired t test) after injection of the lowest dose of enalaprilat (Figure 1B). There were additional small increases in heart rate at 0.5 and 1.0 mg/kg of enalaprilat and also during infusion of 0.32 and 0.64 mg/kg/minute of SCRIP. These latter increases were smaller by comparison to the initial rise in heart rate (all less than 10 beats/min). The total increase in heart rate observed during the combined treatment with enalaprilat and SCRIP was 48 ± 10 beats/minute.

The response of MAP to increasing infusion rates of SCRIP into conscious Na-deficient dogs is illustrated in Figure 2A. Control MAP averaged 111 ± 2 mm Hg
and decreased in a dose-related fashion as the SCRIP infusion was increased. The MAP during infusion of the highest dose of SCRIP was 76 ± 4 mm Hg, a decrease of 35 ± 2 mm Hg from pretreatment levels. With imposition of a supramaximal dose of enalaprilat on the existing supramaximal dose of SCRIP, MAP fell an additional 11 ± 2 mm Hg (p < 0.01, paired t test) to 65 ± 4 mm Hg. On discontinuing the SCRIP infusion, MAP remained at 64 ± 4 mm Hg for at least 30 minutes.

Heart rate during the pretreatment period averaged 114 ± 16 beats/minute and did not change significantly during infusion of 0.04 to 0.64 mg/kg/minute of SCRIP (maximum change from control at 64 mg/kg/minute was 5 ± 9 beats/min). When enalaprilat was injected, the heart rate increased 23 ± 7 beats/minute above the level observed while the dogs were receiving the highest dose of SCRIP (p < 0.05, paired t test) and remained essentially at this level after the SCRIP infusion was discontinued (Figure 2B).

In the group of animals that received enalaprilat first, all three doses were associated with very high levels of PRA (Figure 1C). Infusion of 0.32 mg/kg/minute of SCRIP resulted in a prompt decrease to levels near the limit of detectability for the assay system (0.2 ng of ANG l/ml/hr). This low level of PRA was sustained during infusion of 0.64 mg/kg/minute. On discontinuation of the SCRIP infusion, PRA had begun to rise by the end of recovery period and was within the range of measurement (0.7 ± 0.1 ng of ANG l/ml/hr) but still highly suppressed compared with the pretreatment levels or the very high levels observed during the enalaprilat administration.

In the group of dogs that received SCRIP first, PRA was suppressed during the lowest level of infusion (0.04 mg/kg/min) from 12.8 ± 3.1 to 0.90 ± 0.40 ng of ANG l/ml/hr (Figure 2C). During the 0.08 mg/kg/minute infusion PRA was 0.4 ± 0.1 ng of ANG l/ml/hr, and at the higher doses, PRA remained maximally suppressed. Injection of 1 mg/kg of enalaprilat did not alter the PRA, but discontinuation of the SCRIP infusion resulted in a small increase to 1.3 ± 0.5 ng of ANG l/ml/hr 30 minutes after ending the infusion.

Circulating irANG II levels were reduced in a dose-related manner from control levels of 126 ± 40 fmol/ml to 81 ± 39, 66 ± 26, and 44 ± 02 fmol/ml after injection of 0.1, 0.5, and 1.0 mg/kg of enalaprilat (Figure 1D). Although MAP (see Figure 1A) appeared to be maximally suppressed after the middle dose of enalaprilat, there were still measurable levels of circulating irANG II. When the SCRIP infusion was administered to these dogs, plasma irANG II concentration fell to essentially zero levels (4 of 5 dogs showed zero levels. 1 dog had low but detectable levels). On discontinuing the SCRIP infusion, plasma irANG II levels recovered slightly to 15 ± 9 fmol/ml.

In the dogs that received SCRIP first, plasma irANG II levels averaged 206 ± 45 fmol/ml during the control period before SCRIP infusion, and at the lowest dose of SCRIP, irANG II levels were suppressed to 52 ± 13 fmol/ml (Figure 2D). Higher doses decreased plasma irANG II levels somewhat further, and these suppressed levels were maintained during the period in which enalaprilat was injected and in the 30-minute recovery period when the SCRIP infusion was discontinued.

Discussion

The ACE inhibitors are effective blood pressure lowering agents and their mechanism of action generally is assumed to be inhibition of the formation of circulating ANG II. Certain aspects of the pharmacology of these agents suggest that other factors may be involved. For instance, ACE and kininase II are the same enzyme, and inhibition of kininase II results in increased levels of kinins, potent hypotensive agents. Kinins also are thought to stimulate prostaglandin production, and it has been suggested that formation of vasodilatory prostaglandins could contribute to the blood pressure lowering actions of ACE inhibitors.

The recent synthesis and characterization of potent renin inhibitors have provided another avenue for inhibiting the formation of ANG II that provides for greater specificity. Angiotensinogen is the only known substrate for renin under physiological conditions, and inhibition of renin should not directly affect other enzyme systems such as the kininases.

The present study compares the blood pressure lowering efficacy of a specific renin inhibitor and a potent ACE inhibitor. If ACE inhibitors exert their blood pressure lowering effects by inhibiting the formation of circulating ANG II, then administering a supramaximal dose of an ACE inhibitor during maximal suppression of renin activity should result in no further lowering of MAP. On the other hand, if additional lowering of MAP occurred, it would suggest that ACE inhibitors have hypotensive actions in addition to inhibition of the formation of ANG II.

We used Na-deficient dogs because of their highly renin-dependent blood pressure. Use of conscious animals obviated activation of the sympathetic nervous system that is frequently observed, especially with barbiturate anesthetics.

When enalaprilat was administered before SCRIP, MAP was lowered to approximately 70 mm Hg. This level of blood pressure is similar to that found by others using Na-deficient dogs or rats with maximally effective doses of ACE inhibitors. Thus, 70 mm Hg would appear to be the floor to which MAP can fall in Na-deficient dogs that received ACE inhibitors. Infusing SCRIP into these dogs after having reached this level of MAP produced no further decline in pressure. When SCRIP was administered first, MAP fell to approximately 76 mm Hg at a maximally effective dose, and doubling that dose produced no additional fall in MAP. Thus, maximum MAP lowering with SCRIP appeared to differ from that associated with enalaprilat treatment. Injection of enalaprilat while renin was maximally inhibited resulted in a further statistically significant fall in MAP of approximately 11 mm Hg. Thus, maximally effective doses of SCRIP were not as
hypertensive as those of enalaprilat, and when enalaprilat was added, MAP fell to a level that was characteristic for the ACE inhibitor.

Enalaprilat, whether administered alone or with SCRIP, was associated with a statistically significant moderate tachycardia, in contrast to administration of SCRIP alone, which showed no effect on heart rate. Blood pressure lowering due to renin-angiotensin system blockade is usually notable for its lack of attendant changes in heart rate. The largest single increase in heart rate occurred when 0.1 mg/kg of enalaprilat was administered and was associated with a fall in MAP of approximately 28 mm Hg. When SCRIP was administered alone, the MAP changes were smaller for any given dose and no significant tachycardia was observed. The greater rate of fall of MAP after enalaprilat as compared to SCRIP could have been important in the greater heart rate response. A similar interpretation could be applied also to the heart rate response seen when enalaprilat was added to the SCRIP treatment. A significant captopril-induced tachycardia was observed in a similar study that used Na-deficient dogs, while H77, another peptidal renin inhibitor, had only minor effects on heart rate.

Changes in PRA after administration of enalaprilat were characteristic of blockade of the formation of ANG II and inhibition of the "short loop" feedback suppression of renin secretion. Administration of SCRIP resulted in a prompt decrease in PRA to very low levels. This change is dramatic but not unexpected, given the relative molar concentrations of renin and SCRIP in the circulation. We have observed similar suppression of PRA during 48-hour continuous infusions of SCRIP into conscious Na-deficient dogs. When SCRIP was infused first, PRA was not completely inhibited at the lowest dose, but at higher doses, PRA was virtually undetectable. These data support our previous observation of a dissociation between inhibition of PRA and blood pressure lowering in Na-deficient dogs.

A more specific index of inhibition of the renin-angiotensin system is a decrease in circulating levels of ANG II. Measurements of plasma ANG II concentration are suspect in any study in which ACE inhibitors are used because of the large increase in circulating ANG I concentration that results from inhibition of ACE. Even a small degree of cross-reactivity with ANG I of the antibody used in the ANG II radioimmunoassay can result in a falsely elevated apparent ANG II concentration; however, the ANG I cross-reactivity is only 0.1% with the antibody used here to quantitate ANG II. The unincubated plasma samples collected for PRA measurements (ANG I radioimmunoassay) at the highest dose of enalaprilat averaged 800 pg of ANG I/ml. This level of circulating ANG I would contribute only about 1 fmol/ml to the observed plasma ANG II levels. Morton et al. using a specific ANG I assay, detected 416 fmol/ml of ANG I in Na-deficient dogs during maximal ACE inhibition. At 0.1% cross-reactivity, this would contribute only 0.5 fmol/ml to the observed plasma ANG II levels.

Nussberger et al. recently have developed a powerful new technique to precisely determine circulating levels of ANG II. This method employs high-performance liquid chromatography separation of the various angiotensin metabolites and eliminates any possibility of cross-reactivity. Using this method it was found that ANG II levels could be slightly overestimated because of cross-reactivity with various angiotensin metabolites that exist in plasma despite ACE inhibition. Because of this new observation, we have elected to refer to our measurements as irANG II. As shown by Nussberger et al., the amount of cross-reactivity is small (<10 fmol/ml) and would not alter the conclusions based on the angiotensin measurements.

Enalaprilat produced a dose-related decrease in plasma irANG II concentration but not in MAP. It is not clear why there appears to be a dissociation between fall in MAP and plasma irANG II levels, but the observation is reminiscent of the dissociation between PRA and MAP seen after SCRIP treatment. Although MAP was suppressed maximally by enalaprilat at the two highest doses, plasma irANG II levels were not reduced maximally. Only when SCRIP was added were plasma irANG II levels reduced to near zero. This additional decrease in plasma irANG II levels, however, was not associated with a further decline in MAP.

Plasma irANG II levels in these short-term experiments may not report accurately the ANG II levels in other compartments. The renin-angiotensin system has been shown to be permissive for kidney, brain, and vascular walls. Inhibition of ACE at these other sites could be an important component of the blood pressure lowering effect of enalaprilat. Alternatively, or in addition, the renin-angiotensin-system mechanisms of action, such as bradykinin potentiation, may account for a substantial part of the blood pressure effect.

Infusion of the renin inhibitor SCRIP reduced irANG II to very low levels, even at doses that were not maximally hypotensive (e.g., 0.08 mg/kg/min). The dissociation between plasma irANG II levels and blood pressure seen with SCRIP, where low levels did not correspond to a blood pressure fall, like the dissociation seen with enalaprilat, where a maximal blood pressure fall was attained with moderate remaining plasma ANG II levels, suggests the involvement of some extraplasma renin-angiotensin system, the status of which is not measured accurately by plasma irANG II values. At the highest dose of SCRIP, 0.64 mg/kg/minute, plasma irANG II levels were more than 90% suppressed, and when 1.0 mg/kg of enalaprilat was injected, there was no change in plasma irANG II concentration, but MAP fell an additional 11 mm Hg.

These data demonstrate clearly that after maximal suppression of PRA and plasma irANG II concentration with SCRIP, addition of enalaprilat results in further lowering of MAP. This result suggests either that enalaprilat operates to lower blood pressure by additional mechanisms, such as bradykinin potentiation, or that this ACE inhibitor, but not the renin inhibitor...
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