Converting Enzyme Inhibitor and Saralasin Infusion in Rats

Evidence for an Additional Vasodepressor Property of Converting Enzyme Inhibitor

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SUMMARY Converting enzyme inhibitor (CEI) produced a greater fall in blood pressure (BP) than the competitive antagonist of angiotensin II, sarcosine-alanine angiotensin II (saralasin), when administered to salt-depleted rats; there was no difference in BP response in salt-loaded rats. Thus, two widely used methods of inhibiting the renin-angiotensin system give discrepant effects. The renin-angiotensin system was blocked in anesthetized rats by an infusion of saralasin, and then a bolus injection of CEI was given. A further significant fall in blood pressure occurred in salt-depleted rats (−45.7 ± 6.8 mm Hg; mean ± se). There was only a small change in blood pressure of salt-loaded rats (−11.2 ± 3.1 mm Hg) and no change in blood pressure of salt-depleted rats 1 hour after bilateral nephrectomy. Saralasin infusion after CEI did not influence BP. Bradykinin infusion potentiated the depressor effect of CEI in bilaterally nephrectomized rats. Renin infusion restored the depressor response of nephrectomized rats to saralasin, but administration of CEI then caused no further fall in BP. BP of saralasin-infused rats with Goldblatt two-kidney hypertension given CEI fell by 11.1 ± 5.4 mm Hg; after dietetic salt depletion, BP fell by 20.5 ± 9.9 mm Hg with CEI. Bilateral nephrectomy abolished this response to CEI. Hence, CEI has a significant vasodepressor action in addition to inhibition of angiotensin II production. Since it can be restored in nephrectomized animals by bradykinin infusion, the component of the vasodepressor action of CEI which cannot be blocked by saralasin may be identical with its bradykinin-potentiating action. If so, changes in the renal kallikrein-bradykinin system may have an important role in BP control in response to changes in sodium balance and in hypertension.

TWO RECENTLY DEVELOPED peptides have been used extensively both in man and in laboratory animals to inhibit the renin-angiotensin system. These are the competitive antagonist of angiotensin II, [Sar¹, Ala⁸]angiotensin II (saralasin), and the nonapeptide-converting enzyme inhibitor (CEI).¹⁴ These agents have been used to define the role of the renin-angiotensin system in various physiological and pathological states. There are several theoretical criticisms of such an approach. First, an agent may have different inhibitor properties, in vivo and in vitro as has been demonstrated for angiotensin II antisera;⁷ thus the observation of in vitro antagonism may not be applicable in vivo. Second, the administration of an inhibitor may stimulate compensatory mechanisms which attempt to maintain a falling blood pressure; thus administration of saralasin⁸ or CEI⁹ usually is associated with elevation of circulating renin, although, in the presence of a gross excess of the antagonist or inhibitor, this is not likely to be physiologically important. A third major problem is presented by unwanted properties of the inhibitor. Thus, saralasin has intrinsic agonist actions,¹ and CEI inhibits the degradation of bradykinin which has a vasodepressor action.² A recent study has shown that plasma bradykinin levels nearly triple after the administration of a small intravenous dose of CEI to hypertensive patients.¹⁰ It is theoretically possible, therefore, that CEI might produce a greater fall in blood pressure than saralasin. Another recent paper has demonstrated such a discrepancy, which was attributed to the agonist action of saralasin.¹¹ Therefore it was concluded that CEI is superior as a scientific tool. Of crucial relevance is the significance of the bradykinin-potentiating action of CEI. It has been argued that in hypertension in both man¹⁰ and dogs,⁸ this action is not significant. We have investigated this problem further by comparing the effect of saralasin and CEI in salt-loaded and salt-depleted rats. We also have compared the properties of the two agents by administering CEI to rats in which the renin-angiotensin system had been blocked by saralasin infusion. In addition, we have studied the effect of bilateral nephrectomy on the blood pressure response to this combination of inhibitors.

Methods

White Wistar rats of either sex weighing 150–250 g were used for these studies. Bilateral nephrectomy was performed in some rats through loin incisions under ether light or intraperitoneal pentobarbital (5 mg/100 g body weight) anesthesia 1–24 hours before the infusion study. Other rats were made hypertensive by the application of a silver clip (internal diameter, 0.2 mm) to the left renal artery under ether anesthesia, the contralateral kidney

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being undisturbed (Goldblatt two-kidney hypertensive model). Hypertensive rats were selected on the basis of the blood pressure measured indirectly by light plethysmography. All hypertensive rats were studied within 28 days after renal artery clipping with hypertension of less than 14 days duration.

**Experimental Groups (Table 1)**

*Group 1* received a low salt diet containing sodium, 0.004 mmol/g, and deionized water to drink for 21 days prior to infusion. *Group 2* rats were given normal rat chow containing sodium, 0.166 mmol/g, with 1% saline to drink for 10 days. *Group 3* received the same dietary regime as *Group 1*, but both kidneys were removed 1 hour before study. *Group 4* rats were nephrectomized 24 hours before study and were allowed access to tap water but not food during the postoperative course. *Groups 5–7* consisted of rats with Goldblatt two-kidney hypertension. *Group 5* rats were given normal rat chow and tap water. *Group 6* received low salt diet and deionized water both before and after renal artery clipping. *Group 7* rats were treated as was *Group 5*, but both kidneys were removed 1 hour before the infusion study.

**Infusion Study**

In every case the procedure was carried out under intraperitoneal pentobarbital anesthesia. A tracheostomy was performed before the jugular vein and carotid artery were cannulated. The mean arterial blood pressure was measured with a Statham P23gb transducer connected to a Grass polygraph. When a stable baseline recording was obtained, the pressor response to [Val5]angiotensin II was determined. After the blood pressure returned to the basal level, seven rats each from groups 1 and 2 were given 250 μg of converting enzyme inhibitor (CEI SQ 20881) as a single rapid injection. The dose of CEI was that shown by Engel et al.2 to produce more than 80% inhibition of the pressor response to a test dose of 100 ng of angiotensin I/kg.

Seven rats in groups 1–4 and eight in groups 5 and 6 were given a continuous infusion of saralasin, 10 μg/min. Rats in groups 1 and 7 were infused with saralasin at doses of 1 and 10 μg/min. The maximum stable decrease in blood pressure was obtained after 10–15 minutes, at which time the pressor response to [Val5]angiotensin was determined once more, and then a single rapid injection of CEI (250 μg) was given. Six rats in *Group 1* were given CEI, and saralasin was given when blood pressure had stabilized.

Seven bilaterally nephrectomized rats in *Group 4* were given hog renin (0.2 Goldblatt U.; iv) followed by a continuous saralasin infusion. When a stable blood pressure had been obtained, a single rapid dose of CEI was given. Five rats were infused with bradykinin at rates from 4 to 10 μg/min before, during, and after CEI administration. Significance of results was assessed by unpaired Student’s t-test, whichever was appropriate.

**Results**

**Effect of Converting Enzyme Inhibitor or Angiotensin Antagonist on Blood Pressure**

Infusion of the antagonist produced blockade of the pressor response to a 50-ng test dose of exogenous angiotensin II (i.e., blood pressure rose <2 mm Hg). Pilot studies indicated a flat dose-response curve for angiotensin II after antagonist infusion, with 200-ng doses of angiotensin II producing a pressor response of less than 10 mm Hg.

The CEI produced a significantly greater fall in blood pressure of the low-salt (*Group 1*) rats from 117.2 ± 2.80 to 75.1 ± 5.7 (mean ± SEM) mm Hg compared with an infusion of saralasin (10 μg/min) which reduced the blood pressure from 129.9 ± 4.5 to 104.2 ± 3.6 mm Hg (*P < 0.01*). The fall in blood pressure was 6.0 mm Hg less in five rats infused with a lower dose of saralasin (1 μg/min). However, when these peptides were given to the high-salt rats (*Group 2*), a similar response was observed, with a fall of 120.6 ± 3.37 to 106.1 ± 2.4 and 120.1 ± 8.3 to 107.9 ± 7.3 mm Hg for CEI and antagonist, respectively (*P < 0.05; Fig. 1*).

**Combined Converting Enzyme and Angiotensin II Blockade**

**Normotensive Rats**

Mean blood pressure fell in the three groups of rats with a reduction of 24.8 ± 2.14 mm Hg in *Group 1* (low salt), (*P < 0.01*) 12.10 ± 2.58 mm Hg in *Group 2* (high salt) (*P < 0.01*) and 6.1 ± 8.49 mm Hg in *Group 3* (nephrectomy low salt) (*P > 0.5*) with saralasin infusion.

After CEI was administrated, a further marked fall of 45.7 ± 6.8 mm Hg was observed in *Group 1* (*P < 0.01*), but there was no further change in the blood pressure of *Group 3* (*P > 0.5*). When the sequence of experiments was reversed by infusing CEI before saralasin into salt-depleted rats, saralasin had no significant effect on blood pressure (*Fig. 3*) (*P > 0.5*).

**Hypertensive Rats**

Saralasin infusion caused a marked fall in blood pressure of 47.4 ± 10.9 mm Hg in *Group 5* (hypertensive, normal diet) (*P < 0.01*) and 37.5 ± 6.8 mm Hg in *Group 6* (hypertensive, low-salt diet) (*P < 0.01*). When CEI was...
FIGURE 1 Fall in blood pressure (mean ± SEM) in (a) salt-restricted (a) and salt-loaded rats (b) after the administration of saralasin or converting enzyme inhibitor.

infused, blood pressure fell by 11.1 ± 5.4 mm Hg in the normal-salt hypertensive rats (group 5) (P < 0.05) and by 20.5 ± 9.9 mm Hg in salt-depleted (group 6) rats (P < 0.05). The difference in the response to either agent of the normal and low-salt pretreated rats was not significant (P > 0.1).

One hour after the kidneys were removed (Group 7), the antagonist infusion at a rate of 10 μg/min produced an initial marked pressor response in two rats which, however, was not sustained. Accordingly, the effect of a lower dose (1 μg/min) was studied. This produced a fall of 19.7 ± 6.7 mm Hg, but subsequent injection of CEI had no effect on the blood pressure (Fig. 4). In three rats infused with both doses of saralasin, there was a fall of 17.5 mm Hg during the infusion of 1 μg/min and of 13.6 mm Hg at 10 μg/min.

Bilateral Nephrectomy, Renin, and Bradykinin Infusion

The initial blood pressure of seven bilaterally nephrectomized rats rose from 82.7 ± 9.81 mm Hg to 141.0 ± 7.54 mm Hg after administration of hog renin. Saralasin induced a rapid reduction in the blood pressure to 85.7 ± 9.80 mm Hg (P < 0.001) and, when CEI was given, there was a nonsignificant fall to 81.7 ± 7.02 mm Hg (P > 0.5).

CEI produced a substantial depressor response in bradykinin-infused rats and, likewise, barely vasoactive doses of bradykinin became depressor in CEI-infused rats. Thus three rats infused with bradykinin (4 μg/min) showed a transient acute fall in blood pressure with a sustained although nonsignificant fall from 72.2 ± 2.83 to 65.9 ± 4.62 mm Hg (P < 0.05). CEI was given to these rats during bradykinin infusion and produced a further sustained fall to 43.2 ± 6.93 mm Hg (P < 0.01). In two other rats, bradykinin, 4 μg/min, reduced mean pressure from 63.3 to 60.0 mm Hg. No additional decrease was observed when the rate of infusion was increased to 10 μg/min. Injection of CEI produced no blood pressure fall; however, with a dose of bradykinin of 4 μg/min, blood

FIGURE 2 Mean arterial blood pressure of salt-restricted (a), salt-loaded (b), and nephrectomized salt-restricted (c) rats after saralasin infusion alone or combined with converting enzyme blockade.
FIGURE 3 Change in blood pressure of salt-depleted rats in response to converting enzyme inhibitor and converting enzyme inhibition and saralasin.

pressure fell from 74.7 to 53.8 mm Hg, and a further fall to 46.7 mm Hg occurred with 10 μg/min.

Discussion

The interpretation of data based on inhibition of the renin-angiotensin system is complicated by the additional properties of currently used inhibitors. The present work emphasizes the importance of such properties, since in salt-depleted rats, CEI produces a greater fall in blood pressure than saralasin. Furthermore, when CEI was given to rats in which activity of the renin-angiotensin system had been blocked by saralasin, a further fall in blood pressure could be demonstrated in all those with intact kidneys, although the fall was small in salt-loaded rats. Doses of both agents approximated those used in experimental studies of hypertension in the rat, but although the dose of saralasin was slightly higher, the dose of CEI was the same as that usually employed in man. When saralasin was given to rats pretreated with CEI, no further fall in blood pressure was produced. It seems unlikely, therefore, that the lesser vasodepressor action of saralasin is due solely to its agonist action. Instead, these results point to a vasodepressor property of CEI in addition to its effect on the generation of angiotensin II. It is interesting to observe that the fall in blood pressure could no longer be demonstrated 1 hour after bilateral nephrectomy. This finding is consistent with the recent report that CEI produces no fall in blood pressure in anephric subjects. At the same time the undoubted pressor action of saralasin which also can be observed in hypertensive rats when renin levels are low indicates that this property does have relevance in certain clinical and experimental situations.

The evidence presented here is consistent with the view that the bradykinin-potentiating action of CEI accounts for some, or all, of the vasodepressor effect of the compound in saralasin-infused rats. Thus, infusion of bradykinin into bilaterally nephrectomized rats restored the vasodepressor response to CEI. On the other hand, when such rats were infused with renin and blocked with saralasin, CEI produced no vasodepressor effect. The fall in blood pressure produced by CEI administration to saralasin-infused rats was significantly greater in salt-depleted than in salt-loaded rats. This fact is also consistent with the known properties of the kallikrein-bradykinin system, since plasma bradykinin and urinary kallikrein are significantly elevated by salt depletion. All studies were performed in anesthetized rats; although this does not affect the comparisons between groups, it may account for the small vasodepressor action of saralasin in salt-loaded rats. It is also possible that anesthesia may elevate
plasma bradykinin levels and so enhance the vasodepressor properties of CEI.

The abolition of the vasodepressor action of CEI by bilateral nephrectomy points to a physiological system which has a role in blood pressure maintenance and which is under renal control. The two known systems dependent on the presence of functioning renal tissue, i.e., the renin-angiotensin and fluid volume systems, cannot be invoked to explain the present findings. The most likely hypothesis is that renal formation and secretion of kallikrein are responsible. If so, the kallikrein-bradykinin system may be important in blood pressure control in relation to changes in sodium balance and plasma renin activity, as has been suggested previously.14 We cannot, of course, on the basis of the present experiments, exclude the theoretical possibility that CEI potentiates another renal vasodepressor system or inhibits another renal pressor system. However, such a property of CEI has not yet been demonstrated.

It is of interest that final blood pressure after CEI and saralasin was lower than after either agent alone; indeed, the effects of the two agents appeared to be additive. However, these two groups of experiments were carried out on different occasions so that the degree of salt depletion may not have been identical. Since saralasin produced no further fall in blood pressure in CEI-pre-treated rats, it is difficult to postulate an interaction between the two drugs.

Rats with Goldblatt two-kidney hypertension infused with saralasin showed a depressor response to CEI administration similar to that of salt-loaded normal rats. This is in contrast to the observation of Johnston et al.17 that urinary kallikrein levels are elevated to approximately the same extent in rats with Goldblatt two-kidney hypertension and in salt-depleted normotensive rats, and despite the fact that sodium balance is negative in this model.18 In this respect, therefore, the response to CEI after saralasin blockade is anomalous. As in a previous study, salt restriction did not prevent the rise in blood pressure in this model.18 Salt depletion slightly enhanced the response to CEI after saralasin blockade, but the fall in blood pressure still was substantially less than that of salt-depleted normotensive rats. Just as in the case of normal rats, bilateral nephrectomy of Goldblatt two-kidney hypertensive rats abolished the pressor response to CEI after blockade with saralasin. The findings in the hypertensive rats suggest therefore that the vasodepressor system potentiated by CEI is less active than in normotensive rats.

In addition to a possible role for the renal kallikrein-bradykinin system in blood pressure control, a more practical conclusion can be drawn from the present studies. When a depressor response to CEI is used to assess the role of the renin angiotensin system in hypertension, there is a danger of overestimating such a role.10 Thus, in a recent study, renin was implicated in blood pressure maintenance in 91% of hypertensive patients with "normal" renin levels, using the response to CEI as an indication of such renin involvement. In contrast, with saralasin infusion renin could be implicated in only 10% of these patients.11 In the light of the present work, the observation that CEI infusion produces no fall in blood pressure in patients with low renin levels and in anephric patients14 cannot be interpreted as indicating that CEI modifies blood pressure only by its effect on angiotensin II production.

In certain situations, the bradykinin potentiating property may indeed be insignificant. Thus Miller et al. (1975)15 showed that CEI infusion prevented renal hypertension in the one-kidney Goldblatt dog and, further, that plasma bradykinin levels were below detectable limits in these animals. Even in this case, however, it cannot be assumed that plasma levels of bradykinin reflect concentrations of vasoactive peptide at the vascular receptor site. Therefore, caution should be exercised in interpreting studies based upon CEI infusion only, as a means of inhibiting the renin-angiotensin system.

References

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