Laboratory Studies

Chronic MK421 Fails to Modify Evolution of Hypertension in Neonatally Coarcted Pups

Susan P. Bagby and Eugene F. Fuchs

In inbred dogs with neonatally induced coarctation hypertension, prior serial studies during the first year after aortic banding showed extracellular volume excess with normal plasma renin activity (PRA). The present studies test the hypothesis that slowly evolving aortic constriction in this model will yield intrarenal angiotensin II excess, peripherally undetectable, with continuous slightly positive sodium balance, and thus that chronic blockade of angiotensin II formation will prevent generation of hypertension. Accordingly, we used MK421 (enalapril, 3 mg/kg twice daily), a long-acting angiotensin converting enzyme inhibitor, or placebo, administered orally, from the time of banding through 4 months after banding in sex-matched littermates randomly assigned to one of four groups: coarcted/MK421; control/MK421; coarcted/placebo; control/placebo. Results indicate that MK421 caused identical lowering of absolute forelimb systolic blood pressure in coarcted and control pups but failed to modify evolution of a significant (p<0.005) systolic blood pressure difference in coarcted versus control dogs. Thus, neither temporal course nor final magnitude of relative hypertension was altered by MK421. Efficacy of MK421 was documented by 83% inhibition of the pressor response to angiotensin I at nadir of drug effect and by sustained increases in angiotensin I and renin concentration throughout the period of study. Coarcted and control pups responded similarly to MK421 for all measured variables. Glomerular filtration rate and extracellular volume (measured by [14C]inulin disappearance) did not differ among groups. Thus, chronic administration of MK421 failed to prevent hypertension and did not impair maintenance of normal renal function in the evolving phase of neonatally induced coarctation hypertension. We conclude that, although angiotensin II may participate in the untreated model, it does not appear essential to generation of hypertension. We propose that the renal pressure-natriuresis mechanism regulates distal pressure, that stenosis-related resistance independently determines the proximal-distal difference, and that chronic converting enzyme inhibition lowers the set point of the former without influencing stenosis evolution, thus secondarily lowering proximal pressure by an equal degree. (Hypertension 1989;13:91-101)

Aortic coarctation has been studied as an experimental model of renovascular hypertension¹ and classified with other models wherein all functional renal mass lies distal to a vascular stenosis (e.g., renal artery stenosis present bilaterally or affecting a single kidney).² Acute aortic constriction is followed by transient activation of the renin-angiotensin system as blood pressure rises,³⁻⁵ whereas the phase of established hypertension exhibits normal plasma renin activity (PRA) (see References 1, 6, and 7) coupled with expanded extracellular fluid volume.⁶,⁸

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In neonatally induced coarctation hypertension (NICH) in the inbred Labrador dog, a model designed to mimic clinical coarctation hypertension, thoracic aortic banding that is initially nonconstrictive causes slowly progressive stenosis as normal growth proceeds.¹,⁶,⁷ We have previously shown that evolution of proximal hypertension in NICH during the first year after aortic banding is characterized by persistently normal PRA, normal plasma renin concentration, and normal renal function during both basal and stimulated conditions.⁶,⁷ These "normal" indexes of renin-angiotensin activation in NICH were coupled with 4–5% increases in extracellular fluid volume and plasma volume and were thus inappropriately high relative to the expanded volumes.¹,⁶,⁷ Although this shift of the renin:volume relation satisfied our predictions for the established phase of renin-mediated hypertension, our original hypothesis also held that the volume excess observed
at steady state is generated by an early, transient phase of decreased renal perfusion and increased renin–angiotensin II (Ang II). However, frequent sampling in the NICH model provided evidence neither for an increased basal PRA nor for an exaggerated PRA response to stimuli during the first year after banding. Thus, our prior results could neither support nor refute the thesis that the hypertension and volume excess were Ang II dependent.

To accommodate these new observations, we next proposed that, in contrast to acute coarctation, the slow constriction of the NICH model may cause persistent, low-grade intrarenal renin–Ang II stimulation that, although undetectable in peripheral plasma, mediates the observed volume expansion and generates hypertension. We further reasoned that continuous blockade of Ang II formation throughout evolving aortic constriction should both abolish the hypertension and volume excess observed in the untreated model and induce renal hypoperfusion and exaggerated hyperreninemia. The present study tests this hypothesis in the evolving phase of the NICH model by chronic administration of MK421 (enalapril), a long-acting converting enzyme inhibitor (CEI). The study design, based on random assignment of sex-matched littermates from an inbred dog colony to drug versus placebo groups, introduces CEI before aortic constriction and permits comparison of the difference in blood pressure between the coarcted group versus control group given CEI with the difference between the coarcted group versus control group given placebo.

**Materials and Methods**

**Experimental Subjects**

From the colony of inbred Labrador dogs maintained at Oregon Health Sciences University, four sets of sex-matched litters (one female and three male sets) from separate litters were randomly assigned at birth to primary or alternate status for four experimental groups: 1) coarcted/placebo, 2) control/placebo, 3) coarcted/CEI, and 4) control/CEI. CEI groups received MK421 (enalapril), 3 mg/kg orally every 10-14 hours (6 mg/kg/day) beginning 48 hours after aortic banding; placebo groups received an empty capsule on the same schedule. In the coarcted/placebo and coarcted/CEI groups, nonconstrictive aortic banding was performed at 17 days of age as previously described. One pup randomly assigned to the control/CEI group sustained injury at 2 weeks after aortic banding and was replaced with the littermate originally assigned to alternate control/CEI status; this delayed drug initiation by 2 weeks in the final subject.

**Efficacy of Angiotensin Converting Enzyme Inhibition**

Assessment of the degree of angiotensin converting enzyme (ACE) inhibition at 6 and at 10–12 hours after administration of the CEI during chronic MK421 administration was performed both in preliminary studies and during the present protocol. In conscious pups, intravenous Ang II infusion for 10 minutes (thus allowing the response to plateau), in a dose providing a 30–50 mm Hg pressor response, was followed (after return to baseline) by an equimolar infusion of angiotensin I (Ang I) for a similar period. The ratio of the pressor responses (Ang I: Ang II) was used as an index of ACE inhibition. This method precludes underestimation of ACE inhibition due to enhanced vascular reactivity to Ang II in chronically treated subjects.

MK421 in powder form (generously supplied by Dr. Charles S. Sweet of Merck, Sharp & Dohme Research Laboratories, West Point, Pennsylvania) was weighed into capsules twice weekly; individual doses were based on body weights projected from growth curves. Due to the predictable capriciousness of these puppies, we chose to leave placebo capsules identifiably empty to avoid errors during dosing.

**Protocol**

CEI or placebo was started at 48 hours after aortic banding and continued until study termination at 4 months after banding (Figure 1). Serial measurements at 2–4-week intervals, all in conscious pups, included indirect forelimb systolic blood pressure, pulse rate, plasma renin-angiotensin components, plasma urea nitrogen and creatinine, glomerular filtration rate, and extracellular fluid volume. Body weight was monitored thrice weekly.

**Assay Methods**

**Euvulatory measurements.** Indirect forelimb systolic blood pressure was measured in awake pups by Arteriosonde (model 1020, Kontron Instrs. Inc., Everett, Massachusetts) as previously described.
and validated. We have previously documented excellent agreement between indirect and direct systolic blood pressure in this model; indirect diastolic blood pressure systematically underestimates directly measured values and was not measured. Pulse rate was obtained from the audible Arteriosonde signal after systolic blood pressure was recorded. Amplitude of the femoral systolic pulse wave was subjectively estimated as a crude index of banding efficacy. With pups relaxed in the decubitus position, systolic pulse force was estimated by palpation as 0–4+ as compared with simultaneously assessed noncoarcted controls (designated as 4+). Femoral pulse pressure in coarcted dogs was first detectably diminished between 2 and 2½ weeks after banding and, by 5 weeks, declined to scores ranging from 0 to 2+.

Renin-angiotensin determinations. Free-flowing blood from a forelimb vein was collected in disodium EDTA (1 mg/ml blood), iced, spun at 4°C, and the plasma frozen at −20°C until incubation and assay. Plasma was pretreated by addition of diisopropyl fluorophosphate (DFP, to 0.01 M) and citrate (0.38 M, to pH 6) by previously validated methods. After further processing, as described below for each component, Ang I was measured by radioimmunoassay; the assay sensitivity is 0.6 pg Ang I/ml of original sample with recent interassay and intra-assay coefficients of variation of 16.9±10.5% and 9.4±7.9% (n=21), respectively. Unincubated pretreated plasma was assayed for endogenous Ang I concentration (ng Ang I/ml). Plasma for PRA was incubated at 37°C for 30 minutes; results are corrected for endogenous Ang I levels (ng Ang I/ml/hr). Renin substrate concentration was measured indirectly by addition of excess dog renal renin (5 μl, 8 Goldblatt Units [GU]/ml) to 195 μl plasma and incubation for 1 hour at 37°C.CA Because of prior studies in this13 and other laboratories14,15 showing decreased renin substrate during CEI, and because of our prior finding of a linear dependence of renin reaction rate on renin substrate, we also measured plasma renin concentration by a method independent of renin substrate: 10 μl pretreated experimental plasma was added to 190 μl substrate-rich (1,186 ng Ang I/ml), Ang I−, nephrectomized-dog plasma pool (also pretreated) and incubated for 1 hour at 37°C; results were referenced to a three-point renin standard curve (hog renal renin, Medical Research Council), corrected for dilution, and expressed as GU×10−4/ml. Under these conditions, the lowest detectable renin concentration is 10 GU×10−4/ml of experimental plasma. (Nondetectable levels were considered zero for analysis.)

Blood chemistries. Plasma for measurement of urea nitrogen, creatinine, sodium, and potassium levels was collected into heparinized tubes and measurements were made by a Cobas Bio autoanalyzer (Roche Diagnostics, Nutley, New Jersey) and flame photometer (IL 943, Fisher, Kent, Washington), respectively.

### Results

**Efficacy of Angiotensin Converting Enzyme Inhibition**

In six infusions in six dogs given placebo, Ang I (0.24±0.08 μg/kg/min) increased forelimb systolic blood pressure by 30±10 mm Hg versus 33±12 mm Hg during an equimolar Ang II infusion (Ang I vs. Ang II, p=NS), a pressor ratio of 0.92 (Figure 2). In 13 infusions in eight dogs during chronic treatment with MK421 (3 mg/kg/10–14 hr) performed at 10.6±1.5 hours after MK421 dose, Ang I infusion (0.31±0.15 μg/kg/min) increased forelimb systolic blood pressure by 7±5 mm Hg versus 45±11 mm Hg during an equimolar Ang II infusion (Ang I: Ang II pressor ratio was 0.156). Relative to dogs given placebo, the pressor ratio in CEI-treated dogs represents an 83% inhibition of Ang I pressor response at the nadir of drug action. Two infusions at 6 hours after administration of CEI showed 100% ACE inhibition.

**Hemodynamic Indexes**

Forelimb systolic blood pressure is presented graphically in Figure 3 and summarized with statistical details in Table 1. Coarcted dogs exhibited significantly higher systolic blood pressure and a greater rate of rise than control dogs (band×time interaction, p<0.001); CEI lowered systolic blood...
pressure significantly in both coarcted and control groups. Importantly, there was no significant difference between the responses of coarcted and control dogs to CEI. Thus, the difference in systolic blood pressure between coarcted and control dogs on CEI was identical to the pressure difference between coarcted and control dogs on placebo. Heart rate (Table 1) was not significantly different among the four groups.

Renin-Angiotensin Indexes

Results are summarized with statistical details in Table 1 and presented graphically in Figures 4–7. Adjusted PRA (adjusted for endogenous plasma Ang I level), plasma Ang I, and plasma renin concentration were each significantly increased by CEI throughout the period of study. Coarcted and control dogs did not differ in their responses to CEI. Renin substrate concentration was significantly and persistently decreased by CEI; again, coarcted and control dogs responded similarly.

Renal Function

Results are summarized with statistical data in Table 2; glomerular filtration rate results are presented graphically in Figure 8. Glomerular filtration rate, adjusted for body weight within the analysis of variance, was comparable among the four experimental groups (Figure 8). Similarly, plasma creatinine and urea nitrogen did not differ among the groups (Table 2). Significant time-dependent change was observed for all three variables: a decrease for glomerular filtration rate (Figure 8) and increases for creatinine and urea nitrogen (not shown).

Extracellular Volume

Results are summarized in Table 2. Extracellular volume, adjusted for body weight within the analysis of variance, did not differ significantly among the four groups. There was significant time-dependent decrease (not shown).

Discussion

Chronically administered CEI failed to modify either temporal evolution or magnitude of systolic blood pressure excess in coarcted dogs, yet it lowered absolute systolic blood pressure similarly in coarcted and control groups. The interpretation of these observations must at least address the possibility that Ang II is not required for generation of NICH.

Was Angiotensin II Availability Impaired During Evolution of Hypertension?

The issue most critical to valid interpretation is whether chronic administration of MK421 signifi-

![Figure 2](image)

**FIGURE 2.** Bar graph showing efficacy of angiotensin converting enzyme (ACE) inhibition at 10 hours after administration of enalapril (3 mg/kg twice daily). Pressor effect of infused angiotensin I (AI) was compared with that of an equimolar angiotensin II (AII) infusion; the pressor ratio served as an index of ACE inhibition. Measured at nadir of drug effect, treatment with converting enzyme inhibitor (CEI) (enalapril 3 mg/kg twice daily) provided 83% inhibition of the angiotensin I pressor effect that was observed in group receiving placebo.

![Figure 3](image)

**FIGURE 3.** Line graph showing forelimb systolic blood pressure. Values (mean±SD) are referenced to days after aortic banding. Statistical details are shown in Table 1. CEI, converting enzyme inhibitor (enalapril, 3 mg/kg twice daily); BP, blood pressure.
TABLE 1. Summary of Serial Hemodynamic and Renin-Angiotensin Indexes During Chronic Treatment With MK421 (Enalapril) or Placebo in Neonatally Induced Coarctation Hypertension

<table>
<thead>
<tr>
<th>Study group</th>
<th>Forelimb systolic BP (mm Hg)</th>
<th>Heart rate (beats/min)</th>
<th>Adjusted PRA (ng Ang I/ml/hr)</th>
<th>Plasma Ang I (ng Ang I/ml)</th>
<th>Plasma RC (GU x 10^-6/ml)</th>
<th>Plasma RS (ng Ang I/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CEI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coarcted</td>
<td>129±21</td>
<td>187±20</td>
<td>32±11</td>
<td>6.5±3.7</td>
<td>160±467</td>
<td>342±227</td>
</tr>
<tr>
<td></td>
<td>(60)</td>
<td>(60)</td>
<td>(36)</td>
<td>(36)</td>
<td>(36)</td>
<td>(36)</td>
</tr>
<tr>
<td>Control</td>
<td>110±14</td>
<td>183±22</td>
<td>41±28</td>
<td>6.3±3.3</td>
<td>77±95</td>
<td>359±178</td>
</tr>
<tr>
<td></td>
<td>(60)</td>
<td>(60)</td>
<td>(36)</td>
<td>(36)</td>
<td>(36)</td>
<td>(36)</td>
</tr>
<tr>
<td><strong>Placebo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coarcted</td>
<td>146±20</td>
<td>189±22</td>
<td>6±4</td>
<td>1.0±0.6</td>
<td>10±9</td>
<td>737±180</td>
</tr>
<tr>
<td></td>
<td>(60)</td>
<td>(60)</td>
<td>(36)</td>
<td>(36)</td>
<td>(36)</td>
<td>(36)</td>
</tr>
<tr>
<td>Control</td>
<td>126±15</td>
<td>187±28</td>
<td>4±4</td>
<td>0.8±0.9</td>
<td>7±9</td>
<td>736±187</td>
</tr>
<tr>
<td></td>
<td>(60)</td>
<td>(60)</td>
<td>(36)</td>
<td>(36)</td>
<td>(36)</td>
<td>(36)</td>
</tr>
</tbody>
</table>

Values are mean±SD averaged across time. Numbers in parentheses are total number of observations for the four dogs of each group. BP, blood pressure; PRA, plasma renin activity adjusted for angiotensin I; Ang I, angiotensin I; RC, renin concentration; GU, Goldblatt Units; RS, renin substrate; CEI, angiotensin converting enzyme inhibitor (enalapril [MK421] 3 mg/kg twice daily); NS, not significant.

*See text for significant interaction between main effects.

significantly impaired effective Ang II availability. This can be addressed from four perspectives: 1) completeness of ACE blockade in vascular and tissue sites; 2) response patterns of renin-angiotensin components known to be sensitive to decreased Ang II; 3) change in plasma and tissue levels of Ang II; and 4) alterations in vascular and renal reactivity to Ang II.

First, we documented efficacy of ACE inhibition within the vascular space by demonstrating nearly complete blockade of the Ang I pressor response at the nadir of the MK421 effect (Figure 2). Further, comparison of Ang I pressor response with an equimolar Ang II pressor effect precluded potential overestimation of Ang I-to-Ang II conversion as a result of increased vascular reactivity to Ang II during chronic treatment with CEI. Published studies of ACE activity in tissue during chronic administration of CEI indicate more complete and prolonged inhibition than that observed for intravascular ACE via Ang I infusion. Further, we used high doses of enalapril (6 mg/kg/day) as compared with an estimated maximum of 0.4 mg/kg/day in human studies. It is therefore likely that, in the present study, inhibition of tissue ACE (including renal) was even more profound than that demonstrated for intravascular ACE.

Secondly, in the present study, responses of renin-angiotensin components during treatment with CEI, monitored as biologic indicators, provide strong indirect support for a sustained lowering of Ang II. Thus, persistent increases in adjusted PRA, renin concentration, and Ang I during CEI treatment, each sustained for the full period of study, exclude tachyphylaxis to MK421 and suggest continuing lack of Ang II direct negative feedback on juxtaglomerular cell renin secretion. The sustained lowering of renin substrate concentration may reflect lack of Ang II positive feedback on hepatic or extrahepatic renin substrate synthesis; however, it more likely indicates an increased renin-substrate utiliza-
tension rate secondary to the high renin concentration, since protracted sodium restriction can also lower renin substrate despite increased Ang II level.24) Numerous other studies have similarly documented lack of tachyphylaxis with chronic use of enalapril.25–28 However, it should also be noted that no studies have yet excluded a direct pharmacological effect of CEI to stimulate renin secretion independently of Ang II availability. Thus, responses of renin-angiotensin components cannot be taken as definitive evidence of lowered Ang II availability.

Although appropriate samples were collected, we chose not to attempt measurement of plasma Ang II in the present studies, a decision based on well-documented methodological problems. Recently, Nussberger et al21,29 thoroughly dissected these complexities in man; results indicate that failure of some prior studies27 to demonstrate decline of plasma Ang II during enalapril administration likely derives from 1) substances that cross-react with anti-Ang II antibody (Ang I plus metabolites of Ang I and Ang II), and 2) in vitro generation of Ang II in plasma samples despite presence of inhibitors and cooling of samples.21 These observations further suggest that, during chronic enalapril administration with high circulating levels of Ang I, elimination of in vitro Ang II generation may require high levels of CEI or immediate extraction of samples.21 Nonetheless, even without this precaution, the same investigators were able to demonstrate disappearance of Ang II from plasma at the peak of enalapril action during acute studies, as well as a 58% maximum decrease during chronic treatment.21 In support of these findings, Rasmussen et al14 observed a
markedly decreased Ang II level during enalapril treatment in immediately extracted plasma samples. Also, the dose of enalapril used in the present study is over 10-fold greater than that used by Nussberger et al.\textsuperscript{21} (6 mg/kg/day vs. estimate of 0.4 mg/kg/day). It therefore seems probable that we achieved a substantial decrease in plasma Ang II level in pups treated with CEI for a major fraction of each day.

Finally, enhanced vascular reactivity to Ang II, via up-regulation of Ang II receptors, could offset effects of lowered plasma Ang II levels in CEI-treated groups.\textsuperscript{12} Our protocol did not specifically address this issue. Imai et al.\textsuperscript{12} have described increased vascular reactivity during chronic ACE inhibition with captopril. The persistent hypotensive effect in CEI-treated pups could be considered supportive of a persistent lowering of effective Ang II availability if non-Ang II mechanisms could be excluded. In fact, Given et al.\textsuperscript{30} found no change in catecholamines, norepinephrine, bradykinin, or prostaglandin levels after enalapril administration in normal subjects. Similarly, Millar et al.\textsuperscript{31} and Stroh et al.\textsuperscript{32} found no effects of enalapril on autonomic indexes. While a decreased vascular reactivity to norepinephrine has been reported during captopril treatment, this likely reflects loss of Ang II facilitation of sympathetic neural function\textsuperscript{33} rather than a non-Ang II-related action of CEI. Nevertheless, depressor mechanisms unrelated to Ang II cannot be fully excluded in the present series. However, based on sustained responses of both blood pressure and renin-angiotensin components, it seems reasonable to conclude that any change in vascular reactivity to Ang II was modulating rather than eliminating effective decrease in Ang II availability.

From these pharmacological and biological considerations, it seems highly probable that effective Ang II availability in pups given CEI was substantially lowered over the course of evolving hypertension.

### Table 2. Summary of Serial Renal Functional and Volume Indexes During Chronic Treatment With MK421 (Enalapril) or Placebo in Neonatally Induced Coarctation Hypertension

<table>
<thead>
<tr>
<th>Study group</th>
<th>GFR (cc/min)</th>
<th>Creatinine (mg%)</th>
<th>BUN (mg%)</th>
<th>ECV (cc)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CEI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coarcted</td>
<td>65±10</td>
<td>0.5±0.1</td>
<td>18±5</td>
<td>4,610±496</td>
</tr>
<tr>
<td></td>
<td>(16)</td>
<td>(32)</td>
<td>(32)</td>
<td>(16)</td>
</tr>
<tr>
<td>Control</td>
<td>70±12</td>
<td>0.5±0.1</td>
<td>19±5</td>
<td>4,829±314</td>
</tr>
<tr>
<td></td>
<td>(16)</td>
<td>(32)</td>
<td>(32)</td>
<td>(16)</td>
</tr>
<tr>
<td><strong>Placebo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coarcted</td>
<td>67±10</td>
<td>0.5±0.1</td>
<td>18±5</td>
<td>4,540±566</td>
</tr>
<tr>
<td></td>
<td>(16)</td>
<td>(32)</td>
<td>(32)</td>
<td>(16)</td>
</tr>
<tr>
<td>Control</td>
<td>72±16</td>
<td>0.4±0.1</td>
<td>17±5</td>
<td>4,928±517</td>
</tr>
<tr>
<td></td>
<td>(16)</td>
<td>(32)</td>
<td>(32)</td>
<td>(16)</td>
</tr>
<tr>
<td><strong>Significance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coarcted</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Drug</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Time</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.002</td>
</tr>
</tbody>
</table>

Values are mean±SD averaged across time. Numbers in parentheses are total number of observations for the four dogs of each group. Glomerular filtration rate (GFR) and extracellular fluid volume (ECV) are adjusted for body weight within the analysis of covariance. BUN, plasma urea nitrogen; CEI, angiotensin converting enzyme inhibitor (enalapril [MK421] 3 mg/kg twice daily); NS, not significant.

*See text for significant interaction between main effects.
If One Accepts Impaired Angiotensin II Availability, Could Angiotensin II Nonetheless Account for Generation of Systolic Blood Pressure Excess?

It can be assumed that, despite effective ACE inhibition, measurable Ang II is present. It could be argued that, despite impaired availability of Ang II, the cumulative nature of direct and indirect effects of Ang II on renal sodium excretion could eventually yield the observed pressure excess. However, the temporal development of forelimb systolic blood pressure excess in response to aortic banding was clearly unmodified by chronic ACE inhibition. The results thus seem more compatible with the view that factors other than (or in addition to) Ang II contribute significantly to the evolution of pressure excess in neonatally induced coarctation during ACE inhibition.

Implications for Neonatally Induced Coarctation Hypertension

Based on averages across time, the systolic blood pressure excess of 19 mm Hg in coarcted over control dogs given CEI was identical to the 20 mm Hg excess of coarcted over control dogs given placebo. For the reasons outlined, these findings introduce the possibility that Ang II is not required for development of the systolic blood pressure difference between coarcted and control dogs. It should be emphasized that these results do not speak for or against the participation of Ang II in the untreated model: blockade of Ang II could induce recruitment of alternative pressor mechanisms not operative in the absence of ACE blockade. However, results do suggest that Ang II may not be essential for genesis of systolic blood pressure excess in the NICH model. Similarly, the maintenance of normal renal function in coarcted dogs during chronic treatment with CEI, supported directly by demonstration of normal glomerular filtration rate and indirectly by normal plasma creatinine and urea nitrogen, provides further evidence for the operation of Ang II-independent mechanisms capable of protecting the renal circulation and maintaining intrarenal hemodynamics. Numerous studies document the capacity of chronic enalapril treatment to induce sustained decrease in glomerular filtration rate in states of renal circulatory compromise.

Distal blood pressure, which is not accessible to indirect measurement in coarcted dogs once pulse pressures fall, was not measured in the present series. Based on prior results in this model, we can infer that distal mean blood pressure in placebo-treated coarcted dogs was equal to that in placebo-treated controls. Also from prior studies, acute and chronic manipulations of blood pressure have yielded similar absolute blood pressure changes in proximal and distal sites (e.g., Ang II or norepinephrine infusion [S. Bagby, unpublished observations]). We thus consider it likely that distal blood pressure in CEI-treated coarcted dogs is comparable to blood pressure in CEI-treated controls. In view of the sensitivity of glomerular filtration rate to renal circulatory compromise in the presence of enalapril, the normal glomerular filtration rate observed in CEI-treated coarcted dogs makes it further unlikely that distal blood pressure was lower than in CEI-treated controls.

Our confidence in the extracellular fluid volume findings in the present study is limited. Our prior studies, using sodium-24 space of distribution, documented small but statistically significant and physiologically relevant extracellular fluid volume excess in coarcted dogs in a larger series spanning 2–12 months after banding; similar trends (not always significant) were apparent in smaller series in older dogs. The present results using [14C]inulin fail to show extracellular fluid volume excess. This could reflect differing spaces measured by the two methods, insufficient numbers of subjects (Type II error), or milder aortic stenosis due to the younger age of pups reported here. However, experience with both methods also suggests that the [14C]inulin assay is less sensitive: [14C]inulin level is actively declining during the sampling period and thus small errors in plasma radioactivity translate to large changes in the extrapolated intercept used for extracellular fluid volume calculation; in contrast, sodium-24 levels are stable during the period of sampling.
and permit replicate sampling for more accurate estimation of extracellular fluid volume. (Note that this concern does not apply to glomerular filtration rate calculation, since the formula incorporates a half-life term that offsets the intercept variation.) Since subsequent observations have shown that the $[^3H]$mulin method is capable of detecting decrease in extracellular fluid volume in these pups after sodium restriction, we conclude that the present results can reasonably exclude large extracellular fluid volume changes in response to chronic CEI treatment but cannot meaningfully address the subtle extracellular fluid volume excess previously shown in coarcted dogs nor whether this feature is abolished by chronic CEI treatment.

**If Angiotensin II Is Not Essential for Generation of Coarctation Hypertension, What Non-Angiotensin II Mechanisms Are Additionally Operative?**

Early theories of coarctation hypertension included the view that the mechanical resistance of the stenotic site contributed significantly to the increased vascular resistance. This concept was inappropriately discarded on the basis of results of infrarenal coarctation; thus, the absence of proximal hypertension after infrarenal aortic stenosis was seen as excluding a role for the stenotic resistance factor. In fact, this view failed to account for a potentially active antihypertensive role of the kidneys in offsetting the pressor effect of the mechanical resistance. Recently, Anderson and colleagues have reestablished the validity of the "mechanical" theory in the one-kidney, one clip model (1K/1C) of hypertension in the dog, documenting that the stenotic vascular resistance accounts for up to 25% of the total vascular resistance increase. Young et al and Korner have further clarified the importance of distal vascular resistance and distal tissue flow demand as determinants of the functional severity of arterial stenoses for a given degree of anatomic narrowing. It seems likely that the mechanical resistance in the thoracic coarctation model plays an even greater role given the larger number of vascular beds located distal to the vascular stenosis (c.f., the 1K/1C model).

To exclude an essential role for Ang II does not necessarily diminish the importance of a renal factor. Careful review of prior studies on the etiology of acute coarctation hypertension provides convincing evidence that kidney mass present distal to the vascular stenosis is essential to development of proximal hypertension and, importantly, also to correction of distal hypotension. Thus, the work of Scott and coworkers in the 1950s demonstrated resolution of proximal hypertension and recurrence of distal hypotension after unilateral nephrectomy and transplantation of the second kidney to a site proximal to the coarctation; conversely, unilateral nephrectomy with transplantation of the second kidney to a distal vascular site allowed persistence of proximal hypertension and distal normotension. These findings support operation of an unspecified renal factor and also suggest that renal nerves are not critical to its operation.

Hall et al have recently reviewed evidence supporting the pivotal role of the renal pressure-natriuresis mechanism in the control of arterial blood pressure as it functions in the service of sodium balance. In the case of aortic coarctation, the pressure-natriuresis mechanism operates to control the distal (renally perceived) circulation, and is no longer directly sensitive to the proximal vasculature. The renal factor of Scott and coworkers may in fact represent the pressure-natriuresis phenomenon.

We propose that two relatively independent factors interact to determine proximal pressure in coarctation hypertension: 1) the renal pressure-natriuresis mechanism, operating to restore and sustain a normal pressure in the vascular segment perceived by the kidney (i.e., the distal vasculature) and modulated by neural and humoral factors that influence sodium excretion capacity; and 2) a set of mechanical factors related to functional severity of the stenosis (stenosis area/length and distal tissue blood flow demand/distal resistance), which independently determine the relation of proximal to distal pressure. If so, then the explanation for vascular mediately mediated renal hypertension is simply the introduction of a mechanical resistance between heart and kidney, wherein an intact renal pressure-natriuresis mechanism operates to normalize blood pressure within the distal vascular segment and secondarily obligates proximal pressure rise as a function of stenosis severity.

According to this view, the absolute pressure of the distal vasculature can be varied by changing one or more modulators of renal sodium excretion capacity (Ang II, sympathetic neural, atrial natriuretic factor, etc.) to alter the set point of the renal pressure-natriuresis mechanism without necessarily changing the difference in pressure between proximal and distal vascular segments.

The model proposed is compatible with our finding that CEI lowered absolute systolic blood pressure identically in coarcted and control dogs. Ang II is but one factor modulating the renal pressure-natriuresis mechanism; subtraction of its net pressor effect is evident in sustained lowering of systolic blood pressure in normal pups given CEI. The quantitatively similar lowering of proximal systolic blood pressure in coarcted pups given CEI, assuming the absence of major non-Ang II depressor mechanisms operative during enalapril treatment, is compatible with the postulate that CEI failed to alter stenosis-dependent factors, instead causing a primary fall in distal (renally regulated) pressure and a secondary equal fall in proximal pressure with no effect on the evolving pressure gradient.

The similar systolic blood pressure response to CEI in coarcted and control dogs further suggests that Ang II accounts for a distinct and quantita-
tively normal component of steady-state systolic blood pressure in coarcted dogs. This is in agreement with our prior finding of normal depressor responses to acute CEI (SQ20881) administration in evolving neonatally induced coarctation. The normal renal functional and renin-angiotensin responses of coarcted pups to chronic CEI treatment further indicate that additional nonvasoconstrictive actions of Ang II acting chronically on renal function and sodium excretion in NICHI are also normal. This also is in accord with earlier studies documenting normal renal functional, renin-angiotensin, and extracellular fluid volume responses to low sodium diet, and normal renin-angiotensin responses to acute CEI administration, during the first year after aortic banding.

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References


**KEY WORDS** • enalapril • angiotensinogen • angiotensin I • glomerular filtration rate • converting enzyme inhibitors • natriuresis • plasma renin activity • coarctation • renal pressure-natriuresis
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