Abnormalities of Renal Perfusion and the Renal Pressor System in Dogs with Chronic Aortic Coarctation

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ABSTRACT
To clarify the role of the renin-angiotensin system in coarctation hypertension, 2-year-old inbred dogs with chronic neonatally induced thoracic aortic coarctation were subjected to 6 days of rigorous salt restriction. The following parameters were then measured: glomerular filtration rate, renal plasma flow, plasma renin activity, plasma renin concentration, renin reactivity, and renin substrate concentration. Glomerular filtration rate and renal plasma flow were significantly lower in salt-restricted coarcted dogs: 3.0 ± 0.2 and 9.0 ± 1.5 ml/min kg⁻¹, respectively, compared with values of 4.0 ± 0.2 (P < 0.005) and 13.2 ± 0.9 (P < 0.025) ml/min kg⁻¹ in salt-restricted controls. Plasma renin activity was abnormally high in experimental dogs: 13.5 ± 2.5 vs. 4.5 ± 1.5 ng angiotensin I/ml hour⁻¹ in controls (P < 0.005). In addition, a significant elevation of renin reactivity (indicating a relative increase in circulating accelerators or a relative decrease in inhibitors of the renin reaction) was apparent in the plasma of coarcted dogs. Plasma renin concentration was elevated but to an insignificant degree in coarcted dogs, and renin substrate concentration was comparable with that of controls. The impaired renal perfusion and abnormal elevation of plasma renin activity during salt restriction is analogous to clinical and experimental observations in hypertensive states associated with total renal underperfusion and supports a major role for the renal pressor system in the pathogenesis of coarctation hypertension. The insignificant elevation of plasma renin concentration is not incompatible with this view. The demonstration of increased renin reactivity in coarctation hypertension provides additional evidence that acceleration of the renin reaction is common to all hypertensive states.

Despite extensive investigation, the pathogenesis of hypertension in aortic coarctation has not been clearly defined. The participation of a renal factor was documented in early studies (1, 2), but subsequent attempts to demonstrate decreased renal perfusion (3–6) or increased plasma renin activity (7–9) have been generally unsuccessful. Importantly, these studies were performed during unrestricted sodium intake.

Recent reports suggest that, in hypertensive states characterized by reduced blood supply to the entire kidney mass (total renal underperfusion), depletion of extracellular volume may be necessary to unmask an abnormality in peripheral plasma renin activity (10, 11). Although aortic coarctation is potentially a state of total renal underperfusion, there has been no systematic application of sodium depletion in this setting. Accordingly, our first objective was to determine the response of the renin-angiotensin system to sodium restriction in dogs with neonatally induced chronic aortic coarctation.

Although the conventional measurement of plasma renin activity is often assumed to reflect only plasma renin concentration, other components of the renin reaction may independently influence this value (12). These components include renin substrate concentration and "renin reactivity," a measure of inhibitors or accelerators of the renin reaction. Accelerating factors (or the lack of inhibitors) producing an increase in renin reactivity have been observed in a variety of primary and secondary hypertensive states (12–16) and thus far appear to be common to all forms of hypertension studied. Our second objective was to examine these components of the renin reaction in coarctation hypertension, to assess their contribution to the conventional plasma renin activity, and to seek further insight into the pathophysiological significance of the in vitro renin reactivity measurement in hypertensive states.

Methods
Ten inbred Labrador puppies underwent thoracic aortic banding in the first week of life; the surgical
technique has been detailed elsewhere (Bonchek et al.: Normal baroreceptor sensitivity and enlargement of the carotid sinus in experimental coarctation of the aorta, submitted for publication). At 2 years of age, coarcted dogs and ten age-matched controls from the same colony, all on a liberal sodium intake, underwent placement of carotid and femoral arterial cannulas under anesthesia. On the following day, carotid and femoral blood pressures were recorded in the conscious dogs. Mean blood pressures were calculated as the diastolic pressure plus one-third of the pulse pressure. Coarcted dogs and eight additional controls were then subjected to 6 days of salt restriction (mean sodium intake 0.07 mEq/kg day\(^{-1}\)). On the fifth day, peripheral venous blood was obtained (in ethylenediaminetetraacetic acid [EDTA]) from conscious dogs, and plasma was frozen at \(-20^\circ\)C until it was assayed for renin-angiotensin parameters. A 24-hour urine specimen was also collected on the fifth day for measurement of sodium excretion. On the sixth day, the dogs were anesthetized for measurement of insulin and para-aminodhippurate (PAH) clearances by previously described techniques (17).

Renin Purification.—Renin from mongrel dog kidneys was extracted according to the method of Lucas et al. (18) and standardized in our laboratory: 10 \(\mu\)l of a 1:10,000 dilution in 100 \(\mu\)l of normal dog plasma generated roughly 5 ng angiotensin I (AI)/ml plasma hour\(^{-1}\); this concentration was arbitrarily designated 1 \(\times\) 10\(^{-4}\) units/ml. When excess renin (0.25 units/ml) was added to 50 \(\mu\)l of plasma, total AI generation was complete by 40 minutes, and AI levels remained constant when the incubation was extended to 2 hours. Thus, angiotensinase activity was undetectable even at the highest renin concentrations used.

Plasma Renin Activity.—Plasma pretreated with EDTA, diisopropylfluorophosphate, and citric acid (pH 5.5) was incubated for 1 hour at 37°C. Plasma renin activity was measured in triplicate samples by radioimmunoassay of AI using the method of Cohen et al. (19). The AI standard utilized in the assay was obtained from the Medical Research Council (Research Standard A, code 71/328). The results are expressed as ng AI/ml plasma hour\(^{-1}\).

Renin Substrate.—To 50 \(\mu\)l of plasma (pretreated like the plasma used to measure plasma renin activity) was added 20 \(\mu\)l of renin (final concentration 0.25 units/ml). Incubations for 40, 60, 90, and 120 minutes yielded similar concentrations of substrate to AI under these conditions. The results are expressed as ng AI/ml plasma.

Renin Reactivity.—Renin reactivity is defined as the increment in renin reaction velocity produced in a given plasma sample by the addition of a 1 \(\times\) 10\(^{-4}\) units/ml of renin. This measurement reflects relative inhibitor or accelerator activity in plasma samples containing equal concentrations of substrate (12). The method is illustrated in Figure 1. Three 100-\(\mu\)l aliquots of pretreated plasma containing 0, 1 \(\times\) 10\(^{-4}\) units/ml, and 2 \(\times\) 10\(^{-4}\) units/ml of exogenous dog renin, respectively (added in a 10-\(\mu\)l volume), were incubated for 1 hour at 37°C. The velocity of AI generation (ng/ml plasma hour\(^{-1}\)) in tube A (\(V_0\), no added renin) represents plasma renin activity. The velocity in tube B (\(V_0 + \text{PRA}\)) reflects the effect of endogenous renin plus added renin. Subtraction of the endogenous plasma renin activity value, \(V_A\), from \(V_0\) yields the velocity of AI generation in response to 1 \(\times\) 10\(^{-4}\) units/ml of added renin—by definition, the renin reactivity. Tube C (concentration of added renin = 2 \(\times\) 10\(^{-4}\) units/ml) was included to ensure that the renin reactivity was linear with respect to added renin, i.e., that substrate concentration during the observed period of reaction did not decrease sufficiently to alter the reaction velocity. Renin reactivity values, expressed as ng AI/ml plasma hour\(^{-1}\) (1 \(\times\) 10\(^{-4}\) units/ml of added renin)\(^{-1}\), were derived from linear regression analysis. The slope of the line in Figure 1 corresponds to the renin reactivity.

Although renin reactivity is unaffected by variations in endogenous renin levels (12, 19), variations in renin substrate will influence the observed reaction rate. However, if renin substrate concentrations are similar in the plasma samples to be compared, differences in renin reactivity then reflect differences in the relative activity of accelerators or inhibitors.

Plasma Renin Concentration.—Determinations were made according to a modification of the method of Haas et al. (19). An external renin standard eliminated the influence of variations in renin substrate and unidentified plasma modifiers. By defining the AI-generating potential of a known concentration of exogenous renin in a given plasma sample (via the renin reactivity (RR) measurement) and by assuming that exogenous and endogenous renin molecules behave comparably, one can then extrapolate the calculated regression line to the horizontal axis in Figure 1 to derive the negative value for endogenous plasma renin concentration (PRC), i.e., PRC = PRA/RR (units \(\times\) 10\(^{-4}\)/ml). See Methods for explanation.
TABLE 1

Renal Hemodynamics and Renin-Angiotensin Parameters on Days 5 and 6 of Salt Restriction

<table>
<thead>
<tr>
<th>Group</th>
<th>CIN* (ml/min kg⁻¹)</th>
<th>CPAH* (ml/min kg⁻¹)</th>
<th>PRA (ng AI/ml hour⁻¹)</th>
<th>RR (ng AI/ml hour⁻¹ [x 10⁻⁴ units renin/ml⁻¹])</th>
<th>PRC (units x 10⁻⁵/ml)</th>
<th>RS (ng AI/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coarcted</td>
<td>3.0 ± 0.2</td>
<td>9.0 ± 1.5</td>
<td>13.5 ± 2.5</td>
<td>3.12 ± 0.28</td>
<td>4.7 ± 1.0</td>
<td>824 ± 60</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(10)</td>
<td>(10)</td>
<td>(6)</td>
<td>(6)</td>
<td>(6)</td>
</tr>
<tr>
<td>Control</td>
<td>4.0 ± 0.2</td>
<td>13.2 ± 0.9</td>
<td>4.5 ± 1.5</td>
<td>2.21 ± 0.41</td>
<td>2.3 ± 0.3</td>
<td>727 ± 55</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(5)</td>
<td>(6)</td>
<td>&lt; 0.005</td>
<td>(6)</td>
<td>(8)</td>
</tr>
</tbody>
</table>

All values are means ± se; number of dogs tested is given in parentheses. CIN = inulin clearance, CPAH = PAH clearance, PRA = plasma renin activity, RR = renin reactivity, PRC = plasma renin concentration, RS = renin substrate concentration, AI = angiotensin I, and NS = not significant.

* Measured under anesthesia.

Results

There were no recognized instances of cardiac decompensation or limb paralysis in the coarcted dogs, and these dogs appeared to be as healthy as the normal control dogs.

All data were obtained approximately 2 years after neonatal aortic banding; hypertension was presumably well-established. Blood pressures and body weights were measured on an unrestricted sodium intake; all other data were collected during salt restriction. Values are means ± se. On an unrestricted sodium intake, the mean carotid arterial blood pressure in the coarcted dogs averaged 128 ± 8 mm Hg, significantly higher than the value of 97 ± 5 mm Hg measured at the same site in control dogs. Mean femoral arterial blood pressure averaged 83 ± 9 mm Hg in the coarcted dogs. Femoral blood pressures in the control dogs were not measured, but the average mean femoral arterial blood pressure in the experimental dogs was not significantly different from the average carotid mean blood pressure in the control dogs. Mean pressure gradients across the coarcted segment ranged from 19 to 105 mm Hg with an average of 45 ± 8 mm Hg. The average body weight on the unrestricted sodium intake was greater in the coarcted dogs, averaging 22.7 ± 1.1 kg compared with 18.5 ± 0.6 kg for the age-matched controls (P < 0.025).

In 13 dogs, salt balance was achieved by the fifth day of sodium restriction. Urinary sodium excretion on day 5 was similar in both the control and the experimental group: 2.0 ± 0.6 and 2.8 ± 1.0 mEq/24 hours, respectively.

Results of inulin clearances (CIN) and PAH clearances (CPAH), obtained under anesthesia on day 6 of salt restriction, are shown in Table 1. Both CIN and CPAH in the coarcted group were significantly lower than they were in the control dogs. The calculated filtration fractions of 0.43 ± 0.07 in coarcted dogs and 0.31 ± 0.02 in controls were not significantly different.

Values for renin-angiotensin parameters obtained in awake dogs on day 5 of salt restriction, are also shown in Table 1. Mean plasma renin activity in the coarcted dogs was significantly higher than that in controls. Mean renin substrate concentrations were similar in the two groups. With the demonstration of comparable substrate levels, the renin reactivity values become a relative measure of circulating accelerators or inhibitors of the renin reaction (see Methods). That differences in substrate concentration do not contribute to differences in renin reactivity is further supported by the absence of a correlation between renin reactivity and renin substrate in both coarcted and control groups. The increase in renin reactivity in coarcted dogs is significant at the 95% confidence level. Plasma renin concentration tended to be higher in the experimental dogs (mean 4.7 x 10⁻⁴ units/ml compared with a control mean of 2.3 x 10⁻⁴ units/ml), but the difference was not statistically significant (0.05 < P < 0.10).

Discussion

Neonatal banding of the thoracic aorta provides a model of aortic coarctation unique in its close simulation of the human congenital lesion. In contrast to the acute aortic constriction in adult animals employed by most investigators, this model produces gradual aortic narrowing essentially from birth, permits hemodynamic adjust-
ments and the development of collateral circulation in the setting of a rapidly growing animal, avoids cardiac decompensation, and should more closely reproduce the renal hemodynamic changes that occur clinically.

In 1938, Rytand (20), drawing from the classic work of Goldblatt et al. (21), proposed that aortic coarctation causes renal hypoperfusion and subsequent release of a renal pressor substance. Convincing evidence came first from Scott and co-workers (1, 2), who demonstrated in dogs with chronic thoracic aortic constriction that transplantaion of the entire renal mass to a site proximal to the constriction leads to normalization of blood pressure. Evidence against a role for the renal pressor system has centered around the inability to demonstrate renal hypoperfusion in the majority of subjects (3-6) and the generally normal plasma renin activity (7-9).

Our finding of a significantly depressed renal plasma flow and glomerular filtration rate in chronically coarcted dogs during salt restriction documents an abnormality of renal perfusion in this disease. Previous clinical and experimental studies have shown low-normal to normal renal plasma flows and normal glomerular filtration rates during unrestricted sodium intake (3-6). In light of these facts, our results suggest that some functional stress has unmasked a previously compensated abnormality of renal perfusion. Extracellular volume depletion, although not directly documented in our dogs, seems to be the most probable stress factor considering the degree and the duration of the sodium restriction and the low levels of urinary sodium excretion. If so, our data further imply that extracellular volume is critical to the compensatory adaptations which successfully maintain renal perfusion in unstressed coarctation subjects.

As an alternative explanation, an anesthetic-related stress (e.g., hypotension) may have caused or contributed to the decompensation of adaptive mechanisms with the subsequent manifestation of renal hypoperfusion. Although sodium pentobarbital tends to cause no change or a rise in the mean arterial blood pressure of normotensive dogs (22), we cannot exclude significant hemodynamic alterations in the setting of hypertensive coarcted dogs. Nevertheless, the conclusion that renal plasma flow and glomerular filtration rate in coarcted dogs fall abnormally in response to a given stress remains valid.

The abnormally elevated plasma renin activity in the salt-restricted coarcted dogs also differs from the majority of published reports, which have examined plasma renin activity during unrestricted salt intake and have consistently documented normal values (7-9). Significantly, in the six published cases of coarctation patients subjected to conditions known to stimulate the renin-angiotensin system, plasma renin activity was abnormally increased in three (9, 10, 23).

Recent reports of noncoarctation patients with hypertension and underperfusion of the total renal mass suggest a similar exaggerated rise in plasma renin activity during volume depletion. Kurtzman and co-workers (10) have described an abnormal increase in plasma renin activity from normal basal levels after sodium depletion plus diuretics in two of these high-salt patients. These cases involved stenosis in a transplanted kidney and left renal artery stenosis with a hypoplastic right kidney. Additionally, Bennett et al. (11) have reported in 13 cases of post-replacement transplant hypertension, an abnormally high plasma renin activity and a significantly decreased renal blood flow during salt restriction compared with values in salt-restricted normotensive transplant patients.

Thus, in contrast to the commonly elevated plasma renin activity encountered in the setting of unilateral renal hypoperfusion (24), total renal underperfusion is more typically accompanied by a normal plasma renin activity on ad libitum sodium intake and manifests an abnormally elevated plasma renin activity only during volume-depleting maneuvers. Clinical and experimental studies of total renal underperfusion have further demonstrated that angiotensin II blockade normalizes blood pressure in the salt-depleted state (25), thus providing evidence that hypertension in this setting is mediated by renin.

The abnormally elevated plasma renin activity and the decreased renal perfusion in coarcted dogs during salt restriction parallels the findings in clinical states of total renal underperfusion (10, 11) and invites the conclusion that a similar renin-mediated mechanism underlies coarctation hypertension. Additionally supportive of this view are experimental studies of acute aortic constriction, showing early elevation of plasma renin activity with normalization by 2 weeks (26, 27), a sequence also described in experimental one-kidney hypertension (28).

Integral to the concept of renin mediation in the hypertension of total renin underperfusion (and in contrast to that of unilateral renal hypoperfusion) is the development of compensatory volume expansion with time, thus permitting normalization of
The net reaction velocity (rate of AI generation) in vitro is reflected in the plasma renin activity both present in the plasma. Renin reactivity (the modifiers of the reaction (inhibitors, accelerators, or concentration) but also by renin substrate concentration and by the particular complement of modifiers of the renin release, the renin–renin substrate interaction is complex in its own right and may theoretically yield plasma renin activity changes independently of renin release. The reaction can be viewed as

\[ \text{Renin substrate} \xrightarrow{\text{renin}} \text{angiotensin I} \pm \text{modifiers} \]

The net reaction velocity (rate of AI generation) in vitro is reflected in the plasma renin activity measurement and is determined, not only by renin concentration, but also by renin substrate concentration and by the particular complement of modifiers of the reaction (inhibitors, accelerators, or both) present in the plasma. Renin reactivity (the reactivity of exogenous renin in plasma) is independent of endogenous renin concentration \((12, 19)\). It is therefore a function only of renin substrate concentration and of plasma modifiers. If measured renin substrate concentrations are similar in plasma samples to be compared, renin reactivity then becomes a relative measure of modifying factors alone.

We have shown that renin reactivity is significantly elevated in chronic coarctation hypertension in dogs and that this elevation is not due to an increase in renin substrate concentration, which is similar to control levels. Thus, for any given renin concentration, the plasma of coarcted dogs allows a greater rate of angiotensin I generation than does control plasma, reflecting either relatively more accelerator or relatively less inhibitor activity.

Renin reactivity has not been previously examined in coarctation hypertension. Increases have, however, been reported in a variety of other hypertensive states in man, including essential hypertension of both normal– and low–plasma renin activity categories \((13, 14)\), renovascular hypertension \((12, 15)\), and primary aldosteronism \((16)\). The addition of coarctation hypertension to this group provides further evidence that increased renin reactivity is a common feature of most, if not all, hypertensive states.

The physiological and pathological significance of this acceleration of the renin reaction in hypertension is not yet understood. Theoretically, renin in hypertensive plasma is more effective in generating the pressor angiotensin II. An accelerated renin reaction might therefore accentuate blood pressure elevation in any hypertensive state, regardless of primary etiology. One critical question is, therefore, whether increased renin reactivity produces in vivo a net increase in plasma renin activity and angiotensin II levels.

It was our initial expectation that measurement of each of the three determinants of plasma renin activity in coarctation hypertension (renin substrate concentration, renin reactivity, and plasma renin concentration) would permit assessment of their individual contribution to the abnormally elevated plasma renin activity. If the decreased renal perfusion observed in coarcted dogs was in fact anesthetic related and was thus not temporally coexistent with renin-angiotensin abnormalities, then the elevated plasma renin activity would be due solely to increased renin reactivity. However, if perfusion and renin-angiotensin abnormalities do coexist in time (as we feel more likely), then both increased renin reactivity and hypoperfusion could potentially contribute to the elevation in plasma renin activity. The insignificantly elevated plasma renin concentration in the face of depressed renal perfusion appears to challenge current concepts of renin release \((29)\) but is in fact compatible with the dual abnormality. If the well-documented negative feedback of angiotensin II on renin secretion \((29, 30)\) is considered, then the “normal” plasma renin concentration may reflect a balance between the positive stimulus of hypoperfusion and the negative feedback of high angiotensin II, the latter augmented by increased renin reactivity. The coexistence of the two abnormalities in coarcted dogs precludes clear separation of hypoperfusion effects and accelerator (or lack of inhibitor) effects on plasma renin concentrations and on plasma renin activity. Accordingly, our findings do not permit clarification of the effect of increased renin reactivity on plasma renin activity in hypertensive states.

In summary, we have demonstrated in dogs with neonatally induced chronic aortic coarctation that salt restriction is accompanied by renal hypoperfusion and by an abnormal elevation of the plasma renin activity. This finding suggests that a renin-mediated mechanism is operative, analogous to that observed in other hypertensive states associated with total renal underperfusion. We, therefore, favor a major role for the renal pressor system in coarctation hypertension. The additional demonstration of increased renin reactivity provides further evidence that this abnormality is indeed a
common feature of most, if not all, hypertensive states.

Acknowledgment

The authors wish to express special thanks to Dorothy MacFarlane for her invaluable technical assistance.

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Circulation Research, Vol. 37, November 1975
Abnormalities of renal perfusion and the renal pressor system in dogs with chronic aortic coarctation.

S P Bagby, W J McDonald, D W Strong, G A Porter, W M Bennett and L I Bonchek

_Circ Res._ 1975;37:615-620
doi: 10.1161/01.RES.37.5.615

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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