Physiopathogenesis of Acute Aortic Coarctation Hypertension in Conscious Rats

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Abstract We investigated the role of vasopressin, angiotensin II, and catecholamines in the onset of acute (45-minute) aortic coarctation hypertension in conscious rats. Partial aortic constriction was performed by means of a pneumatic cuff placed around the abdominal aorta above the renal arteries for 15 or 45 minutes. A sham-operated group was used as control. Mean carotid pressure before aortic constriction did not differ between rat groups. Aortic constriction produced a similar increase of mean carotid pressure during 15 minutes (36±3 to 37±3 mm Hg above basal levels) and 45 minutes (37±2 to 39±3 mm Hg). Plasma vasopressin concentration after 15 minutes of coarctation (4.4±0.5 pg/mL) did not differ from that observed in control rats (3.0±0.8 pg/mL), whereas after 45 minutes, it was significantly higher (14.3±3.3 pg/mL). Plasma renin activity increased significantly after coarctation (21.7±4.1 and 29.9±2.9 ng angiotensin I/mL per hour, at 15 and 45 minutes, respectively) when compared with control rats (3.9±0.5 ng angiotensin I/mL per hour). After coarctation, plasma norepinephrine concentration was consistently reduced, whereas plasma epinephrine concentration did not differ from control rats. In conclusion, these data provide evidence for an effective vasopressor role for vasopressin in the genesis of acute (45-minute) aortic coarctation hypertension in conscious rats. In addition, although the results confirm that the renin-angiotensin system participates earlier in the onset of coarctation hypertension, they rule out a significant vasopressor role for catecholamines in the early development of hypertension. (Hypertension. 1994;23[suppl] I: I-78-I-81.)

Key Words • vasopressin • renin-angiotensin system • angiotensin II • blood pressure • norepinephrine • epinephrine

Methods

Experiments were performed on male Wistar rats (260 to 320 g). On the day before the experiment, the rats were anesthetized with pentobarbital sodium (40 mg/kg IP) after pretreatment with atropine (0.5 mg/kg IP) for implantation of polyethylene catheters (Clay Adams, Parsippany, NJ) into the femoral artery (PE-10 connected to PE-50) and the ascending aorta (PE-10 connected to PE-50) and the ascending aorta via the left carotid artery (PE-50) for arterial pressure measurement and blood collection. After laparotomy, a pneumatic cuff was placed around the aorta immediately below the diaphragm, and the tubing connected to the cuff was exteriorized through the animal’s back together with the catheters, as described elsewhere.34 Sham-operated (control) rats were prepared as described above except for cuff implantation around the aorta.

The experiments were conducted with the animals unrestrained in individual cages. Both carotid and femoral arterial pressures were recorded continuously with a Statham pressure transducer (model P23Db, Hato Rey, Puerto Rico) attached to a Grass recorder (model 7D, Needham, Mass). After basal measurements of the arterial pressures (15 to 20 minutes), the balloon inside the cuff was filled with liquid to partially constrict the aorta to keep the arterial pressure distal to the cuff (mean femoral pressure) at precisely 50 mm Hg throughout the experiment.

The animals were divided into the following groups: 10 rats were submitted to 15 minutes of aortic constriction; 10 rats were submitted to 45 minutes of aortic constriction; and 11 rats (control) were submitted only to basal measurement of arterial pressure. Near the end of the period of coarctation or basal measurements of arterial pressure, 3 mL of blood was collected from the carotid artery while saline was simultaneously infused ("push-pull" method) into the femoral artery at the same rate (1 mL/min). Three blood samples were taken with microcapillary tubes for the determination of hematocrit, plasma osmolality, and plasma protein. Plasma osmolality was determined by vapor pressure osmometry (model 5300C, Wescor, Inc, Logan, Utah), and plasma protein was deter-
Hormone Assays
AVP was determined in plasma after deproteinization with acetone. The samples were dried and the residue reconstituted in water and analyzed using a sensitive radioimmunoassay as described previously.8-9 PRA was determined by the quantitation of generated angiotensin I (Ang I) using a modification of the radioimmunoassay method of Sealey and Laragh.10 The antibody was kindly provided by Dr Sealey. Plasma norepinephrine and epinephrine levels were determined using a radioenzymatic method that was a modification of the method of Peuler and Johnson.11 Using catechol-O-methyltransferase produced in our laboratory, we measured the products resulting from the transfer of the 3H-methyl group from S-adenosyl-L-methyl-3H-methionine (Du Pont, Wilmington, Del). The products of this transfer are extracted and isolated by thin-layer chromatography and the derivatives converted and extracted. The radioactivity of these extracts is proportional to the amount of norepinephrine and epinephrine in the sample.

Statistical Analysis
All values are expressed as mean±SEM. Statistical analysis of the hypertensive response after AoC was performed using one-way analysis of variance for repeated measures. Comparisons among the different groups were made using one-way analysis of variance and the Bonferroni procedure. Differences were considered significant at a value of $P<.05$.

Results
Hypertensive Response
Mean carotid pressure before AoC (Fig 1) did not differ between rat groups (116±4 and 115±2 mm Hg, 15 and 40 minutes of AoC, respectively) or from the control rat group (122±3 mm Hg, not shown in Fig 1). Mean carotid pressure increased rapidly after AoC in both rat groups, reaching a plateau in 5 minutes and remaining near this level (152±2 to 155±3 mm Hg) over the remaining period of constriction. The mean aortic gradient (mean carotid pressure minus mean femoral pressure) of both groups was within 102 to 105 mm Hg throughout the experiment.

Plasma Vasopressin
The plasma AVP concentration (Fig 2) at 15 minutes after AoC (4.4±0.5 pg/mL) did not differ significantly from that found in control rats (3.0±0.8 pg/mL). However, at 45 minutes after the start of AoC, the rats displayed a significantly higher plasma AVP level (14.3±3.3 pg/mL) compared with both 15 minutes of constriction and control rats.

Plasma Renin Activity
After AoC, PRA levels (Fig 2) were significantly elevated at both 15 minutes (21.7±4.1 ng Ang I/mL per hour) and at 45 minutes (29.9±2.9 ng Ang I/mL per hour) when compared with control rats (3.9±0.5 ng Ang I/mL per hour).

Plasma Catecholamines
Partial AoC elicited a fall in norepinephrine (Fig 2) at 15 minutes (151±41 pg/mL), which was also sustained at 45 minutes after constriction (193±36 pg/mL) compared with control rats (373±27 pg/mL). On the other hand, plasma epinephrine concentration (Fig 2) of control rats (189±26 pg/mL) did not differ significantly
from values found after 15 minutes (133±26 pg/mL) or 45 minutes (317±67 pg/mL) of sustained coarctation, although a significant difference was observed between the values obtained at 15 and 45 minutes.

**Hematocrit, Plasma Osmolality, and Protein**

No difference was observed for the hematocrit of control rats (0.4±0.01) and rats submitted to 15 minutes (0.37±0.002) or 45 minutes (0.34±0.02) of coarctation. Plasma protein after 15 minutes (5.0±0.1 mg/100 mL) and after 45 minutes (4.9±0.1 mg/100 mL) of AoC differed significantly from control rats (5.5±0.1 mg/100 mL). Plasma osmolality after 45 minutes of AoC (295±2.0 mOsm/kg) was slightly but significantly higher than after 15 minutes (289±1.2 mOsm/kg) and in control rats (288±0.8 mOsm/kg).

**Discussion**

Only recently has pharmacologic evidence from conscious rats shown that AVP shares with the renin-angiotensin system an important pathophysiological role in the maintenance of the acute hypertensive response after partial AoC. In the present study we measured in conscious rats the responses of plasma AVP, PRA, and plasma catecholamine (norepinephrine and epinephrine) concentrations after 15 and 45 minutes of a partial aortic constriction.

** Vasopressin Responses**

The present studies clearly show that AoC brings about a slow but substantial increase in plasma AVP. Plasma AVP concentration was unchanged 15 minutes after partial AoC compared with control rats but was fivefold higher 45 minutes after constriction. This contribution of AVP to the pressor response of AoC has been shown only indirectly in our previous studies. In these previous studies pharmacologic blockade of the pressor effect of AVP with [d(CH2)5 Tyr(Me) AVP] and Ang II with saralasin indicated that Ang II was important for the prompt (5 to 15 minutes) rise in proximal pressure after AoC, whereas AVP was responsible for the maintenance (15 to 45 minutes) of arterial pressure elevation.

The major physiological stimuli for AVP release are increased plasma osmolality and reduced arterial blood pressure and blood volume. Dunn et al demonstrated in conscious rats a twofold to threefold increase in plasma AVP levels when osmolality and blood volume had changed by less than 2% from the basal level of 294±1.4 mOsm/kg. However, plasma AVP levels of 15 pg/mL were obtained only with plasma osmolality as high as 310 mOsm/kg. In the present study, the remarkable increase in plasma AVP level of 14.3 pg/mL (Fig 2) displayed at 45 minutes of AoC should be ascribed to other mechanisms triggered by narrowing of the aorta. Hypovolemia is known to be a potent nonosmotic stimulus of AVP release, acting via left atrial receptors. Although blood volume and left atrial pressure were not monitored in the present study, control of AVP release by cardiac receptors appears to be weak or absent in the rat. Inhibition of AVP release during aortic constriction should also be expected from activation of the arterial baroreceptors because of the pronounced proximal hypertension. Therefore, it appears that the inhibitory mechanisms of AVP release, ie, cardiopulmonary and arterial baroreceptors, were presumably overridden by stimulatory mechanisms triggered by aortic constriction.

The changes in plasma AVP observed in the present study provide support for a pathophysiologic role of AVP in acute (45-minute) AoC hypertension. Although there is evidence that circulating Ang II can stimulate AVP secretion, there is also evidence that afferent renal fibers carry mechanical and chemical information from the kidney to specific brain nuclei that may influence neurohumoral control of the kidney itself or the circulation in general. Our laboratory studies of acute AoC in renal denervated rats demonstrated, by means of the V1 vascular AVP receptor antagonist, that AVP was not involved in the hypertensive response elicited by aortic constriction. In addition, we have also shown that the integrity of the median eminence of the hypothalamus plays a pivotal role in the maintenance (30 to 45 minutes) of acute AoC hypertension involving the release of AVP from the neurohypophysis. Therefore, these findings lend support to the notion that the neuroendocrine axis is indeed involved in the cardiovascular response to AoC. Even though direct evidence of renin hemodynamic influences in plasma AVP concentration is still lacking (see Robertson), this possible mechanism should be taken into consideration to explain the increased plasma AVP level at 45 minutes of coarctation.

**Renin-Angiotensin Responses**

The present data confirm previous observations that AoC above the renal arteries promotes overactivity of the renin-angiotensin system due to the low renal perfusion pressure. Our data (Fig 2) show that PRA was increased approximately sixfold 15 minutes and sevenfold 45 minutes after AoC.

**Catecholamine Responses**

Compared with control rats, plasma norepinephrine levels were significantly lower at both 15 and 45 minutes after AoC, with values 60% and 48% below levels of control rats, respectively. Similar findings were obtained in conscious dogs submitted to 24 to 48 hours of AoC. Because norepinephrine is not released from the adrenal medulla in appreciable amounts, plasma norepinephrine concentration mostly reflects release from peripheral nerve endings. In fact, rodents have approximately 10% of norepinephrine released from the adrenal medulla at rest versus 90% of epinephrine, whereas this ratio in humans and dogs is 20% of norepinephrine versus 80% of epinephrine. It is well known that a number of factors determine the concentration of plasma norepinephrine. Nevertheless, the marked decrease in plasma norepinephrine concentration observed in conscious rats (present study) and dogs can probably be attributed to reflex inhibition of the sympathetic outflow elicited by arterial baroreceptors subjected to elevated levels of pressure proximal to the coarctation.

Plasma epinephrine concentrations at 15 and 45 minutes of AoC were not significantly different from control rats, despite the fact that they were significantly higher after 45 minutes of coarctation than after 15 minutes. One can hypothesize from these data that lengthening of the duration of the experimental proto-
col over 45 minutes may substantially enhance plasma epinephrine levels. In fact, anesthetized dogs and patients exhibited a marked increase in epinephrine secretion rate when submitted to cross-clamping of the aorta. The mechanisms by which the lower pressure distal to the coarctation affects adrenal medullary function remain unclear. However, both neurogenic spinal cord excitation and a direct effect of underperfusion on the adrenal gland may be involved.

In conclusion, in the present study, hormone assays (AVP, PRA, epinephrine, and norepinephrine) carried out on blood collected from conscious rats at 15 and 45 minutes of AoC provided results that support a role for PRA in the early stage and a combined role for both PRA and AVP in the maintenance (45 minutes) of proximal hypertension. In addition, the results rule out a significant role for catecholamines in the onset (up to 45 minutes) of hypertension.

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References


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