Plasma Atrial Natriuretic Factor During Chronic Thoracic Inferior Vena Caval Constriction

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The effects of chronic constriction of the thoracic inferior vena cava (TIVCC) on plasma atrial natriuretic factor (pANF) were studied in conscious dogs (n = 5). TIVCC decreased left and right atrial pressure and led to a decrease in pANF concentration from 199 ± 12 to 104 ± 14 pg/ml while plasma renin and vasopressin concentrations increased. These hormonal changes were associated with a significant fall in sodium excretion to <5 meq/day. pANF remained suppressed during chronic TIVCC as the dogs expanded their extracellular fluid volume and developed ascites. Acute release of TIVCC resulted in abrupt increases in left and right atrial pressure but only a modest rise in pANF from 96 ± 16 to 185 ± 45 pg/ml. The magnitude of the rise in pANF (twofold) contrasted sharply with the eightfold increase in sodium excretion that occurred over the first 24 hours. Our data suggest that decrease in atrial pressure below normal results in a decline in pANF, which, acting in concert with the activated renin-angiotensin system and vasopressin, may contribute to sodium retention. On the other hand, during acute release of TIVCC, which markedly increased atrial pressure and sodium excretion, pANF only returned to control levels. These data suggest that ANF release may be attenuated during chronic reduction in atrial pressure and also raise a question concerning the magnitude of the primary role of ANF in this natriuretic response. (Circulation Research 1988;62:279–285)

Although the relation between increased atrial filling pressure and pANF levels has been studied in vitro and in vivo in anesthetized and awake animals, few studies have addressed the question of whether pANF levels decrease when atrial pressures are reduced below normal levels. Furthermore, sequential studies examining chronic pANF responses to decreased atrial pressures over a period of days to weeks have not been performed. The conscious dog with chronic thoracic inferior vena cava constriction (TIVCC) provides an ideal model for such an experiment. Longitudinal studies in the conscious dog have made important contributions to the understanding of complex pathophysiologic processes.

This study evaluates the effects of acute and chronic changes in atrial pressure on plasma immunoreactive ANF and their relation to daily sodium excretion in the conscious dog before, during, and after prolonged TIVCC.

Materials and Methods

Five male mongrel dogs were used in the chronic studies described here. All dogs underwent an initial training period, during which time they were trained to lie on their right side on a padded table. After training, the dogs were premedicated with intravenous morphine sulfate (1–2 mg/kg) and then anesthetized with intravenous sodium pentobarbital (25 mg/kg). Through a left thoracotomy under sterile conditions, catheters were placed in the descending aorta, left and right atria, and thoracic inferior vena cava just above the diaphragm. An inflatable constrictor cuff (Hazen-Everett, Teaneck, New Jersey) was implanted around the thoracic inferior vena cava proximal to the caval
blood samples were obtained as described for the
studies. On the following day, hemodynamic
monitoring was performed, with blood samples taken
later the dog was brought back to the experiment room
and blood samples were obtained at 15, 30, and 45
minutes after constriction. The animal was then re-
and plasma volume was measured every
other day by the Evans blue method.
Constriction of the vena cava was performed in two
stages on the first and second experimental days. After
an initial control period of 15 minutes, blood samples
were drawn. The thoracic inferior vena cava was then
constricted by slowly injecting saline into the caval cuff
over a 5–10-minute period to produce a 5–6-mm-Hg
increase in inferior vena cava pressure (IVCp) distal
to the cuff. Hemodynamic monitoring was continued,
and blood samples were obtained at 15, 30, and 45
minutes after constriction. The animal was then
returned to its cage with the cuff still inflated, and 5 hours
later the dog was brought back to the experiment room
where a further 45-minute period of hemodynamic
monitoring was performed, with blood samples taken
at the midpoint of the session. If necessary, the inflation
of the caval cuff was adjusted to maintain the distal
IVCp set earlier in the day. On the following day, an
identical protocol was followed so that the IVCp distal
to the constriction cuff was increased by an additional
5–6 mm Hg. All hemodynamic measurements and
blood samples were obtained as described for the
previous day. After the afternoon measurements were
made on day 2, the cuff inflation tube was sealed and
no further adjustments were made until release of caval
constriction 10 to 12 days later. Daily measurements
were continued throughout the constriction period. On
the day of release of constriction, control measure-
ments were made, and then the cuff was rapidly
deflated. Blood samples were obtained at 15, 30, and
45 minutes after release while pressures were continu-
ously recorded. Five hours later, further pressure
measurements were made and blood samples obtained.
Daily measurements were continued for 3 additional
days following release.

**Plasma Assays**

We found that use of heparin as an anticoagulant in
our blood sample tubes resulted in a 10–15% loss of
apANF immunoreactivity compared with EDTA.13
Therefore, blood samples were drawn into chilled
vacutainer tubes containing 1.5 mg/ml EDTA. Plasma
was separated by centrifugation at 3,000 rpm for 15
minutes and stored at –20° C. Plasma concentration
of immunoreactive pANF was measured by radioim-
munooassay using a rabbit antibody prepared against
synthetic human ANF-(99-126), whose sequence is
identical to that of canine ANF-(99-126). Since the
sequence of dog and human ANF is identical, 100%
cross-reactivity should be observed between dog ANF
and the antibody used in this study. Synthetic ANF
standards and [125I]ANF were also obtained from
Peninsula Laboratories, Belmont, California. Assay
was performed by addition of 0.1 ml rabbit antiserum
to 0.1 ml of standard or unknown plasma followed by
overnight incubation at 4° C. Next, 0.1 ml of human
[125I]ANF (99-126) was added to the reaction mixture,
and the tubes were again incubated overnight. Bound
human [125I]ANF was precipitated by addition of goat
anti-rabbit immunoglobulin G (IgG) serum and normal
rabbit serum. After a 2-hour incubation, 0.5 ml of assay
buffer was added to each tube, the mixture was
centrifuged, and the supernatant aspirated. The amount
of bound [125I]ANF was determined by counting each
tube for 10 minutes or 5,000 counts. After appropriate
corrections for background and nonspecific binding,
the values for ANF in picograms per milliliter were read
from a standard curve. To ensure uniformity of results
within any one dog, all samples from each animal were
run together within a single assay; the intra-assay
variability was 7.3%. In this study, we have reported
ANF levels as total measurable immunoreactivity in
dog plasma (pANF).

Since many investigators have reported pANF levels
after extraction of plasma samples, we compared ANF
values obtained from assay of unextracted and Sep-Pak
extracted samples. One hundred and one plasma
samples covering a wide range of ANF values were
analyzed by both methods. Plasma samples were
applied to C-18 Sep-Pak cartridges that were pre-
ashed with acetonitrile and distilled water. ANF
peptide was eluted with 80% acetonitrile in water
containing 0.1 M trifluoracetic acid. The eluate was
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**Results**

A series of six experiments was performed on five conscious dogs prepared as described above for TIVCC. The sequential hemodynamic, hormonal, and urinary changes with caval constriction are illustrated in Figure 2. At 45 minutes after onset of TIVCC, IVCp rose from 2.0 ± 0.2 to 7.9 ± 0.3 mm Hg (p < 0.001) and left atrial pressure decreased from 4.8 ± 0.3 to 0.5 ± 0.4 mm Hg (p < 0.003). Right atrial pressure fell from 2.0 ± 0.3 to −1.4 ± 0.5 mm Hg (p < 0.02). Heart rate remained relatively unchanged (from 66.7 ± 4.6 to 69.7 ± 3.4) while arterial blood pressure fell slightly from 95.3 ± 2.6 to 89.5 ± 3.0 mm Hg (p < 0.03). Accompanying the fall in atrial pressures, pANF decreased rapidly from a control average of 199 ± 12 to 154 ± 15 pg/ml (p < 0.001) when measured after 45 minutes of decreased atrial pressure. The acute changes in pANF correlated with left atrial pressure (LAP) (r = 0.9, n = 15) and right atrial pressure (RAP) (r = 0.88, n = 15). Within 45 minutes of caval constriction, plasma renin activity (PRA) increased from 0.2 ± 0.02 to 1.6 ± 0.46 ng/ml/hr (p < 0.04), and plasma vasopressin concentration rose in all except one animal; the rise in plasma arginine vasopressin (AVP) from 1.7 ± 0.3 to 7.4 ± 3.5 was statistically significant (p < 0.05, Wilcoxon signed rank), when this animal was excluded from analysis. Five hours after the initial constriction, left and right atrial pressures rose toward control levels but still remained significantly reduced (2.3 ± 0.5 and 0.4 ± 0.2 mm Hg, respectively [p < 0.002]) when compared with control. Plasma ANF concentration did not rise with partial restoration of atrial pressure but remained decreased at this time (155 ± 16 pg/ml [p < 0.001]). On the second day, additional cuff inflation produced a fall in left atrial pressure from 3.6 ± 0.6 to 0.6 ± 0.4 mm Hg, a decrease in right atrial pressure from 0.8 ± 0.6 to −1.2 ± 0.4 mm Hg, and a small decrease in arterial pressure from 87.7 ± 3.0 to 82.0 ± 4.7 mm Hg. This additional decrease in atrial pressure was associated with a further decrease in pANF levels to 142 ± 12 pg/ml when compared with control (p < 0.05).

By the morning of the third day of constriction, atrial pressure remained depressed at 0.0 ± 0.6 and −1.1 ± 0.5 mm Hg for left and right atrial pressures, respectively. pANF was 104 ± 17 pg/ml at this time; this value was significantly lower than control (p < 0.001) and was also significantly lower than the ANF level observed on the previous day prior to the second constriction. Arterial pressure reached its nadir on day 3, falling to 76.0 ± 1.5 mm Hg (p < 0.001), while PRA was elevated to 8.7 ± 1.3 ng/ml/hr (p < 0.05, Wilcoxon signed rank). Plasma AVP remained significantly elevated above control at 4.0 ± 0.7 pg/ml (p < 0.01).

On day 4 and thereafter, arterial and atrial pressures rose toward control levels as the dogs retained sodium and water and expanded their extracellular fluid volume. This was associated with a gradual fall in PRA toward control levels. Plasma vasopressin concentration also fell toward basal levels. Interestingly, pANF concentration remained low throughout the remainder of inferior vena caval constriction despite near normalization of atrial pressures. Overall, the pANF during the 12-day chronic TIVCC did not correlate with left or right atrial pressures (r = 0.3 and 0.22, respectively). Twenty-four hour sodium excretion decreased dramatically from 67.7 ± 5.6 meq per day on the final control day to less than 15 ± 4.9 meq per day by day 3 (p < 0.001) and less than 4 ± 1.0 meq per day by day 4. Thereafter, sodium excretion remained below 16 meq per day until day 7, when two of the experimental dogs began to return to normal sodium balance. This resulted in a drift upward in the group mean to 21.0 meq per day for sodium excretion for days 7–11, although...
on average, sodium excretion was below control throughout the entire constriction period. Overall, the dogs developed positive sodium balance as estimated by the difference between daily sodium intake and urinary sodium excretion. The average sodium retained during this period was 466 ± 102 meq. The initial fall in daily urinary sodium output over the first several days of TIVCC closely paralleled the fall in pANF and atrial pressure and was mirrored by the rise in PRA and AVP.

There was a good correlation between plasma ANF levels and urinary sodium excretion (r = 0.65) during TIVCC as illustrated in Figure 3 (top panel). In addition, with the decrease in sodium excretion and development of a markedly positive sodium and water balance, plasma volume rose from 1,234 ± 68 to a peak of 1,583 ± 117 ml (p<0.01), body weight increased by an average of 3.2 ± 0.7 kg, and the dogs developed ascites.
Abrupt release of TIVCC resulted in large changes in systemic hemodynamics (Figure 2). Left atrial pressure increased by an average of 7.0 mm Hg, rising from 2.9 ± 1.0 to 9.9 ± 1.2 mm Hg within 45 minutes of release (p < 0.002). Right atrial pressure similarly rose from 0.8 ± 1.1 to 6.1 ± 0.4 mm Hg over this time period. Release of constriction led to a modest rise in pANF from 96 ± 16 to 185 ± 45 pg/ml within 45 minutes (p < 0.05), a value close to control levels of 199 ± 12. Concomitantly, mean arterial pressure increased from 88 ± 4 to 107 ± 7 mm Hg (p < 0.03), and heart rate rose slightly from 81 ± 9.7 to 89 ± 12.8 beats/min. Plasma renin activity decreased significantly with TIVCC release from 2.8 ± 1.3 to 1.3 ± 0.8 ng/ml/hr; there also was a slight fall in plasma vasopressin concentration from 2.3 ± 0.2 to 2.2 ± 0.2 pg/ml (p < 0.02). These changes all occurred within 45 minutes after deflation of the hydraulic constriction cuff.

Twenty-four hours later, atrial pressures were still markedly elevated (LAP, 9.6 ± 1 mm Hg; RAP, 4.9 ± 0.6 mm Hg; see Figure 2) but pANF remained at preconstriction levels, 221 ± 42 pg/ml (p = 0.90). Although pANF did not rise above control, 24-hour sodium excretion had risen eightfold on the first day after release. The natriuresis was also accompanied by a large diuresis as urine volume increased threefold to 1,737 ± 272 ml/day. There appeared to be little relation between the pANF and urinary sodium excretion during this period (Figure 3, bottom panel). The total urinary sodium excreted after release of TIVCC was 437 ± 76 meq. At this time, PRA, AVP, and arterial pressures had nearly returned to control levels. Plasma volume at the end of recovery was 1,355 ± 86 ml, which was not significantly different from the control value. Average body weight returned to control level (i.e., 23.0 ± 1.8 kg).

Discussion

The present experiments were performed to evaluate the physiologic regulation of pANF during the onset, maintenance, and release of TIVCC. In this model of low cardiac output, large changes in renal sodium, and water excretion are produced in conjunction with alterations in cardiac filling pressures. This model provides the opportunity to examine the relation of ANF with these physiologic changes as well as its possible role in mediating the diuresis and natriuresis.

In these experiments, several observations were made: 1) constriction of the thoracic inferior vena cava decreased atrial pressure and pANF while PRA and AVP both increased. It is quite possible, therefore, that the decrease in pANF may well have contributed, along with the rise in PRA and AVP, to the increased retention of sodium and water seen with TIVCC. Indeed, recent evidence has suggested that pANF may serve as a counterregulatory hormone opposing the vasopressin and renin-angiotensin systems.6-13 Our data support this viewpoint and demonstrate that during TIVCC, AVP and PRA change reciprocally with changes in pANF. Further, the finding that sodium excretion changes correlated with changes in pANF levels during TIVCC suggests that pANF participates in the regulation of extracellular fluid volume during caval constriction. Proof for a role of pANF in sodium regulation awaits studies using ANF inhibitor, which is not currently available. 2) The low pANF despite an increase in AVP suggests that changes in atrial pressure play a more dominant physiologic role in the control of ANF secretion than plasma AVP concentration. 3) Release of caval constriction invariably resulted in elevated atrial pressure, and a large natriuresis and diuresis but only a modest rise in pANF to control levels. Previous reports have demonstrated that acute atrial distension produced by volume expansion or inflation of a balloon in the atrium leads to a rise in pANF concentration.12-14 However, the rise in pANF that accompanied release of TIVCC in our experiments was rather small compared with previous reports that have described much larger increases in pANF in response to increases in atrial pressure of similar magnitude.13 In fact, during the chronic phase of TIVCC, when atrial pressures have increased toward normal, pANF remained suppressed (Figure 2). This led us to hypothesize that there might be a blunting of the ANF release in response to increased atrial pressure in dogs with chronic caval constriction and prompted us to perform an additional series of experiments in conscious dogs in which we selectively raised right atrial pressure by acutely constricting the pulmonary artery (PAC) with an inflatable cuff. Results of these experiments have shown that for equivalent increase in right atrial pressure, PAC raised pANF more than release of chronic TIVCC.15 This difference between acute PAC and release of chronic TIVCC was even more striking when one considers that acute PAC only increased right atrial pressure, while release of chronic
TIVCC markedly increased left and right atrial pressures. Since data from several laboratories indicate that stretch of either the right or left atrium will increase pANF concentration,13,14 our experiments suggest that ANF release in response to elevated atrial pressure was attenuated in the chronic TIVCC dogs. No ready explanation for this phenomenon is available at this time, although one might suggest that chronic TIVCC may reduce atrial synthesis of ANF and therefore the content of releasable ANF and thus diminish the amount of ANF released into the blood during rapid atrial stretch. 4) It has been suggested that an increase in pANF may play a role in the natriuretic response to atrial stretch or saline infusion.7,8 However, we remain puzzled that after cuff deflation, pANF did not exceed control values despite the significantly elevated atrial pressure and that the pANF level was not commensurate with the ensuing natriuresis. How, then, may these modest changes in pANF affect sodium excretion? One possible explanation may be that in these studies, ANF release occurred in conjunction with a marked increase in arterial and atrial pressures. Restoration of renal perfusion pressure can promote natriuresis. Furthermore, such a pressure rise would be expected to stimulate high and low pressure baroreceptors to reflexively decrease renal sympathetic nerve activity and to produce a natriuresis.20 The rising plasma ANF may have contributed to this effect since it has been reported that ANF may inhibit renal sympathetic nerve activity through activation of inhibitory vagal cardio-pulmonary receptors.21 Thus, ANF may facilitate withdrawal of renal sympathetic tone and thereby augment the natriuretic response to release of TIVCC. PRA and AVP fell in response to TIVCC release. These changes may have been due to improved systemic and renal hemodynamics. In addition, rise in ANF may have contributed to the fall in PRA and AVP. ANF infusion has been reported to decrease renin release,20 inhibit aldosterone secretion,24,25 and suppress the plasma vasopressin response to hemorrhage or dehydration.21 Effects such as these may also contribute to the natriuresis and diuresis that follow deflation of the caval cuff. Finally, an upregulation in ANF receptor number and/or increased responsiveness to ANF might have occurred during chronic TIVCC.26 Therefore, although we observed that pANF levels rose only modestly with release of TIVCC, one may argue that in the physiologic range, ANF may function in concert with other hemodynamic and hormonal stimuli to regulate renal sodium and water excretion. There are, however, other evidences that do not support a major role for pANF in regulation of sodium excretion when pANF levels are in the range seen in conscious dogs. In our experiment, the increase in urinary sodium excretion did not correlate with the increase in pANF after release of TIVCC (Figure 3, bottom panel). For example, Goetz et al19 and Verburg et al27 found that infusion of synthetic ANF, which produced pANF levels even greater than those that we observed after constriction release, did not cause a significant natriuresis. At higher infusion rates, ANF increased sodium excretion, but the pANF levels were much greater than those seen after release of TIVCC. Indeed, ANF may not be a potent natriuretic agent at physiologic concentrations. Freeman et al21 and Scriven and Burnett26 have reported that ANF infusion into dogs with caval constriction did not lead to an appreciable increase in sodium excretion, despite a large decrease in plasma renin activity and rise in the filtered load of sodium. It was suggested that ANF was ineffective because the animals were in a preexisting state of avid sodium retention induced by the caval constriction. However, in our study, the changes in sodium excretion paralleled the changes in PRA, AVP, and renal hemodynamics. Therefore, we propose that ANF may act as a modulator of renal and systemic responses to vasoconstrictive/antinatriuretic hormones. When plasma ANF changes in opposite direction to these hormonal levels, it works in concert with these hormones. However, in states when both ANF and antinatriuretic hormones are activated (e.g., congestive heart failure), ANF's effect is masked or overridden by the renin-angiotensin system and AVP and perhaps by the renal hemodynamic state. This postulate is supported by the observations that ANF infusions are ineffective in animal models of renal sodium retention characterized by the activation of vasoconstrictive/antinatriuretic neurohormonal mechanisms.11,23

In summary, results of the present study indicate that pANF is physiologically regulated during states of low and high atrial filling pressure associated with caval constriction and release. These observed changes in pANF may contribute to renal salt and water homeostasis during the onset, maintenance, and release of TIVCC. However, based on the modest pANF response to release of caval constriction and data from other laboratories, we propose that ANF plays only a modulatory role in sodium and water homeostasis. Further experiments and development of selective ANF antagonists may help answer this question.

References

KEY WORDS • atrial natriuretic factor • low cardiac output • thoracic caval constriction • natriuretics
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