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.
catheter, and the chest was closed and evacuated; analgesics were administered postoperatively. A seven-
day course of oral antibiotics (Keflex 1.5 g/day) was
given, and the catheters were flushed daily and filled
with heparin (1,000 U/ml). Two weeks were allowed
for recovery from surgery before experiments were
started. These experiments were approved by Harvard
Medical School Animal Care Committee and were
performed in accordance with the guidelines of the
United States Public Health Service.

Experimental Procedures

Dogs were fed each day at 4 P.M. with a diet that
provided 70–80 meq of sodium per day and were
allowed water ad libitum. Experiments were performed
in the morning after at least a 12-hour fast. On each
experimental day the dog was brought into the experi-
ment room, where it rested quietly on its side for 30
minutes on the padded table. The indwelling catheters
were attached to Statham P23 Dc transducers (Hato
Rey, Puerto Rico), which were zeroed at the level of the
spinous processes. A Grass Model 7b polygraph (Quincy,
Massachusetts) was used to record aortic, left
and right atrial, and thoracic inferior vena cava
pressures as well as a Lead II electrocardiogram and the
tachograph signal derived from the ECG. The data were
recorded on an FM tape recorder (Hewlett-Packard
3968A, Palo Alto, California) and analyzed on-line by
computer.

Caval Constriction Experiments

During the three-day control period, daily hemody-
amic measurements were made on each animal. After
30–45 minutes of recumbency, blood samples were
drawn from the aortic catheter for measurement of
pANF, plasma vasopressin, serum osmolality, serum
sodium and potassium concentrations, and hematocrit.
Blood samples were replaced with an equal volume of
isotonic saline. 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 caval 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 re-
turned 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
pANF immunoreactivity compared with EDTA.37
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 radioi-
munnoassay using a rabbit antibody prepared against
synthetic human ANF-(99-126), whose sequence is
equal 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
teptide was eluted with 80% acetonitrile in water
containing 0.1 M trifluoracetic acid. The eluate was
Acute changes in atrial pressure were associated with a decrease in atrial natriuretic factor (ANF) values obtained by direct analysis and analysis of Sep-Pak extracted plasma.

**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,
FIGURE 2. Hemodynamic and hormonal data for control, chronic constriction, and release phases of chronic thoracic inferior vena cava constriction. Multiple time points for the first and second day of constriction and first day of release are for 0, 15, 30, and 45 minutes and 5 hours following constriction or release. The data are presented as mean ± SEM of 6 experiments performed on 5 conscious dogs.

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 AVP and PRA changed reciprocally with changes in right atrial pressure from 2.8 ± 1.3 to 13 ± 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. 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. 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. 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. 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. 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, 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. It has been suggested that an increase in pANF may play a role in the natriuretic response to atrial stretch or saline infusion. However, we remain puzzled that after cuff deflation, pANF did not exceed changes may have been due to improved systemic and 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. 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. Thus, ANF may facilitate withdrawal of renal sympathetic tone and thereby augment the natriuretic response to release of TIVCC. Therefore, we propose that ANF may act as a modulator in sodium and water homeostasis.

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. 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
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