Bilateral Atrial Appendectomy Abolishes
Increased Plasma Atrial Natriuretic Peptide Release and Blunts Sodium and Water Excretion During Volume Loading in Conscious Dogs

Julian M. Stewart, Roosevelt Dean, Miriam Brown, Donna Diasparra, Guillermo A. Zeballos, Mary Schustek, Michael H. Gewitz, Carl I. Thompson, and Thomas H. Hintze

The atrial appendages contain most of the atrial natriuretic factor (ANF) in the mammalian heart, and atrial appendage mechanical function predicts ANF secretion during volume loading. To demonstrate the crucial role of the atrial appendages in ANF release, we first measured hemodynamics and changes in plasma ANF after injection of 1,000 ml i.v. normal saline in conscious dogs and again after bilateral atrial appendectomy; we next measured changes in renal function using infusions of atriopeptin 24 to achieve plasma levels corresponding to levels achieved during volume loading; and we lastly measured renal function during acute volume expansion and also after atrial appendectomy. Plasma ANF increased from 65±11 to 246±54 pg/ml after volume loading but did not increase after atrial appendectomy. Atrial appendectomy did not alter the tachycardia or hemodynamic effects of volume loading. Infusion of 10 ng/kg/min atriopeptin 24 increased plasma ANF from 50±9 to 234±54 pg/ml, increased urine output 34±10%, and increased sodium excretion 62±10% in dogs with intact atrial appendages. Renal function was decreased in dogs before atrial appendectomy: 20, 40, and 60 minutes after volume loading, urine flow rate increased by 5.9±0.5, 6.9±0.4, and 4.4±0.8 ml/min, while sodium excretion increased by 717±60, 839±84, and 582±57 μeq/min. After atrial appendectomy urine flow rate increased 2.1±0.7, 2.7±0.7, and 2.0±0.6 ml/min, and sodium excretion increased only by 327±110, 324±77, and 340±92 μeq/min ( p<0.01) during volume loading. The total fraction of water and sodium, 63±5% and 60±4%, respectively, of the load excreted in 4 hours was significantly reduced to 30±5% and 35±3% of the load ( p<0.01) after bilateral appendectomy. We conclude that bilateral atrial appendectomy eliminates ANF release and blunts renal excretion of sodium and water after a large acute volume load in conscious dogs. (Circulation Research 1992;70:724–732)

Key Words • atrial function • Bainbridge reflex • atriopeptin 24 • natriuresis • atrial natriuretic factor • diuresis • atricectomy

Atrial natriuretic factor (ANF) is normally secreted by the atria in response to atrial stretch.1–5 In previous studies6,7 we have estimated atrial stretch by developing methods to measure atrial appendage wall function in awake animals. This is primarily due to the ability to study atrial appendage function and evidence that suggests that the greatest concentra-
responses with right or left appendectomy; Sakata et al. demonstrated volume-dependent ANF release in anesthetized rats, in which atrial appendectomy blunted the renal response to volume loading independent of ANF; and Kaneko et al. showed only a small increase in plasma ANF in volume-loaded anesthetized rats. However, later studies in rats by Villareal et al., Schwab et al., Hirth et al., and Hoffman and Keiser demonstrated increased plasma ANF, increased natriuresis, and increased diuresis with volume loading, and these responses were significantly attenuated by atrial appendectomy. In the monkey, Benjamin et al. have shown that bilateral atrial appendectomy greatly attenuates the rise in plasma ANF and the natriuresis after acute volume expansion.

The effects of atrial appendectomy in dogs have been more controversial. Benjamin et al. indicated that removal of the atrial appendages from dogs does not eliminate the rise in plasma ANF and does not affect the salt and water excretion that occurs after acute volume expansion using whole blood. Cowley et al. indicated that removal of the atrial appendages from dogs reduces salt and water excretion after volume expansion with isotonic saline, but this is independent of changes in plasma ANF. Moreover, Cowley et al. found in the same study that ANF did not increase with volume loading whether or not atrial appendectomy had been performed. Still other experiments indicated increased ANF with volume loading, but this was unrelated to changes in renal function, and atrial appendectomy had no effect on sodium excretion.

Some of the variability in results arises from the diverse methods used to increase intravascular volume and the inability to elicit significant ANF secretion in intact dogs. Preliminary data from our studies suggested that relatively high physiological levels of ANF had to be achieved to exert significant natriuretic and diuretic effects, and to achieve such ANF levels, a large volume load had to be administered with sufficient rapidity so as to produce a large increase in atrial pressure and in atrial stretch. We believe that ANF is released from the atrial appendages in amounts sufficient to produce diuresis and natriuresis only when there is a large deformation of the wall of the atrial appendages.

With these constraints in mind, our first goal was to measure atrial function, hemodynamics, and the change in plasma ANF after a large acute volume load in the normal conscious dog and to determine whether the increase in ANF is blunted after bilateral atrial appendectomy. Our second goal was to determine whether levels of plasma ANF, comparable to those achieved during volume loading, could increase salt and water excretion. Our third goal was to determine the effects of bilateral atrial appendectomy on the renal excretion of sodium and water after a large acute volume load.

Materials and Methods

General Methods and Surgical Preparations

Male dogs were used for initial experiments to determine the effects of bilateral atrietectomy during volume loading, whereas female dogs were used to determine the effects of plasma ANF or atrietectomy on renal function. All dogs were trained to lie quietly on the laboratory table, and female dogs also became accustomed to the placement of a urinary bladder catheter (Foley, 12F) for urine collection. Experiments were always started in the morning. Dogs were fed Purina laboratory canine chow mixed with Kennel Ration canned foods (0.42% sodium) on the afternoon preceding experiments. Water was available ad libitum except during the experiments, when no water was given. Estimated sodium intake was 65 meq/day. On the day of initial experiments the dogs were instrumented using local lidocaine anesthesia with a femoral artery catheter. After the initial volume-loading experiment, each dog was anesthetized with thiamylal sodium, and the femoral artery catheter was tunneled subcutaneously to the neck.

Dogs used for volume-loading experiments then underwent thoracic surgery for removal of the atrial appendages. All dogs undergoing surgery were sedated with acepromazine (3 mg/kg), anesthetized with pentobarbital sodium (25 mg/kg), intubated, and placed on a positive pressure ventilator (Harvard Apparatus, South Natick, Mass.). An incision was made in the fourth left intercostal space. Bilateral atrial appendectomy was performed: the right and left atrial appendages were clamped, sutured behind the clamp, and excised. Excised right and left atrial appendages were weighed. In the male dogs, Tygon catheters were placed in the remaining left atrium for pressure measurement.

In all operated dogs, the chest was closed in layers, the pneumothorax was reduced, and the dog was allowed 10–14 days to recover. During recovery, all dogs received antibiotics. Volume-loading or infusion experiments were repeated after recovery was complete.

Recording and Instrumentation

In all of the dogs, mean arterial pressure, heart rate, and hematocrit were measured. Recordings were made for at least 40 minutes before all experiments. In some of the dogs, left atrial pressure was also measured. Hemodynamic data were recorded on a 14-channel magnetic tape recorder (model 3700B, Bell and Howell, Rutherford, N.J.) and played back on a direct-writing oscillograph (model 2800S, Gould Instruments, Inc., Rahway, N.J.). Pressures were measured using the previously implanted catheters attached to strain-gauge manometers (model P23ID, Statham, Rahway, N.J.). Heart rate was derived from the pressure–pulse interval using a cardiotochometer (Beckman Instruments, Inc., Fullerton, Calif.). Mean pressures were derived using 2-Hz low-pass filters. All the procedures involving the use of dogs were approved by the Institutional Animal Care Use Committee of New York Medical College and conform to the guidelines of the American Physiological Society and to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Urine Collection, Electrolytes, and Osmolarity

Urine was collected continuously from female dogs during experiments through the indwelling bladder catheter, which was placed acutely on the day of the experiment. In all experiments urine was collected for at least two 20-minute control periods. Urine volume was measured, and the concentration of urine sodium and potassium was measured along with measurements of...
serum sodium and potassium using a flame photometer (model 343, Instrumentation Laboratories, Chicago). Average urine flow rate was calculated from the urine volume excreted over a 20-minute time interval. Urine osmolality was determined using a vapor pressure osmometer (model 5100B, Wescor Inc., Logan, Utah). We have used all the techniques previously.25

**Blood Collection, ANF Assay, and Plasma Electrolytes**

Blood was withdrawn from the aortic catheter for measurement of plasma ANF and placed in chilled tubes containing EDTA and 5 µl/ml aprotinin (Sigma Chemical Co., St. Louis, Mo.). Plasma was separated and frozen at −70°C until used. Plasma was extracted using a C-18 column (Sep-Pak) and a radioimmunoassay kit for human ANF (Peninsula Laboratories, Inc., Belmont, Calif.). The percent recovery was calculated in each sample by adding 2,000 cpm radioactive ANF to each plasma sample. Intra-assay variability is 4%. Interassay variability is 8%. Percent extraction was measured in every sample. Reported values were corrected based on the percent recovery. We have used all these techniques previously.6,7,9,25,26

**Volume-Loading Protocol**

On the day of the experiment, a large-bore catheter (19-gauge Intracath) was placed in a peripheral vein in all dogs and attached to a warmed bag of saline (1,000 ml, Travenol). The saline was infused as rapidly as possible, over 2–5 minutes, using an arterial pressure bag inflated to 200–300 mm Hg as previously reported.6,7 Hemodynamics were recorded continuously. Blood samples were taken at control and after 500 and 100 ml saline had been infused. In female dogs, blood was also taken at 20-minute intervals continuing for 240 minutes after volume loading.

**Effects of Atrial Appendectomy on Plasma ANF Levels**

We measured arterial plasma ANF in conscious male dogs during volume loading (n=6) before and after bilateral atrial appendectomy.

**Effects of Administered ANF on Plasma ANF and Salt and Water Excretion**

Female dogs (n=11) were used to determine the effects of ANF infusion on salt and water excretion. On the day of the experiment, a catheter was placed in a peripheral vein. Atriopeptin 24 (Monsanto Chemical Co., St. Louis, Mo.) was infused at a rate that increased from 0 to 10, 30, and 50 ng/kg/min as a cumulative dose for 30 minutes at each step. We have used this protocol previously.9 Blood and urine samples were obtained, and hemodynamics were measured during the last 20 minutes of each infusion.

**Effects of Atrial Appendectomy on Sodium and Water Excretion**

Female dogs (n=6) were used for the experiments. Volume loading was performed before and after atrial appendectomy in each of these female dogs. Blood and urine samples were collected before and after volume loading as described above.

**Statistical Analysis**

Mean±SEM was calculated, and difference from control was determined by a one-way analysis of variance (ANOVA). Two-way ANOVA was used to detect differences between the experimental groups. A Scheffe’s test was incorporated when ANOVA was used. A microcomputer-based system (PDP 11/73) was used for the analyses. Significance was determined at p<0.05.

**Results**

**Effects of Atrial Appendectomy on Plasma ANF Levels**

The effects of volume loading on cardiovascular function in intact dogs and in atrial-appendectomized dogs with left atrial catheters are shown in Table 1. Volume expansion caused a significant increase in mean arterial pressure in all the dogs. This was accompanied by a marked increase in left atrial pressure and a significant tachycardia. Hematocrit fell in both groups studied. There were no significant differences in the hemodynamic responses to volume loading in operated and intact dogs.
TABLE 2. Atrial Appendage Weights Removed at Surgery and at Postmortem Examination

<table>
<thead>
<tr>
<th></th>
<th>Weight of removed atrial appendage (g)</th>
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<tr>
<td></td>
<td>n</td>
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<tr>
<td>At appendectomy surgery</td>
<td></td>
</tr>
<tr>
<td>Male dogs</td>
<td>6</td>
</tr>
<tr>
<td>Female dogs</td>
<td>7</td>
</tr>
<tr>
<td>At postmortem examination</td>
<td>14</td>
</tr>
</tbody>
</table>

Values are mean±SEM. The weights of the atrial appendages removed from dogs during appendectomy surgery and from dogs during normal postmortem examination are shown above. The body weights of the two groups of dogs were not significantly different.

To determine the extent of removal of the atrial appendages at surgery, we weighed the appendages obtained from atrial-appendectomized dogs and compared those weights with the weights of atrial appendages obtained during careful anatomic dissection at postmortem examination in similar dogs. The weights at surgery and at postmortem examination are shown in Table 2. Using the data shown in the table, we removed ~69% of the left atrial appendage and ~52% of the right atrial appendage during the current experiment. It is probable that these are underestimates of the extent of functional atrial appendectomy because they do not include the tissue damage from the placement of the clamp or from the suture line that was proximal to the clamp.

The change in plasma ANF during volume loading before and after bilateral atrial appendectomy is shown in Figure 1. These data indicate that plasma ANF increased significantly in intact dogs but failed to increase in dogs after atrial appendectomy. We did not measure atrial pressure in the intact group because those dogs did not have atrial pressure catheters. However, hematocrit decreased while arterial pressure and heart rate increased in a similar fashion in unoperated and in operated dogs after atrial appendectomy, indicating a similar and significant degree of atrial stretch.

Effects of ANF Infusion on Plasma ANF and Salt and Water Excretion

The effects of atriopeptin 24 infusion on plasma immunoreactive ANF and on cardiovascular and renal function are shown in Table 3. The increase in urine flow rate and urine sodium excretion during ANF infusion are shown in Figure 2. Administration of a lower infusion rate of ANF (e.g., 5 ng/kg/min) had no significant effect on cardiovascular or renal function. ANF increased significantly at all infusion rates shown in the table, achieving essentially the maximum of human physiological range (i.e., 600 pg/ml) at an infusion rate of 30 ng/kg/min. There were no significant changes in mean arterial pressure, hematocrit, and heart rate from resting values of 98±4 mm Hg, 39±2%, and 85±6 beats per minute, respectively. Urine flow rate increased by >100% from 0.56±0.10 to 1.21±0.19 ml/min; sodium excretion increased similarly (p<0.05) from 81±20 to 209±34 μeq/min. Urine sodium concentration increased significantly from 144±19 to 173±13 μeq/ml during the atriopeptin infusion. Figure 2 indicates that plasma ANF levels of >200 pg/ml produce only a relatively modest, although statistically significant, 34±10% increase in urine output and a 62±10% increase in sodium excretion.

Effects of Atrial Appendectomy on Sodium and Water Excretion

The effects of volume loading with 1,000 ml saline on plasma ANF levels, cardiovascular function, and renal function in dogs before and after atrial appendectomy are shown in Table 4. Volume expansion caused mean arterial pressure to increase significantly in intact dogs from 102±2 to 128±5 mm Hg, which was similar to the increase in atrial-appendectomized dogs from 101±4 to 123±7 mm Hg. There was a similar increase in heart rate (from 93±7 to 180±16 beats per minute in intact dogs and from 104±7 to 176±13 beats per minute in atrial-appendectomized dogs) and a similar decrease in hematocrit (from 40±2% to 27±2% in intact dogs and from 36±2% to 25±1% in atrial-appendectomized dogs). These data are also similar to data obtained from our volume-loading studies reported in Table 1. ANF increased significantly with volume loading from 69±5 to 237±27 pg/ml but did not increase significantly from 46±5 pg/ml after atrial appendectomy.

The time course of changes in plasma ANF during the experiment is shown in Figure 3. ANF in intact dogs initially increased rapidly and then fell off rapidly during the remainder of the experiment. No significant increase in ANF occurred in atrial-appendectomized dogs. The increases in plasma ANF at 500 and 1,000 ml of volume expansion are similar to the results obtained during our initial volume-loading experiments (Table 1) and demonstrate once again that ANF increases in response to volume loading only if the atrial appendages are intact. At surgery we again weighed the atrial appendages and compared the values from atrial-appendectomized dogs with normal postmortem values from a large group of dogs (Table 2). Our conservative

![Figure 1](http://circres.ahajournals.org/)

**Figure 1.** Graph showing the change in plasma atrial natriuretic factor (ANF) during volume loading in intact dogs (●) and bilateral-appendectomized dogs (▲). Plasma ANF increased significantly in intact dogs but failed to increase after atrial appendectomy. *p<0.05 compared with appendectomized dogs.
estimates indicate that we removed 71% of the left atrial appendage and 81% of the right atrial appendage at bilateral atrial appendectomy.

The data for sodium, potassium, and osmolar excretion and for urine flow rate are summarized in Table 4. The most striking difference between salt and water excretion during volume loading occurred during the first 60 minutes; after that there were no significant differences between the two groups. Figure 4 demonstrates the changes occurring in water and salt excretion in response to volume loading and during the 240 minutes after volume loading.

In intact dogs, cumulative urine excretion was 39 ± 3% of the administered water load at 80 minutes (Table 4) and 63 ± 5% at 240 minutes after volume loading. This compares with atrial-appendectomized dogs, in whom only 16 ± 3% and 30 ± 5% of the administered volume load was excreted at 80 minutes and 240 minutes, respectively. Cumulative sodium excretion was 35 ± 3% of the administered sodium load at 80 minutes and 60 ± 4% at 240 minutes after volume loading but only 19 ± 2% and 42 ± 4%, respectively, after atrial appendectomy.

**Discussion**

Our results indicate that a rapidly administered large volume load consistently produces a significant increase in mean left atrial pressure and in mean arterial pressure, a decrease in hematocrit, and an increase in heart rate in conscious dogs. There are no significant differences in hemodynamic effects of volume loading following atrial appendectomy. Plasma ANF increases to a concentration of ~250 pg/ml in dogs with intact atrial appendages but does not increase above baseline levels in atrial-appendectomized dogs during volume loading; i.e., atrial appendectomy abolishes the increase in plasma ANF in conscious dogs caused by volume loading. Plasma ANF levels of ~250 pg/ml, achieved by the administration of exogenous atriopeptin 24, produce statistically and physiologically significant, albeit small, increases in urine sodium and water excretion. Finally, our data indicate that atrial appendectomy not only eliminates the volume-dependent increase in plasma ANF but also reduces by ~50% the early diuresis and natriuresis after volume loading. Thus, atrial appendectomy eliminates the volume-dependent increase in plasma ANF and blunts renal sodium and water excretion to acute large-volume expansion in awake dogs. High physiological plasma concentrations of ANF may in part be responsible for alterations in renal function during acute volume loading in the normal conscious dog.

In our first series of experiments, 1,000 ml saline increased mean left atrial pressure from ~5 to 17 mm Hg. In dogs with intact atrial appendages, ANF increased from normal levels of ~50 to 250 pg/ml. Table 1 and Figure 1 show that 500 ml saline produced an intermediate increase in mean left atrial pressure to 13 mm Hg and a modest increase in plasma ANF to 110 pg/ml; such a plasma ANF level is at or just above the upper limit for ANF in the normovolemic conscious dog\(^6,7,25-29\) and in normovolemic healthy humans.\(^30,31\)
TABLE 4. Effects of Acute Volume Expansion on Hemodynamics, Renal Function, and Plasma Atrial Natriuretic Factor in Conscious Dogs With and Without Atrial Appendages

<table>
<thead>
<tr>
<th></th>
<th>Volume expansion</th>
<th>Change from control</th>
<th>Time elapsed after volume expansion</th>
<th>Time elapsed after volume expansion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>500 ml</td>
<td>1,000 ml</td>
<td>20 min</td>
</tr>
<tr>
<td>Plasma ANF (pg/ml)</td>
<td>Intact</td>
<td>69±5</td>
<td>85±21*</td>
<td>268±29*</td>
</tr>
<tr>
<td></td>
<td>Appendectomized</td>
<td>46±5</td>
<td>9±8†</td>
<td>19±7†</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>Intact</td>
<td>40±2</td>
<td>-8.9±0.8*</td>
<td>-13.4±1.4*</td>
</tr>
<tr>
<td></td>
<td>Appendectomized</td>
<td>35±1</td>
<td>-5.8±0.9*</td>
<td>-8.6±0.9*</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>Intact</td>
<td>93±7</td>
<td>56±9*</td>
<td>87±13*</td>
</tr>
<tr>
<td></td>
<td>Appendectomized</td>
<td>104±7</td>
<td>58±10*</td>
<td>72±10*</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>Intact</td>
<td>102±2</td>
<td>22±3*</td>
<td>26±4*</td>
</tr>
<tr>
<td></td>
<td>Appendectomized</td>
<td>101±4*</td>
<td>21±4*</td>
<td>22±4*</td>
</tr>
<tr>
<td>Urine flow (ml/min)</td>
<td>Intact</td>
<td>0.25±0.04</td>
<td>...</td>
<td>...</td>
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<tr>
<td></td>
<td>Appendectomized</td>
<td>0.33±0.05</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Cumulative urine volume (ml)</td>
<td>Intact</td>
<td>5.3±1.0</td>
<td>...</td>
<td>...</td>
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<tr>
<td></td>
<td>Appendectomized</td>
<td>7.3±1.0</td>
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<tr>
<td>Urine sodium concentration (μeq/ml)</td>
<td>Intact</td>
<td>134±28</td>
<td>...</td>
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<tr>
<td></td>
<td>Appendectomized</td>
<td>142±28</td>
<td>...</td>
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<tr>
<td>Urine sodium excretion rate (μeq/min)</td>
<td>Intact</td>
<td>29±9</td>
<td>...</td>
<td>...</td>
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<tr>
<td></td>
<td>Appendectomized</td>
<td>38±8</td>
<td>...</td>
<td>...</td>
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<tr>
<td>Cumulative urine Na⁺ excreted (meq)</td>
<td>Intact</td>
<td>0.58±0.18</td>
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<tr>
<td></td>
<td>Appendectomized</td>
<td>0.76±0.16</td>
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<tr>
<td>Urine potassium concentration (μeq/ml)</td>
<td>Intact</td>
<td>82±17</td>
<td>...</td>
<td>...</td>
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<tr>
<td></td>
<td>Appendectomized</td>
<td>112±19</td>
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<td>...</td>
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<tr>
<td>Urine potassium excretion rate (μeq/min)</td>
<td>Intact</td>
<td>19±9</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>Appendectomized</td>
<td>29±6</td>
<td>...</td>
<td>...</td>
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<tr>
<td>Cumulative urine potassium excreted (meq)</td>
<td>Intact</td>
<td>0.4±0.1</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>Appendectomized</td>
<td>0.6±0.1</td>
<td>...</td>
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<tr>
<td>Urine osmolality (mosm)</td>
<td>Intact</td>
<td>1,360±181</td>
<td>...</td>
<td>...</td>
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<tr>
<td></td>
<td>Appendectomized</td>
<td>1,412±199</td>
<td>...</td>
<td>...</td>
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<tr>
<td>Urine osmolar excretion rate (mosm/min)</td>
<td>Intact</td>
<td>0.31±0.05</td>
<td>...</td>
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<tr>
<td></td>
<td>Appendectomized</td>
<td>0.35±0.07</td>
<td>...</td>
<td>...</td>
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<tr>
<td>Cumulative osmolar excretion (mosm)</td>
<td>Intact</td>
<td>6.1±1.1</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>Appendectomized</td>
<td>7.5±1.2</td>
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</table>

Values are mean±SEM in dogs with intact atrial appendages and in dogs after bilateral atrial appendectomy. The control value is the absolute value, whereas the data for 500 and 1,000 ml and 20, 40, 60, and 80 minutes are changes from control. ANF; atrial natriuretic factor; bpm, beats per minute.

*p<0.05 vs. control value.

†p<0.05 vs. intact value.

Thus, a very large increase in intravascular volume is required to produce a large enough atrial stretch to produce an important increase in plasma ANF above normal. Lesser volume loads, although still producing a statistically significant increase in ANF, are not always successful in producing a physiologically important change in ANF; i.e., ANF may not increase much above the normal range and therefore may not exert a direct demonstrable effect on renal function.

After atrial appendectomy, a similar increase in intravascular volume, as documented by the fall in hematocrit, did not produce an increase in ANF, although mean left atrial pressure, mean arterial pressure, and heart rate all increased. The Bainbridge reflex--induced tachycardia characteristic of the awake dog, which has been previously described,32,33 was preserved in the atrial-appendectomized group, indicating that the atrial vagal receptors responsible for initiating the Bainbridge
reflex were intact. This is supported by previous studies summarized by Linden\textsuperscript{44} that indicate that the stretch receptors responsible for the tachycardia occurring during volume expansion are located at atrial–venous junctions and not in the atrial appendages. Our data indicate that ANF is primarily released by atrial appendages in response to volume loading. This is supported by our previous study,\textsuperscript{6} a preliminary report by Cernacek et al.,\textsuperscript{8} and a study in rats.\textsuperscript{35}

Data during volume loading in dogs with intact atrial appendages are comparable to data previously reported by Bishop and Peterson,\textsuperscript{36} Sit and Vatner,\textsuperscript{37} and Boetcher et al.\textsuperscript{38} with respect to changes in heart rate, arterial pressure, left atrial pressure, and hematocrit in conscious dogs subjected to a similar degree of volume loading. Also the increases that we measured in plasma ANF in intact dogs in response to volume loading are comparable to data previously reported\textsuperscript{23,27,29} and to our own studies.\textsuperscript{5,7,9} Therefore, in addition to sparing atrial neuronal reflexes, surgery did not cause major changes in the cardiac dynamic response to volume expansion, and atrial appendectomy effectively eliminated the rise in ANF resulting from a large increase in atrial stretch without disturbing atrial reflexes or systemic hemodynamics. These data indicate that the intravascular volume-sensitive pool for ANF resides in the atrial appendages, that a sufficiently large atrial stretch will cause its release, and that this release of ANF is eliminated by atrial appendectomy.

Our second set of experiments was performed to answer the following question: Can the increased levels of ANF that are achieved during volume loading affect renal function? Atriopeptin 24 was infused to increase steady-state plasma ANF to \(\sim 250\) pg/ml (i.e., levels comparable to those achieved during volume loading). Our data indicate that there is a dose-dependent change in sodium and water excretion with changing plasma ANF. Significant changes in renal function first occur at a plasma ANF level of \(\sim 250\) pg/ml. Plasma ANF of \(100\) pg/ml did not produce significant changes in salt and water excretion. ANF infusion rates that we used did not alter mean arterial pressure and therefore did not change renal perfusion pressure. Similar results have been obtained by us previously\textsuperscript{9} and by other investigators.\textsuperscript{28,29,39–41} The contribution of ANF alone to salt and water excretion in the absence of volume loading accounts only for a modest increase in urine output by 0.5 ml/min. Evidence suggests that the renal effects of ANF depend on intravascular volume such that ANF may have more significant effects on renal function after even a small degree of previous volume expansion.

In our final set of experiments, we administered 1,000 ml saline to produce atrial stretch and ANF release while measuring urine flow rate and sodium excretion. Again, there were no significant differences in the hemodynamic response to volume loading in the dogs studied, and the Bainbridge reflex was preserved after removal of the atrial appendages. Atrial appendectomy reduced total sodium and water excretion after volume expansion by \(\sim 50\%\) at 4 hours. As shown in Figure 4, dogs with intact atrial appendages had an early and marked increase in urine flow rate and in sodium excretion that was significantly different for the first 60

**FIGURE 3.** The time course of changes in plasma atrial natriuretic factor (ANF) during the second set of volume-loading experiments. ANF did not increase significantly in atrial-appendectomized dogs (●). ANF in intact dogs (▲) initially increased rapidly and then fell off rapidly during the remainder of the experiment. On the abscissa, 500 and 1,000 indicate milliliters of volume expansion. *p<0.05 compared with appendectomized dogs.

**FIGURE 4.** Graphs showing the change in urine flow rate (\(\Delta UV\), top panel) and the change in urine sodium excretion (\(\Delta U_{Na}V\), bottom panel) during the 240 minutes after volume loading. During the first 60 minutes after volume loading, UV and \(U_{Na}V\) increased by significantly greater amounts in intact dogs (▲) compared with dogs after atrial appendectomy (●). UV and \(U_{Na}V\) were not different in intact and atrial-appendectomized dogs by 100 minutes after volume loading. *p<0.05 compared with appendectomized dogs.
minutes after the volume loading, and the early peak in urine output was essentially eliminated by atrial appendectomy. However, 80 minutes after volume loading, both atrial-appendectomized and intact dogs had comparable diuresis and natriuresis with no significant difference between the two groups. Thus, the enhancement in renal function in dogs with atrial appendages intact occurred early in the course of volume loading. The early peak and falloff in plasma ANF is similar to, although it appears much earlier than, the peak and falloff in sodium and urine excretion. This is consistent with our study in the monkey, which indicated a plasma half-life of ANF of 3 minutes and a half-life for the change in renal function of 6–10 minutes.42 Therefore, increased ANF may partially determine the early time course of salt and water excretion. However, ANF is clearly not the sole determinant of early volume-induced diuresis and natriuresis. Removal of ANF release by atrial appendectomy blunts but does not eliminate the diuresis, and the contribution of ANF alone to urine excretion in our second set of experiments in which atriopeptin 24 was administered to euvoletic dogs amounted to a relatively modest (39%) increase in urine flow. This suggests an interactive role for ANF with other factors (e.g., mean arterial pressure, cardiac output, renal blood flow, and plasma oncotic pressure) in promoting diuresis.43

Control levels of ANF were not statistically different in dogs with intact atrial appendages and in dogs with atrial appendages removed, even though the increase in ANF with volume loading was abolished after atrial appendectomy. This has been noted by other investigators13,14,19,21 and may be explained either by an inability to distinguish quantitatively between different, but low, plasma ANF levels or by the possibility that control levels of ANF originate partially from the ventricles.29,44 Our previous work suggests that we are able to distinguish among low levels of ANF.6,7

Our data confirm and extend observations made in monkeys19 and in rats.10–18 Thus, Benjamin et al19 demonstrated that bilateral atrial appendectomy attenuated the rise in ANF and also the increase in urinary water and sodium excretion with volume loading in conscious monkeys. Villareal et al,13 who performed acute volume-loading experiments in the anesthetized rat, showed that atrial appendectomy suppressed a rise in ANF and blunted diuresis and natriuresis by ~50% compared with rats with intact atrial appendages. Schwab et al16 showed qualitatively similar results in rats after unilateral right atrial appendectomy. Kobrin et al21 also demonstrated similar results in experiments in which bilateral atrial appendectomy reduced sodium and water excretion after a 25% increase in intravascular volume, and Hirth et al27 indicated that the renal response to acute volume load in rats was related to ANF, since sodium and water excretion could be attenuated with an anti-ANF antibody.

Our study contrasts with other studies performed in dogs, however. In general, the differences can be explained by comparing their volume-loading techniques with ours, examining whether a sufficient atrial stretch increased ANF to sufficiently high levels so as to affect renal function. Using 250 ml blood given slowly over 20 minutes, Benjamin et al21 volume-expanded anesthetized normal and atrial-appendectomized dogs. Consequently, they measured only small increases in plasma ANF from 25 to 42 pg/ml, indicating only a small degree of atrial stretch. They did not directly measure atrial stretch by quantitating atrial pressure or diameter changes, and it is difficult to ascertain the magnitude of change in stretch that they achieved. Central venous pressure increased only from -1.5 to 0 cm H2O (-1 mm Hg) in their sham-operated animals and from 0 to 3 cm H2O (-2 mm Hg) in their atrial-appendectomized dogs and was always within the normal, euvoletic range. Our volume-loading experiments have consistently caused large increases in mean left atrial pressures and atrial diameter, indicating a large increase in wall stress.6,7 The small increases in plasma ANF observed by Benjamin et al21 are not physiologically important, since ANF in normal dogs varies between 20 and 80 pg/ml and the normal range for plasma ANF in humans is up to 100 pg/ml.20 Also, anesthesia alters ANF release in dogs8 and in rats.14

Salazar et al,23 while focusing on the effects of acute and chronically administered sodium on ANF release, used a large volume load equal to 5% of the total body weight during acute experiments but gave the saline over a 30-minute period; therefore, ANF only increased from 48 to 119 pg/ml. Atrial stretch was not quantitated in those studies. The relatively small increment of ANF was unrelated to changes in renal function. Similarly Kinter et al24 performed atrial appendectomy in four conscious dogs and compared renal responses to a 25% blood volume increment delivered over 20 minutes. A relatively small diuresis and natriuresis resulted. Neither ANF nor atrial stretch was measured during volume infusion.

Cowley et al,22 using conscious dogs, showed large increases in urine and sodium excretion with a volume load of 400 ml saline that was delivered over 10 minutes. Urine flow increased by >4 ml/min, and urine sodium excretion increased by >400 μeq/min; these results are similar to our own. Atrial appendectomy greatly blunted the diuresis that followed volume loading, similar to our results. Volume loading produced no increase in plasma ANF whether or not atrial appendectomy had been performed. This observation stands in contrast to our experience and to the observations of other investigators who have consistently shown increased plasma ANF with atrial stretch.6,7,23 Since atrial stretch was not measured during the experiments by Cowley et al, it may be that insufficient stimulus for ANF release was achieved; therefore, strictly speaking, these experiments do not test the relation of ANF release to atrial appendectomy or the effects of ANF on renal salt and water excretion because no significant changes in plasma ANF were induced. Cowley et al suggested that atrial appendage–dependent factors other than ANF may be important determinates of renal function. This could be true. However, these factors are unrelated to afferent atrial neuronal stimulation, which was unchanged by appendectomy, and these factors are related to ANF, which was abolished by appendectomy.

In summary, in the normal conscious dog, a large and rapid increase in plasma volume causes atrial stretch, increased plasma ANF, and diuresis and natriuresis. When that same volume load is given to atrial-appendectomized dogs, plasma ANF does not increase, and the early diuresis and natriuresis are significantly re-
duced even though the hemodynamic response and atrial reflexes appear to remain intact. Therefore, atrial appendectomy eliminates the source of ANF during volume loading, causing reduced renal capability to excrete an acute salt and water load.

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