Role of Right and Left Atria in Natriuresis and Atrial Natriuretic Factor Release During Blood Volume Changes in the Conscious Rat

Raul Garcia, Marc Cantin, and Gaétan Thibault

This study investigated whether excision of either the right or left atrial appendage of rats alters their natriuretic response and the release of atrial natriuretic factor during acute blood volume expansion or reduction. These animals were subjected to a thoracotomy and either had their right or left atrial appendages removed or underwent a right or left atrial sham appendectomy for comparative, control purposes. Intrajugular vein, intracarotid artery, and intravesical catheters were installed 3-4 weeks later under sodium pentobarbital anesthesia. Then, when the rats were conscious, blood volume was expanded using blood from donor rats once every 15 minutes in 3 increments of 10% of the calculated total blood volume at a rate of 5 ml/kg/min. Blood and urine samples were collected before volume expansion and at the end of each 15-minute period, with the withdrawn blood being replaced. A maximal fourfold increase in urinary volume, urinary sodium excretion, and plasma atrial natriuretic factor was observed in all but the right-atrial-appendectomized animals. Plasma atrial natriuretic factor, urinary volume, and urinary sodium excretion were correlated in all 4 groups. No significant changes in blood pressure or hematocrit were noted. Plasma vasopressin, measured at the end of volume expansion, was significantly lower in animals subjected to left atrial appendectomy. High-performance liquid chromatography of plasma from the control groups indicated that most of the released ANF during blood volume expansion corresponded to a high molecular weight peptide. Additional rats, processed as above, were subjected to 10% blood volume decrements. Urine and blood were collected before the blood volume reduction and at 15-minute intervals after each of the 3 decrements of 10% volume. A gradual and marked decline in urinary volume and sodium excretion was seen in all 4 groups but only the control groups showed a significant lowering of plasma atrial natriuretic factor. Plasma vasopressin was equally high in all groups. A significant but negative correlation was evident between the plasma levels of atrial natriuretic factor and vasopressin (r = -0.51, p < 0.01). Left atrial appendectomy induced a chronic elevation of systemic arterial blood pressure, which may represent a new model of experimental hypertension whose mechanisms remain to be investigated. It can be concluded that the right atrium, probably by releasing atrial natriuretic factor, may be involved in the short-term regulation of blood volume, but other neural and hormonal systems (such as vasopressin) may play more important roles in volume conservation during hemorrhage. (Circulation Research 1987;61:99-106)

It has been reported that left atrial distension produces diuresis and natriuresis, which are attributed to stretch receptors in the left atrium responsible for a homeostatic mechanism linking changes in circulatory volume with renal responses. However, distention of the right atrium and of both atrial appendages also exerts similar effects, which are enhanced in blood volume-expanded animals.

The diuresis elicited by left atrial distension has been partially ascribed to an inhibition of arginine vasopressin (AVP) release, but the natriuretic response is more difficult to explain. The discovery of atrial natriuretic factor (ANF) secretory-like atrial-specific granules has provided new perspectives in understanding the mechanisms controlling diuresis and natriuresis induced by increased intra-atrial pressure. More recently, it has been demonstrated that stimulation of left atrial receptors releases ANF into the circulation via a mechanism more related to atrial pressure than to a reflex triggered by vagal or sympathetic efferents, thus reinforcing the possibility that ANF may indeed be involved in the natriuresis caused by atrial distension.

We have reported previously that in adrenalectomized rats undergoing steroid treatment, sodium chloride administration produces a decrease in tissular ANF content in the right but not in the left atrium. Furthermore, in spontaneous and nonspontaneous models of experimental hypertension with high levels of plasma ANF, the left atrium is found to have a lower tissular ANF content than the right. These observations suggest that, depending on the stimuli, atria could release ANF independently of each other.

To challenge this hypothesis, we have investigated whether ablation of either the right or left atrial appendage modifies the natriuretic response as well as ANF release during acute blood volume expansion and retraction.

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This work was supported in part by grants from the Medical Research Council (MT-7665), the Ministère de la Science et de la Technologie du Quebec, and the Quebec Heart Foundation
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Received August 7, 1986; accepted February 27, 1987
Materials and Methods

A left parasternal thoracotomy was performed on male Sprague-Dawley rats (180–200 g) under light ether anesthesia without mechanical respiration. Either the left or right atrial appendages were exposed, extended to their maximum length, ligated around their bases, tied, and removed (auricular appendectomy). It can be estimated that the right atrium, because of its proximity with the cava veins, was 50% removed. The left atrium, which is more detached, was about 80% removed. The incision was closed in two planes: one for thoracic muscle and bone structures; the other for skin. No more than 60 seconds were allowed to elapse from the moment the skin was opened to the time it was closed. The immediate postsurgery mortality rate was less than 10%, no late postsurgery mortality occurred.

Two other groups were subjected to thoracotomy, after which the heart was exposed and either the right atrium handled (right appendectomy control group) or the heart lifted and the left atrium handled (left appendectomy control group).

Three to four weeks after surgery, the experiments were continued as described below.

Protocol 1

The rats were anesthetized with sodium pentobarbital (60 mg/kg body weight ip) prior to installation of left intrajugular vein, left carotid artery, and bladder catheters. Once the rats were fully conscious (1–2 hours after surgery), they were placed in restrainer cages, and the intra-arterial catheter was connected to a Gould Statham P23ID (Hato Rey, Puerto Rico) pressure transducer for continuous monitoring of mean blood pressure (BP). Blood samples (1 ml) were withdrawn every 15 minutes, at 0, 15, 30, and 45 minutes, through the arterial catheter, and the blood volume was replaced immediately with blood from donor rats. Urine was collected at 15 minutes before time 0 and every 15 minutes thereafter (i.e., at time 0, 15, 30, and 45 minutes).

At 0, 15, and 30 minutes, after the blood samples were withdrawn, blood from donor rats was infused through the intravenous catheter into the experimental animals in amounts equal to 10% increments of the total blood volume, which was assumed to be 50 ml/kg body weight. The blood infusions were given at a rate of 5 ml/kg/min.

Protocol 2

The rats were prepared and blood and urine samples were collected as described above. Blood volume was reduced in 10% decrements. The withdrawn blood was used to measure ANF, but the volume was not replaced. These decrements were carried out at 0, 15, and 30 minutes, with an additional blood sample being taken at 45 minutes.

To measure immunoreactive ANF, blood was collected in glass tubes containing the following protease inhibitors in a final concentration of 1.0 x 10^-5 M ethylenediaminetetraacetic acid (EDTA), 5 x 10^-6 M pepstatin, 1,000 U/ml aprotinin, and 3 x 10^-5 M phenylmethylsulfonyl fluoride. The samples were immediately centrifuged at 3,000 rpm for 10 minutes at 4°C. ANF was extracted from plasma using Corning glass Vycor beads (Corning, N.Y.), after which the extracts were lyophylized and kept at -20°C until radioimmunoassayed (RIA), as described elsewhere. The detection limit of the assay is 0.75 pg/tube. The inter- and intra-assay coefficients of variance are below 14%.

At the end of both protocols (at 45 minutes), blood was withdrawn from some animals and placed into tubes containing 1 x 10^-3 M EDTA and 100 U/ml aprotinin. AVP was measured by RIA according to the method of Larose et al. The lower limit of detection of this assay is 0.28 pg/tube. The inter- and intra-assay coefficients of variance are below 12%.

Blood samples, taken at 45 minutes from 5 animals of the right appendectomy control group, were processed as for immunoreactive ANF, pooled, and analyzed by reverse-phase high-performance liquid chromatography (HPLC) on a C18 Bondapak column (Waters Associates, Milford, Mass.) (0.39 x 30 cm), using a 15–55% linear gradient of acetonitrile containing 0.1% trifluoroacetic acid at 0.5%/min and a flow rate of 1 ml/min. Two-minute fractions were collected.

Microhematocrit was measured at 0, 15, 30, and 45 minutes. Urinary volume (UV) was calculated by weight, and urinary sodium was assessed by flame photometry.

Statistical Analysis

The results are expressed as means ± SEM. The data presented in Figure 1, 2, 5, 6, and 7 and the hematocrit values were analyzed by two-way analysis of variance with repeated measures to globally test the time effect, the group effect, and the group interaction by time. One-way analysis of variance was employed with repeated measures for each group to globally test the time effect. Dunnet's test was applied whenever a level of significance was found (p<0.05).

The data in Figures 4 and 9 were analyzed by one-way analysis of variance and Dunnet's test.

Mean arterial BP before and after volume expansion was analyzed by the paired Student's t test.

Results

Protocol 1

Figure 1 depicts the UV (upper panel), UoV (middle panel), and plasma ANF values (lower panel) in control and right atrial appendectomized animals before and after graded volume expansion. No significant differences between either group were noted at time 0, although the right atrial appendectomized animals tended to have higher plasma ANF values than the controls. After graded volume expansion, there was an approximately fourfold increase of UV, UoV, and plasma ANF, but only in the control group.

Figure 2 illustrates the same parameters in left atrial appendectomized rats and their controls. Graded blood volume expansion again produced a fourfold rise in UV, UoV, and plasma ANF in the control group.
while rats subjected to left atrial appendectomy responded with a sharp elevation of all 3 parameters after the first 10% blood volume increment and then responded with a rapid decrease in UV and plasma ANF to pre-expansion levels during the following 10% blood volume increments. A positive but poor correlation was registered between plasma ANF, UV, and UNaV in all 4 groups (Table 1).

Figure 3 shows the chromatographic pattern of plasma from the right atrial appendectomy controls after 3 increments of 10% blood volume. An atrial factor eluted at 25–30% of acetonitrile corresponded to a short-form peptide, but the factor eluted at 40% acetonitrile corresponded to a longer, more abundant peptide.

Figure 4 demonstrates the plasma AVP values after blood volume expansion. Because of the small number of samples, both control groups were pooled. Plasma AVP was significantly lower in left atrial appendectomized rats than in either of the controls or the right atrial appendectomized animals.

BP and hematocrit did not change significantly during blood volume expansion (data not shown).

Protocol 2

Figure 5 depicts the UV (upper panel), UNaV (middle panel), and plasma ANF values (lower panel) during graded blood volume reduction in the control and right atrial appendectomy groups, both of which experienced a gradual decline of UV and UNaV. Plasma ANF was decreased significantly at 45 minutes only in the controls.

Similar results were obtained when the left atrial appendectomized rats and their controls were bled (Figure 6). Plasma ANF was usually lower in the control group, this tendency being statistically significant at 45 minutes (p < 0.05). An analogous pattern of diuresis was observed. Plasma ANF did not significantly change in left atrial appendectomized rats during blood volume retraction.

No correlation was evident between plasma ANF levels and either diuresis or natriuresis.

A significant (p < 0.01) decrease of hematocrit (Table 2) and BP (Figure 7) was seen in all groups during the last two experimental periods of blood volume reduction. The plasma AVP values registered at the end of Protocol #2 were similar in all groups, being 44.34 ± 15, 46.11 ± 4.49, 51.17 ± 12.07, and 51.90 ± 12.20 pg/ml for the control and right atrial appendectomized groups and the control and left atrial appendectomized groups, respectively.

A significant negative correlation (r = −0.51, p < 0.01) was evident between hematocrit and plasma ANF during graded blood volume expansion.
Table 1. Correlation Between Plasma ANF and UV or U_{NaV} During Graded Blood Volume Expansion in Right or Left Atrial Appendectomized Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>ANF vs UV</th>
<th>ANF vs U_{NaV}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 38)</td>
<td>0.44</td>
<td>0.46</td>
</tr>
<tr>
<td>Right atrial appendectomy (n = 39)</td>
<td>0.42</td>
<td>0.55</td>
</tr>
<tr>
<td>Control (n = 32)</td>
<td>0.50</td>
<td>0.47</td>
</tr>
<tr>
<td>Left atrial appendectomy (n = 47)</td>
<td>0.52</td>
<td>0.53</td>
</tr>
</tbody>
</table>

*p < 0.01

*p < 0.01) between plasma ANF and AVP was noted when the values from all experimental groups were pooled (Figure 8).

When both protocols were considered together, the basal values of direct mean BP were significantly higher in left atrial appendectomized animals than in the other groups (p < 0.01, Figure 9), being 110 ± 3, 119 ± 3, 116 ± 5, and 146 ± 2 mm Hg for the control and right atrial appendectomized groups and the control and left atrial appendectomized groups, respectively.

**Discussion**

We have reported earlier that the addition of sodium chloride to steroid replacement therapy in adrenalectomized rats produces a relative decrease of ANF content in the right but not in the left atrium. Conversely, only the left atrium seems to be involved in the increased release of ANF in spontaneous and one-kidney, one-clip models of experimental hypertension in the rat. These findings prompted us to investigate whether both atria could participate in blood volume regulation, independent of each other, and we chose graded increments and decrements of blood volume as a model. Homologous whole blood was used instead of saline to produce graded blood volume expansion because whole blood leaves the composition of the recipient animal's blood unaltered, and saline infusion may cause intracompartmental fluid shifts.

In anesthetized rats, saline-induced blood volume expansion equal to approximately 50% of total blood volume elicited a fourfold to fivefold increase of plasma ANF with a rise in right atrial pressure. Left atrial distension releases ANF into the circulation, probably as a result of direct stretch stimulation rather than of reflex activation. It has also been demonstrated recently that right or bilateral atrial appendectomy blunts the natriuresis induced by blood volume expansion. However, these studies did not examine whether the blunting of the natriuretic response is accompanied by changes in plasma ANF or whether the left atrium participates in the diminution of natriuresis.

We have now demonstrated that graded blood volume expansion produces a fourfold increase in diuresis, sodium excretion, and plasma ANF in control groups of atrial appendectomized rats and complete abolition of the diuretic and natriuretic response in...
right atrial appendectomized animals. The plasma ANF levels tend to be higher in right appendectomized rats but are not statistically different from control values. The finding that plasma ANF levels in right atrial appendectomized animals are similar to the values of the control after blood volume expansion (although the right atrial appendectomized group presented lower diuresis and natriuresis, Figure 1), suggests that plasma ANF is not the only factor involved in sodium excretion during volume expansion. Furthermore, significant but poor correlation is obtained between plasma ANF, diuresis, and natriuresis (Table 1).

The pattern of diuresis, sodium excretion, and ANF release in left atrial appendectomized animals (Figure 2) is totally unlike that observed when the right atrial appendage is removed. The rapid increase of diuresis, natriuresis, and plasma ANF after the first 10% blood volume increment is followed, in the case of diuresis and plasma ANF, by a decline to basal levels during subsequent volume increments. The controls display a gradual elevation of diuresis, natriuresis, and plasma ANF, representing, after 3 increments of 10% volume, a fourfold rise in all 3 parameters, similar to the right appendectomy control group.

The blood volume increments used in our study are known to heighten pressure in both atria. Moreover, since systemic veins and lung vascular beds act as a continuous low-pressure system, changes in left atrial pressure should be reflected in the right atrium as well. With the total capacity of the left atrium reduced by removal of its appendage, we may assume that, given a certain blood volume, intra-atrial pressure should be comparatively higher than in an intact atrium. Moreover, the increase in arterial blood pressure observed in left atrial appendectomized animals by increasing end-diastolic left ventricular pressure may also contribute to rising left atrial pressure, and this raised left atrial pressure should be transmitted to the right atrium. An enhanced basal right atrial pressure could explain the observed tendency of left atrial appendectomized animals to have higher basal diuresis, natriuresis, and plasma ANF values, as seen in Figure 2. A small increment in blood volume would further raise atrial pressure, resulting in a sharp renal response and ANF release (Figure 2). In addition, left atrial

### Table 2. Effect of Graded Blood Volume Reduction on Hematocrit

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hematocrit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 minutes</td>
</tr>
<tr>
<td>Control (n = 12)</td>
<td>50 ± 0.6</td>
</tr>
<tr>
<td>Right atrial appendectomy (n = 13)</td>
<td>48 ± 0.9</td>
</tr>
<tr>
<td>Control (n = 12)</td>
<td>47 ± 0.9</td>
</tr>
<tr>
<td>Left atrial appendectomy (n = 12)</td>
<td>48 ± 0.9</td>
</tr>
</tbody>
</table>

*p < 0.01 vs time 0 Values are means ± SEM
appendectomy may by itself induce pulmonary arterial hypertension, which may increase end-diastolic right ventricular pressure and further augment right atrial pressure.

Since removal of the right but not the left atrial appendage abolishes the renal response and the release of ANF following blood volume expansion, our study indicates that, atrial appendages being a rich source of ANF, the right atrium plays an important regulatory role in volume homeostasis, which may be mediated by the peptide. Experiments in dogs designed to raise right or left atrial pressure by atrial stretch have failed to demonstrate a relation between plasma ANF and the renal response. However, the fact that the latter can be abolished in rats during blood volume expansion by the administration of ANF antiserum supports the hypothesis that ANF may be the humoral mediator of the renal response. However, the possibility that these apparent contradictory results could be secondary to species differences cannot, however, be ruled out. Since the atrial pressure reached by distending the atria with a balloon is higher than that generated during moderate blood volume changes, the triggering of a neural mechanism has been postulated by Goetz et al.

There is a consensus that the main circulating form of ANF in the normovolemic rat is a 28-residue peptide (Ser 99-Tyr 126). A similar peptide has been found to be released by the perfused rat heart. The secretory granules of atrial cardiocytes contain pro-ANF (Asn 1-Tyr 126), which is not detected in the circulation and which is very slowly transformed by the blood into a shorter, more active peptide (Thibault et al, unpublished observations). It seems then that blood is not an important factor in the processing of ANF. We now report that volume expansion causes the release not only of a short form of ANF but also of a peptide (as seen in Figure 3) that is eluted where longer forms of ANF usually appear. The significance of this finding is not clear at present. It is possible that during a stressful situation (e.g., acute volume expansion), the release of ANF in a brief time period is of such magnitude that the processing enzymes are not able to transform pro-ANF into a shorter peptide. Whether the pro-peptide, once released, can be processed by another tissue, such as vascular endothelial cells, has yet to be investigated.

The surgical preparation of the rats and their manipulation probably account for the higher than normal plasma AVP levels observed in control and right atrial appendectomy rats after blood volume expansion (Figure 4). Under the same experimental conditions, however, plasma AVP is lower in animals subjected to left atrial appendectomy than in the other 2 groups just mentioned. Brennan et al have reported that plasma AVP is decreased by elevated left but not right atrial pressure, indicating a major role for the left atrium in the control of AVP release during changes in intratral pressure. Moreover, plasma AVP levels can be modified by minor pressure alterations in the left atrial appendage, both being negatively correlated. Furthermore, increases in plasma AVP can be prevented by raising left atrial pressure. Therefore, we suggest that because the volume of the left atria is reduced after appendectomy, volume expansion would augment intra-atrial pressure to a level higher than in the control and right atrial appendectomy groups thus preventing the AVP release induced by surgery and resulting in lower plasma AVP levels in left atrial appendectomized rats.

After graded blood volume reductions by hemorrhage, a gradual decline in urine flow is observed with decreased urinary flow in all groups. However, only the controls present a parallel decrease of plasma ANF, which is significant only after a total of 30% of blood volume is lost and when a significant drop in blood pressure is manifested. The finding of high plasma AVP values in all 4 groups is not unexpected since it is well known that hemorrhage produces a diminution of urinary flow associated with a rise in plasma AVP concentrations. As suggested by Henry et al, the fall in atrial distention and pressure during hemorrhage could be a major cause of the enhancement in AVP release. Since hemorrhage produces a progressive decline in urinary flow in ANF.
both the control and appendectomy groups and since only the controls show a significant decrease of plasma ANF, it may be concluded that this cardiac peptide does not play an important role in volume homeostasis during acute volume losses. The lack of a correlation between either natriuresis or diuresis and plasma ANF also argues against the active involvement of ANF in volume homeostasis. Another humoral system, such as AVP, and renal hemodynamic changes may exert a more important function in sodium and fluid conservation under the present experimental conditions.

As seen in these blood volume expansion experiments, rats whose left atrial appendages are removed usually have higher plasma ANF levels, a tendency that is unmasked when plasma ANF falls very low in the controls (Figure 6). As discussed above, these higher plasma ANF levels in left atrial appendectomized animals could be secondary to the reduction of both the total atrial internal volume and distensibility, which could, with a given blood volume, produce higher atrial pressures than in the controls. Another factor that has to be considered is the high arterial BP observed in left atrial appendectomized animals, which by raising end-diastolic ventricular pressure may further increase left atrial pressure.

When all plasma ANF and AVP concentrations are pooled, a significant but minor correlation is noted between them. It has been reported that ANF administration inhibits hemorrhage-induced AVP release, implicating the cardiac peptide in AVP regulation. The fact remains, however, that in our experiments, right or left atrial appendectomized animals displayed similar plasma ANF levels conjointly with significantly different plasma AVP concentrations, which suggests that the release of both hormones could be more an epiphemomenon derived from their opposite regulating mechanisms rather than a direct dependency.

One surprising observation in our experiments was the direct mean arterial BP recorded in conscious, left atrial appendectomized rats (Figure 9). The mechanism(s) involved in the elevation of BP are not known and no references are found in the literature regarding this particular problem. However, it has been reported that left atrial appendectomy induces a chronic increase of pulmonary vascular resistance in the dog. Acute enhancement of pulmonary artery pressure by means of an inflated balloon raises systemic BP in the rat by means of blood volume expansion, indicating that the right atrium, by secreting ANF, could be involved in the short-term regulation of blood volume during acute changes. ANF seems not to be involved in blood volume conservation during hemorrhage where other neural and hormonal systems, such as AVP, could play a more important role. Finally, left atrial appendectomy elicits a rise in systemic BP whose mechanisms remain to be investigated.

Acknowledgments

The authors thank Ms. Marie-Claude Gaudreau, Ms. Josée Bilodeau, and Ms. Chantal Mercure for their excellent technical assistance, and Ms. Claudine Jutras for her valuable secretarial work. The authors wish to acknowledge the technical assistance of Mrs. Suzanne Diebold and Louise Théroux.

References


Key Words: atrial natriuretic factor • atrial appendages • volume expansion • hemorrhage • blood pressure
Role of right and left atria in natriuresis and atrial natriuretic factor release during blood volume changes in the conscious rat.

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doi: 10.1161/01.RES.61.1.99

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

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