PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **183**, 54–58 (1986)

Effects of Atrial Appendectomy on Circulating Atrial Natriuretic Factor during Volume Expansion in the Rat (42385)

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Abstract. This study examined the changes in the circulating level of endogenous atrial natriuretic factor during diuresis and natriuresis produced by acute volume expansion in anesthetized rats with either bilateral atrial appendectomy ($n = 9$) or sham operation ($n = 9$). Following control measurements in the sham-operated rats, 1% body weight volume expansion with isotonic saline produced an increment in urinary sodium excretion of over 4 μeq/min ($P < 0.05$) while urine volume increased by more than 20 μl/min ($P < 0.05$). These responses were associated with a significant increase in immunoreactive plasma atrial natriuretic factor from a baseline value of 82 ± 10 pg/ml to a level of 120 ± 14 pg/ml ($P < 0.05$). In contrast, in the group of rats with bilateral atrial appendectomy an identical degree of volume expansion increased urinary sodium excretion and urine volume by only 0.61 μeq/min ($P < 0.05$) and 3.07 μl/min ($P < 0.05$), respectively. In this group, immunoreactive plasma atrial natriuretic factor remained statistically unchanged from a control value of 70 ± 12 pg/ml to a level of 82 ± 16 pg/ml ($P > 0.05$). Comparison of the two groups indicates that the natriuresis, diuresis, and plasma atrial natriuretic factor levels during volume expansion were significantly reduced in the rats with bilateral atrial appendectomy. No differences in mean arterial pressure and heart rate were observed between the two groups. These data demonstrate that removal of both atrial appendages in the rat attenuated the release of atrial natriuretic factor during volume expansion; and this effect, in turn, was associated with a reduction in the natriuretic and diuretic responses.

The atria of mammals contain a family of structurally related polypeptides (1–4) collectively referred to as atrial natriuretic factor (ANF). The natural or synthetic cardiac peptides have vasoactive properties as well as potent natriuretic and diuretic effects (5, 6). The remarkable hemodynamic and renal actions of ANF suggest that the cardiac atria may be involved in the regulation of body fluid balance through the release of the ANF peptides. Recent studies in the rat have investigated the hemodynamic signals that induce the release of ANF. Increments in atrial pressure and/or volume have been reported (7, 8) to promote sodium and water excretion. In addition, experiments in anesthetized (9) and conscious (10) rats have indicated that the natriuretic and diuretic responses to acute expansion of the intravascular volume were blunted in rats with prior removal of the atrial appendages. Atrial natriuretic factor is highly concentrated in the atrial appendages (11), and in these studies with atrial appendectomy (9, 10) the decreased ability to excrete sodium and water during acute volume expansion may have been related to a reduction in ANF available for release. However, in these investigations (9, 10) the lack of actual measurements of circulating ANF limits this interpretation. The present study was designed to evaluate the hypothesis that the reduced natriuretic response during acute volume expansion in rats with bilateral atrial appendectomy is mediated by a decrement in the circulating levels of ANF.

Methods. Sprague–Dawley male rats with body weights between 275 and 387 g were used. The animals were fed a commercial chow diet containing 0.11 and 0.23 meq per gram of sodium and potassium, respectively. Rat chow and tap water were available *ad libitum*. For the acute experiments, the rats were anesthetized with 5-*sec*-butyl-5-ethyl-2-thiobarbituric acid (Inactin, 100 mg/kg ip) and placed on a surgical tray over a heating pad to maintain body temperature between 37 and 39°C. A tracheostomy was performed and the animals were maintained on positive-pressure ventilation throughout the experimental pro-

0037-9727/86 $1.50

tocol. Indwelling heparinized polyethylene catheters (PE 50) were positioned in the left jugular vein for the infusion of solutions, in the right carotid artery for blood sampling and measurement of mean arterial pressure (MAP) and heart rate (HR), and in the right femoral vein for replacement of blood. A catheter (PE 90) was inserted into the dome of the urinary bladder for the collection of urine.

Two different groups of experiments were performed. In the rats with bilateral atrial appendectomy (Group 1, $n = 9$) a median sternotomy was produced, and following reflection of the pericardium both atria were exposed. The right and left atrial appendages were gently retracted to their maximum length, loop ligatures (4-O silk) were tightened around the base of the atrial appendages, and both appendages were excised. The chest was then covered with gauze soaked in isotonic saline. In a second group of rats with sham atrial appendectomy (Group 2, $n = 9$) an identical surgical protocol was followed with the exception that the atria were not manipulated. In both groups, after completion of surgery, a maintenance infusion of isotonic sodium chloride at a rate of 25 μl/min was begun. Thirty minutes were allowed for recovery from the preparatory procedures. Mean arterial pressure and heart rate were continuously monitored with a Statham P 23D6 pressure transducer and tracings were made on a Hewlett-Packard 7702B recorder.

Experimental protocol. An identical experimental protocol was performed in the two groups of rats studied. Following the 30 min of equilibration, a 15-min control period (C) was obtained. During this time all urine was collected for the determination of urine volume ($U\dot{V}$) and urinary sodium ($U_{Na}\dot{V}$) and potassium ($U_K\dot{V}$) excretions. At the end of the control period, 2 ml of blood was withdrawn for the measurement of immunoreactive plasma atrial natriuretic factor (iANF) and hematocrit (Hct). All blood samples were replaced with equal volumes of fresh donor blood obtained from normal anesthetized rats by cardiac puncture. Following a second 10-min equilibration period, an intravenous infusion of isotonic sodium chloride in a volume equal to 1% of body weight was administered for 30 min. During this experimental (E) period, the average volume of infusion was 3.24 ± 0.14 ml for Group 1 and 3.12 ± 0.11 ml for Group 2 ($P > 0.05$). Urine collections were made during the last 15 min of the experimental volume expansion period followed by a final blood sample for determination of plasma iANF and Hct.

Analytical methods. Urinary sodium and potassium concentrations were determined by flame photometry. Hematocrit was determined by the microcapillary tube method. Immunoreactive atrial natriuretic factor levels were measured in extracted plasma by radioimmunoassay. Briefly, blood samples were collected in tubes containing EDTA (1 mg/ml blood), aprotinin (500 KIU/ml blood), and soybean trypsin inhibitor (50 BAEE units/ml blood). The blood samples were centrifuged at 1500*g* and 10°C for 20 min. The plasma samples were stored at −20°C for a period not exceeding 2 weeks. Rat plasma (0.75 ml) was applied to a C18 reverse-phase BondElut column (3 ml/200 mg; Analytichem International) equilibrated previously with 0.1 *M* acetic acid (HOAc). The column was then washed with 1 ml of 0.1 *M* HOAc. ANF was eluted from the column with 1 ml of 50% acetonitrile–50 m*M* sodium phosphate, pH 7.4. The extraction of ANF from rat plasma by this procedure is 86.5 ± 7% (mean ± SD; $n = 25$) as estimated by the recovery of radiolabeled ANF. The column eluate containing ANF was evaporated to dryness and stored overnight at −20°C. No loss of ANF immunoreactivity has been observed when the dried plasma ANF extracts are stored at this temperature for up to a 1-week period. Immediately prior to assay, the residue was resuspended with 0.375 ml of assay buffer. The radioimmunoassay of ANF is a modification of the method reported by several groups (12–14). Rabbit anti-human atrial natriuretic factor serum (Peninsula Laboratories) which cross-reacts 100% with rat ANF and (3-[^{125}I]iodotyrosyl^{28}) human atrial natriuretic factor (2000 Ci/mmole; Amersham Corp) were utilized in this procedure. Bound ANF was separated from free ANF by a double-antibody precipitation technique.

Serial dilution of rat plasma extracts resulted in the displacement of radiolabeled ANF that paralleled the displacement curve obtained with authentic human ANF (Bachem). The amount of unlabeled human ANF required to

displace 50% of radiolabeled ANF was 41 ± 19 pg (mean ± SD; 30 assays). The sensitivity of this procedure was 3 pg per 100 μl sample when defined as the least amount of ANF which can be distinguished from blank based on the 95% confidence limits for each value. The intraassay and interassay coefficients of variation were 4.2 and 15.1%, respectively.

The results are presented as means ± SEM. A two factor mixed design analysis of variance with repeated measurements was performed on the raw data and the log transformed data whenever the variances were unequal (15). Significance was determined with the least significance difference test (LSD) within and between appropriate group means. Differences at the 5% level were considered significant.

Results. The hemodynamic, renal excretory, and immunoreactive atrial natriuretic factor data from Group 1 (bilateral atrial appendectomy, $n = 9$) and Group 2 (sham atrial appendectomy, $n = 9$) are presented in Table I. Body weights were not significantly different between groups, averaging 324 ± 14 g in Group 1 and 312 ± 11 g in Group 2 ($P > 0.05$). In both groups of animals MAP and HR remained unchanged from control during volume expansion ($P > 0.05$), and no statistical differences among these hemodynamic functions were noted between the two groups in either the control or experimental periods.

In the bilateral atrial appendectomy rats (Group 1), the control levels of $U_{Na}\dot{V}$ and $U\dot{V}$ were significantly reduced by 58 and 47%, respectively, when compared to the control values of the sham-operated animals (Group 2), $P < 0.05$ for both values). Baseline $U_K\dot{V}$ and Hct were not significantly different between the two groups ($P > 0.05$). Similarly, no difference in the control levels of plasma iANF were noted between the two groups of animals ($P > 0.05$).

In the sham atrial appendectomy rats, 1% body weight intravascular volume expansion with saline significantly increased $U_{Na}\dot{V}$ by more than 11-fold from control ($P < 0.05$), while $U\dot{V}$ was elevated 5-fold ($P < 0.05$) and $U_K\dot{V}$ doubled ($P < 0.05$). These renal excretory changes were associated with a significant increment in plasma iANF from a baseline value of 82 ± 10 pg/ml to a level of 120 ± 14 pg/ml during volume expansion ($P < 0.05$). In contrast, in the bilateral atrial appendectomy rats plasma iANF did not change significantly during volume expansion, from a control value of 70 ± 12 pg/ml to a level of 82 ± 16 pg/ml ($P > 0.05$). In this group, saline infusion increased $U_{Na}\dot{V}$ by only 5-fold from baseline ($P < 0.05$), and $U\dot{V}$ and $U_K\dot{V}$ only doubled ($P < 0.05$ for both values). Comparison between the two groups of animals during volume expansion indicates that the natriuretic and diuretic responses in the bilateral atrial appendectomy rats were markedly attenuated ($P < 0.05$ for both values). Similarly, the level of plasma iANF during saline infusion was significantly reduced in the bilateral atrial appendectomy group compared with the sham group ($P < 0.05$). In both groups of animals the hematocrit was decreased by approximately 7% during volume infusion, and based on these changes the calculated degree (16) of intravascular volume expansion for the two groups was of approximately 13%.

Discussion. The present study demonstrates that acute volume expansion of a modest degree with isotonic saline in rats with intact atria produced significant increments in sodium and water excretion that were associated with significant increases in the circulating levels of immunoreactive atrial natriuretic factor. In contrast, in rats with bilateral atrial appendectomy the magnitude of natriuresis and diuresis following an identical degree of volume expansion was significantly reduced compared with those of the sham-operated group. Associated with these attenuated responses was the failure of iANF to increase significantly during saline infusion. Thus, the composite data indicate that in the intact rats the natriuresis and diuresis observed during acute volume expansion were mediated, at least in part, by the endogenous release of ANF.

These observations confirm and extend the study of Veress *et al.* (9) who reported that removal of the myoendocrine tissue containing ANF altered sodium and water excretion. In anesthetized rats with right atrial appendectomy, these investigators demonstrated that 25% expansion of the estimated blood volume with isooncotic albumin reduced the natriuretic and diuretic responses by approximately 50% compared to the sham-operated controls. These changes occurred in the absence of any alterations in cardiovascular or renal function. In addition, this pattern of re-

TABLE I. EFFECTS OF ACUTE VOLUME EXPANSION IN GROUPS OF RATS WITH BILATERAL ATRIAL APPENDECTOMY (GROUP 1) AND SHAM ATRIAL APPENDECTOMY (GROUP 2)

	MAP (mm Hg)		HR (beats/min)		$U\dot{V}$ (μl/min)		$U_{Na}\dot{V}$ (μEq/min)		$U_k\dot{V}$ (μEq/min)		iANF (pg/ml)		Hct (%)	
	C	E	C	E	C	E	C	E	C	E	C	E	C	E
Group 1 ($n = 9$)	117 ± 2	118 ± 1	390 ± 12	385 ± 9	2.47 ± 0.56	5.54[a] ± 0.89	0.15 ± 0.04	0.76[a] ± 0.19	0.75 ± 0.20	1.25[a] ± 0.23	70 ± 12	82 ± 16	45 ± 1	42[a] ± 1
Group 2 ($n = 9$)	120 ± 3	119 ± 3	384 ± 6	379 ± 7	4.62[b] ± 0.42	24.8[a,b] ± 4.0	0.36[b] ± 0.05	4.39[a,b] ± 0.77	1.09 ± 0.14	2.93[a,b] ± 0.32	82 ± 10	120[a,b] ± 14	44 ± 1	41[a] ± 1

Note. Values are means ± SEM. C = control period. E = experimental period during volume expansion. Abbreviations: MAP = mean arterial pressure. HR = heart rate. $U\dot{V}$ = urinary volume excretion. $U_{Na}\dot{V}$ = urinary sodium excretion. $U_k\dot{V}$ = urinary potassium excretion. iANF = immunoreactive plasma atrial natriuretic factor. Hct = hematocrit.

[a] $P < 0.05$ between control and experimental periods.

[b] $P < 0.05$ between groups.

sponse was not influenced by prior vagotomy. Similar attenuations in sodium and water excretions after acute volume expansion with isooncotic albumin were reported by Korbin *et al.* (10) in the conscious rat with prior removal of both atrial appendages. Although both of these studies are suggestive of a role for endogenous ANF in the regulation of sodium and water excretion during acute volume expansion, lack of plasma ANF measurements limits this interpretation.

In contrast to the experiments of Veress *et al.* (9) and Korbin *et al.* (10), volume expansion was produced with isotonic saline infusion in the present study. It is possible that isotonic saline volume expansion, although minimal, may have resulted in the dilution of plasma protein concentration which in turn could have promoted increases in glomerular filtration and natriuresis. However, the degree of protein dilution would have been similar in both experimental series. Thus, the possible effects of protein dilution and increased glomerular filtration were most likely not different between the two groups of rats.

In the present experiments, the degree of sodium and water excretion was attenuated but not completely abolished in the group with bilateral atrial appendectomy. Therefore, it becomes apparent that other regulatory mechanisms (17, 18) actively participated in the control of sodium and water balance not only during volume expansion, but also in the basal state. Compared to the sham-operated group, basal rates of sodium and water excretion were attenuated in the animals without atrial appendages. Although the basal level of plasma iANF appeared to be reduced in this experimental group, the values between the two groups were not significantly different (Table I). These baseline differences in natriuresis and diuresis cannot be attributed to changes in the hemodynamic functions studied, since MAP and HR were not different among the two groups. The reasons are unclear for the lack of difference in baseline plasma iANF between the two groups. It can be speculated that the amount of ANF available for secretion from the body of the atria was sufficient to maintain similar basal levels of plasma iANF in the two experimental groups. With volume expansion and increased disten-

tion of the atrial appendages, however, additional recruitment of ANF for secretion presumably occurred in the sham-operated rats, but not in the rats with bilateral appendectomy. In the study of Veress *et al.* (9), similar baseline attenuations in sodium and water excretion were observed in the right atrial appendectomy group of rats, although no differences in glomerular filtration rate, central venous pressure, cardiac output, mean arterial pressure, or heart rate were noted between the right atrial appendectomy and the sham-operated animals. Thus, depending on the physiological or pathophysiological state, several integrated neurohumoral mechanisms including the renin–angiotensin–aldosterone system, antidiuretic hormone and cardiorenal reflexes (17, 18) are operative at any given time for the regulation of sodium and water balance. In addition, there is now evidence to indicate that the atrial natriuretic factor endocrine system is another important regulatory mechanism that becomes activated during intravascular volume expansion.

In summary, the present study provides direct evidence that removal of both atrial appendages in the rat blunts the release of endogenous atrial natriuretic factor during volume expansion; and this effect, in turn, is associated with reduced natriuretic and diuretic responses. This investigation further supports the concept that atrial natriuretic factor is partially involved in the regulation of sodium and water balance.

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Received February 19, 1986. P.S.E.B.M. 1986, Vol. 183.
Accepted May 22, 1986.