ATRIAL NATRIURETIC PEPTIDE REPLACEMENT THERAPY IN RATS SUBJECTED TO BIATRIAL APPENDECTOMY

The postoperative fluid retention found in some patients after the Cox maze procedure has been attributed to surgically induced loss of atrial natriuretic peptide. We postulated that exogenous atrial natriuretic peptide could reverse this antidiuresis. A rat model was used to investigate this hypothesis. In group I, the sham group, the atrial appendages were left intact and the animals were then subjected to a fluid challenge equivalent to 1% of the animal's body weight. In group II, after biatrial appendectomy, the animals were subjected to a fluid challenge similar to that in group I. Animals in group III underwent the same protocol as that for group II plus intravenous administration of atriopeptin III at varying concentrations. Urine output and plasma atrial natriuretic peptide levels were significantly decreased after biatrial appendectomies ($p \leq 0.01$). Urine output returned to control levels after biatrial appendectomies with low-dose atrial natriuretic peptide infusion ($0.5 \text{ pmol/min} = 25.5 \text{ pg/min}$), although circulating atrial natriuretic peptide levels were lower. Urine output and plasma atrial natriuretic peptide levels increased with atrial natriuretic peptide infusions between 0.5 and 50 pmol/min. Heart rate and mean blood pressure did not vary significantly with atrial natriuretic peptide infusions. Thus atrial natriuretic peptide can be used effectively in low doses to induce a diuresis after biatrial appendectomies. Atrial natriuretic peptide may have clinical application after the Cox maze procedure. (J THORAC CARDIOVASC SURG 1995;109:976-80)

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Among the reported complications of the Cox maze procedure is postoperative fluid retention in some patients that necessitates the use of osmotic diuretics followed by administration of spiro lactone.^{1,2} Although the mechanism for fluid retention is not known, a blunting of the production of the atrial natriuretic peptide (ANP) has been proposed as a partial explanation.¹ ANP, found in all mammalian species, has natriuretic, diuretic, and vasoactive properties as its principal effects.^{3,4} This hormone, stored in granules in specialized atrial cells called cardiocytes, are most abundant in the right atrium and in the atrial

appendages.³⁻⁶ Prior studies have demonstrated that the loss of the right atrial appendage during heart operations can influence postoperative fluid retention^{5,7}; biatrial appendectomies are done as part of the Cox maze procedure.¹

ANP has been administered in therapeutic doses in animals,⁸⁻¹¹ in normal human subjects,¹²⁻¹⁸ and in patients with congestive heart failure^{15,19,20} and resulted in increased diuresis. Previous studies in human subjects,^{5,21} rats,²²⁻²⁴ and dogs^{25,26} have indicated that either biatrial or right atrial appendectomy blunted diuresis and natriuresis in response to induced hypervolemia. Our hypothesis was that exogenous ANP (atriopeptin III; Peninsula, Belmont, Calif.), administered in replacement doses, could be used to replenish the circulating hormone and achieve adequate diuresis after biatrial appendectomies.

Methods

Experimental preparation. Adult male Sprague-Dawley rats with body weights between 375 and 400 gm underwent one of three surgical procedures. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National

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Society for Medical Research, the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health, and the Veterinary Division of Montefiore Medical Center. All animals received rat chow ad libitum that contained 0.11 and 0.23 mEq per gram of sodium and potassium, respectively. Tap water was available to the animals ad libitum. The rats were anesthetized with sodium pentobarbital (60 mg/kg intraperitoneally)²² and were placed on a surgical tray over a heating pad to maintain body temperature between 37° and 40° C. Intubation of the trachea was done with a 14-gauge, 2¼-inch plastic intravenous cannula (Becton Dickinson & Co., Sandy, Utah); the animals were supported with positive-pressure ventilation (Harvard Rodent Ventilator model No. 683, Harvard Apparatus, Co. Inc., Southnatick, Mass.) with room air. Indwelling heparinized polyethylene catheters (PE 50, Clay Adams, Parsippany, N.J.) were placed in the right internal jugular vein for infusion of fluids (Harvard Infusion pump model No. 600-900, Harvard Apparatus Co., Inc., Dover, Mass.) and in the left carotid artery for blood sampling and measurement of blood pressure and heart rate (Datamedix Guardian model No. GN 1001, Datamedix, Sharon, Mass.). All blood samples were replaced with equal volumes of fresh donor blood obtained from normal anesthetized rats via abdominal aortic cannulation. Polyethylene (PE 50) tubing was placed into the bladder via a suprapubic cystostomy and urine was collected into the modified barrel of a 1 ml syringe.

Surgical procedures. The animals were assigned by use of a random numbers table into the experimental groups. All animals underwent a median sternotomy. Group I was the sham control group. After reflection of the pericardium, there was no manipulation of the atria in four sham rats (subgroup IA) and atrial manipulation without injury in four other sham rats (subgroup IB). In both sham subgroups, the atrial appendages were left intact. The chest was then covered with gauze soaked in isotonic saline solution. During the 30-minute recovery period that followed the operation, a continuous infusion of isotonic saline was administered at a rate of 20 µl/min. After the recovery period, a fluid challenge with isotonic saline solution in a volume equivalent to 1% body weight was given over 30 minutes. Blood samples and urine output were measured at 15, 30, and 45 minutes after the fluid challenge. A maintenance infusion at a rate of 20 µl/min of isotonic saline solution was started after the fluid challenge and was maintained for the duration of the experiment.

In group II ($n = 8$), after sternotomy and reflection of the pericardium, both atrial appendages were gently retracted to their maximum length; 5-0 silk ligatures (Ethicon, Inc., Somerville, N.J.) were placed at the base of each appendage after which atrial appendectomy was performed. As in group I, a fluid challenge was followed by a maintenance infusion.

In group III ($n = 18$), the identical surgical protocol used with the group II animals was followed with the exception that at the end of the fluid challenge, a continuous infusion of atriopeptin III, M.W. 2550, at 20 µl/min was maintained until completion of the experiment. The

Table I. Results 30 minutes after infusion: sham groups IA and IB

	Group IA: no manipulation	Group IB: manipulation
ANP levels (pmol/min)	172.8 ± 51.9	122.5 ± 144.9
Urine output (ml/15 min)	0.15 ± 0.05	0.18 ± 0.05
Heart rate (beats/min)	382.5 ± 39.9	360 ± 39.9
Systolic BP (mm Hg)	113.7 ± 11.9	104.8 ± 10.3

All results are expressed as mean ± standard error of mean. BP, Blood pressure.

concentration of the natriuretic peptide was varied in five subgroups (subgroups A through E: 0.5, 5, 25, 50, and 100 pmol/min, respectively), but the infusion volume was maintained at 20 µl/min.

Blood samples and assay. Blood samples were drawn from the carotid artery catheter in 1 ml aliquots for ANP determination at 15, 30, and 45 minutes after completion of the fluid challenge. The blood samples were added to vacuum blood collection tubes (Terumo Medical, Elkton, Md.) that contained 0.05 ml of 15% liquid ethylenediaminetetraacetic acid and 10 KIU aprotinin (Sigma, St. Louis, Mo.). The samples were kept on ice until the end of the experiment. The tubes were centrifuged at 3500 rpm for 5 minutes and the separated plasma was stored in polypropylene tubes in a liquid nitrogen refrigerator at -196° C until the time of extraction.²⁷ Each sample was coded to prevent identification at the time of assay.

ANP levels were determined by the use of the radioimmunoassay disequilibrium technique method outlined by Stewart and associates.²⁸ The minimal concentration of ANP 99 to 126 amino acids detected ranged from 0.5 to 1.0 pg/ml. Intraassay variability was 4%, and interassay variability was 8%. Percent extraction was 74% ± 3%.

Statistical analysis. Data between groups were analyzed by an analysis of variance. Post hoc evaluation was done with the Scheffe test. Statistically significant difference was determined at $p \leq 0.05$. The mean plus or minus the standard error of the mean are reported.

Results

Results were compared at all time points and between all groups; however, only the results that represented the maximum effect (30 minutes after the end of the fluid challenge) and the 5 pmol/min dose of ANP data are presented. Because there was no significant difference in urine output, ANP levels, heart rates, or blood pressure 30 minutes after a fluid challenge between the subgroups of sham rats (Table I), the data of these subgroups have been pooled and are presented in Table II.

Bilateral appendectomies (group II) resulted in the lowest urine output, which was statistically signifi-

Fig. 1. Urine output (UOP) expressed in milliliters per 15 minutes. BAA, Biatrial appendectomy; ANP, atrial natriuretic peptide. Doses are in picomoles per minute. Urine outputs for sham and ANP infusion groups are significantly different from those of group II ($p \leq 0.01$) at all times.

Table II. Results 30 minutes after fluid challenge

Experimental group	ANP levels	Systolic BP	Heart rate
Sham			
Group I	146.9 $\pm$ 87.1	108.6 $\pm$ 14	371.3 $\pm$ 19
BAA			
Group II	22.9 $\pm$ 10.8	109.5 $\pm$ 13	420 $\pm$ 20
ANP 0.5 pmol/min			
Group IIIA	33.8 $\pm$ 9.7	120 $\pm$ 9	397.5 $\pm$ 33
ANP 5.0 pmol/min			
Group IIIB	187.8 $\pm$ 43.3	111 $\pm$ 8	375 $\pm$ 29
ANP 25 pmol/min			
Group IIIC	652.3 $\pm$ 146.6	103.7 $\pm$ 6	412.5 $\pm$ 11
ANP 50 pmol/min			
Group IIID	1713 $\pm$ 603.8	97.3 $\pm$ 8	480
ANP 100 pmol/min			
Group IIIE	2292 $\pm$ 768.9	98.7 $\pm$ 6	375 $\pm$ 26

All results are expressed as mean $\pm$ standard error of mean. All ANP levels are significantly different from those in group II ($p \leq 0.01$); there is no significant difference in blood pressure or heart rate. BAA, Biatrial appendectomy; BP, mean systolic blood pressure. ANP levels are expressed in picograms per milliliter.

cant when compared with that of all other animals ($p \leq 0.01$; Fig. 1). Additionally, group II rats had the lowest ANP levels when compared with those of all other animals ($p \leq 0.01$; Table II).

After biatrial appendectomies, exogenous admin-

istration of ANP (group III) resulted in an incremental increase in urine output, with peak urine outputs occurring at an infusion concentration of 50 pmol/min (subgroup IIID) and at a time interval between 15 and 30 minutes of infusion. Infusion of ANP at 0.5 pmol/min (group IIIA) resulted in urine output and ANP levels significantly higher than those of group II (Table II and Fig. 1; $p \leq 0.01$). Serum levels of ANP increased correspondingly up to an infusion rate of 100 pmol/min (Table II).

The heart rate and blood pressure did not vary significantly between groups.

Discussion

On the basis of the results of prior series of studies that demonstrated that loss of the right atrial appendage may cause fluid retention and, second, that exogenous ANP can induce diuresis, we postulated that use of exogenous ANP could reverse the antidiuresis noted in some patients after the Cox maze procedure despite good ventricular function.^{1,2} Among the possible causes of the fluid retention is ANP depletion secondary to the multiple atrial incisions and biatrial appendectomies.^{1,2} Clinical postoperative studies have documented

ANP depletion after right atrial or biatrial appendectomies and significantly higher levels of ANP and better diuresis and natriuresis after volume expansion in patients in whom the right atrial appendage was preserved during venous cannulation.⁵ In regard to the Cox maze procedure, we could not find any prior documentation of postoperative ANP levels, although Cox and associates¹ postulated that attenuated serum ANP levels may contribute to postoperative fluid retention.

In previous studies on rats and dogs, in which biatrial appendectomies (but not left atrial appendectomy alone) were done, diuresis and natriuresis were blunted in response to a fluid challenge.²²⁻²⁵ In our study, we did biatrial appendectomies to simulate that portion of the maze procedure. We demonstrated a blunted diuretic response and low ANP levels in group II (biatrial appendectomies), whereas in the sham group a brisk diuresis was noted. With the use of varying concentrations of ANP, diuresis was restored in animals who underwent the same surgical procedure as the animals in group II, which supports our hypothesis. The use of low-dose ANP is important because the side effects in human subjects reported appear to be dose related.^{13-15, 17}

Cody, Atlas, and Laragh¹⁵ reviewed the studies done on human subjects who received synthetic preparations of ANP. The majority of those studied fell into two major categories: normal control subjects and those with fluid overload states. It was noted that a significant diuretic response was elicited in a group of healthy subjects with normal ANP levels. Also noted was a blunted response to ANP infusions in patients with congestive heart failure, who have high endogenous ANP levels.^{19, 29} The attenuated response to exogenous ANP may be due to diminished renal perfusion in congestive heart failure²⁹⁻³¹ or saturation of ANP binding sites. The fluid retention seen after the maze procedure and other cardiac procedures that damage the atrial appendages is not caused by the same mechanism as that in clinical congestive heart failure caused by poor ventricular function. Therefore exogenous ANP may have an important clinical application in postoperative volume overload states. We could not locate a study in which exogenous ANP was administered after heart operations or in patients with diminished ANP levels.

Side effects with ANP administration include bradycardia and hypotension.^{12, 13, 18, 19} Cody, Atlas, and Laragh¹⁵ reported that with steady-state ANP

administration in normal supine subjects, no significant change in blood pressure was noted. In our study, there was no change in heart rate or blood pressure in rats as the dose of ANP increased up to 100 pg/min (Table II). Although natriuresis has been noted with exogenous ANP infusion, serum electrolyte abnormalities have not been noted.^{13, 14, 32, 33}

After biatrial appendectomies, exogenous ANP reversed the surgically created antidiuresis in rats. Exogenous ANP may offer an alternative therapy for the fluid retention seen in some patients undergoing the Cox maze procedure that may result from the atrial appendectomies and multiple atrial incisions. The effects of the maze III modifications in eliminating the problem of postoperative fluid retention seen in some patients after the original maze procedure is not known. The information generated by the dose-response curve may aid in the determination of the appropriate dosage for clinical use.

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