

Is Atriopeptin a Physiological or Pathophysiological Substance?

Studies in the Autoimmune Rat

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Abstract

Atriopeptin (AP), a natriuretic-diuretic and vasodilatory peptide, is synthesized and secreted from mammalian atria. The definitive role of this peptide on cardiovascular physiology and pathophysiology has yet to be determined. We developed a population of autoimmune rats sensitized against their own AP to evaluate the consequences of prolonged AP deficiency on physiological and pathophysiological processes. Natriuresis in response to acute intravenous volume expansion was inhibited in the autoimmune rat, however, natriuresis produced by chronic oral salt loading was not suppressed in these animals. Plasma AP increased threefold in the spontaneously hypertensive rat when evaluated as a function of blood pressure. Immunization of these rats had no effect on the rate of development, magnitude of their developing hypertension, or their daily sodium excretion when compared with nonimmunized controls. Mineralocorticoid escape occurred during desoxycorticosterone acetate administration to rats. The ability of rats to escape from the sodium-retaining effects of this steroid was not affected by prior immunization against AP. These results suggest that AP is an important natriuretic substance in response to acute intravascular volume loading. However, atriopeptin does not appear to be involved in the natriuretic response to chronic intravascular volume loading, blood pressure regulation, or mineralocorticoid escape.

Introduction

The heart and kidney appear to be hormonally linked based on the recent discovery that mammalian atria synthesize, and in response to distension secrete, a 28 amino acid natriuretic-vasodilator peptide AP28 (1–3). The current understanding of atriopeptin (AP)¹ physiology and its presumed role in pathophysiology has been inferred by studying tissue and blood levels of AP in the presence or absence of pharmacological manipulations or cardiovascular disease. AP produces a prompt natriuresis and diuresis in a dose-dependent manner when administered to rats (4). Similarly, a natriuresis and di-

uresis is produced by intravenous fluid administration (5), oral salt loading (6), head-out water immersion (7), or pharmacological elevation of blood pressure (8), maneuvers that also stimulate AP28 secretion. Therefore, it has been suggested that the natriuresis and diuresis produced by volume expansion is a result of AP's effect on renal function.

AP is also a potent vasorelaxant (9). Spontaneously hypertensive rats (SHR) are exquisitely sensitive to the vasorelaxant effects of AP. A dose of AP that normalizes blood pressure in hypertensive rats, but is neither natriuretic nor diuretic, fails to lower blood pressure in normotensive rats (10). Interestingly, endogenous AP plasma levels are elevated in the SHR while atrial stores are depleted. These alterations correlate with the degree of hypertension (11). Therefore it has been hypothesized that AP may play a protective or pathogenic role in the development or maintenance of hypertension in the SHR rat.

It has also been hypothesized that AP is the factor responsible for the escape phenomenon seen during mineralocorticoid administration. AP plasma levels rise coincident to the time when the renal tubules escape from the sodium-retaining effects of the mineralocorticoid (12).

Much has been written about the critical role of AP as a hormone intimately involved in intravascular volume and blood pressure homeostasis (13–15). Whether AP is truly a physiological substance has not yet been determined. A major issue of confusion is that plasma AP levels do not correlate with natriuresis or diuresis in all circumstances. For example, a natriuresis and twofold increase in plasma AP is produced by left atrial distension in conscious dogs (16). In contrast, left atrial distension in conscious dogs after cardiac denervation produced similar AP plasma levels without a resultant natriuresis (16), thus dissociating AP secretion and sodium excretion in this model of atrial stretch-induced natriuresis. Furthermore, administration of pharmacologic doses of AP that produce a prompt diuresis and natriuresis in conscious monkeys (17) or rats (4) results in plasma AP levels far in excess of levels produced by endogenous secretion of the peptide. Thus, it is possible that endogenous secretion of AP may only be temporally related to natriuresis and diuresis, and that volume expansion activates other natriuretic and diuretic mechanisms.

Further understanding of AP's role in physiology will come from studies developed to inactivate the circulating peptide. Intravenous injection of specific antiserum (passive immunization) against AP significantly decreased urine flow and sodium excretion in response to intravenous volume expansion (18, 19). These data demonstrate the role of AP as an important modulator of acute intravascular volume changes. However, because passive immunity is limited to the study of acute volume changes in the animal, we developed a population of autoimmune rats sensitized against their own AP in order to evaluate the consequences of prolonged AP deficiency on physiological and pathophysiological processes.

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1. *Abbreviations used in this paper:* AP, atriopeptin; APir, plasma atriopeptin immunoreactivity; DOCA, desoxycorticosterone acetate; RAP, right atrial pressure; SHR, spontaneously hypertensive rats; $U_{Na}V$, volume of Na in urine.

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Methods

Immunization protocol and titer determination. Female Sprague-Dawley rats weighing 200–250 g were immunized against AP. To render AP immunogenic, rat AP28 was covalently bound to bovine thyroglobulin by the carbodiimide method (20). Animals were initially injected subcutaneously with the conjugated rat AP28 (200 μ g AP28 by weight) in an emulsion of Freund's complete adjuvant (0.4 cm³) (Calbiochem-Behring Diagnostics, American Hoechst Corp., San Diego, CA). Animals were boosted after 1 and 2 mo with the conjugated rat AP28 (100 μ g AP28) again in an emulsion of Freund's complete adjuvant (0.2 cm³). Animals were titered 2 wk after the third immunization. Titers were defined as the dilution of rat serum that bound 50% of iodinated rat AP24 (10,000 cpm). Free iodinated peptide was precipitated with 2.5 mg of activated charcoal (Sigma Chemical Co., St. Louis, MO) and counted on an Apex gamma counter (Micromedex Systems, Inc., Horsham, PA). 67% of all rats undergoing this immunization protocol would achieve titers of 2,000 or greater.

AP24 infusions. Six rats that attained AP titers ranging from 1:500 to 1:2,000 and six nonimmune rats were selected for infusion of rat AP24. Nonimmune rats in these studies were age matched and not subjected to the immunization protocol. Arterial, venous, and bladder access was achieved by surgical placement of PE50 tubing into the femoral artery, femoral vein, and urinary bladder after rats were anesthetized with chloral hydrate (350 mg/kg). Blood pressure was measured with a pressure transducer (type 4-327-0010; Bell and Howell Co., Pasadena, CA), and urine volume was determined gravimetrically by collection into preweighed tubes. Fluids and AP24 were infused into the femoral vein. All animals were volume expanded for 1 h with 5% dextrose and 0.25% sodium chloride (0.4 ml/min per kg) before basal urine collections. AP24 was administered in three (100, 300, 600 ng/kg per min) sequential 15-min infusions.

Intravenous volume expansion. Eight rats with titers to AP ranging from 1:2,000 to 1:20,000 and eight nonimmune rats were selected for use in this study. Rats were anesthetized with chloral hydrate (350 mg/kg) intraperitoneally before PE50 tubing placement into the right atrium via the jugular vein for right atrial pressure (RAP) measurements, into the femoral vein for fluid administration, and into the urinary bladder for timed urine collections. Atrial pressure was measured with a pressure transducer placed at the level of the heart. Urine was collected into preweighed tubes and measured gravimetrically, and sodium was measured using a flame photometer (Instrumentation Laboratory Inc., Lexington, MA). Volume expansion was produced by a 15-min infusion of 0.9% sodium chloride and 4% BSA at 1.2 ml/min per kg. Blood (300 μ l) was collected into 9% sodium citrate (1:9, vol/vol), then immediately replaced with equal volumes of 0.9% sodium chloride and 4% BSA. Plasma AP immunoreactivity was measured as previously described (21).

Oral salt loading. 16 immunized rats with titers ranging from 1:2,000 to 1:20,000 and eight nonimmunized rats were placed in individual metabolic cages in a room with alternating 12-h cycles of light and dark and given free access to food and water. Each sodium diet was administered for 14 d, then on day 15, diets were changed to the next highest sodium concentration. The standard diet consisted of 0.4% NaCl rat chow (Ralston-Purina Co., St. Louis, MO) and tap water. NaCl was added to the diet by the addition of 8.0% NaCl rat chow (Ralston-Purina Co.) and by incrementally increasing the amount of NaCl in the drinking water. Urine volume, urine sodium, and water intake were measured daily. Systolic blood pressures were measured weekly by the indirect tail cuff method using a pneumatic pulse transducer (Narco Bio-Systems, a Heathdyne Co., Houston, TX).

Autoimmunity in the SHR. 15 SHR (Taconic Farms, Germantown, NY) were initially immunized at 4 wk of age and subsequently boosted at 8 wk as previously described. Animals who achieved titers of 1:2,000 to 1:20,000 were used for further study. To determine the plasma AP immunoreactivity (APir), tail vein blood was collected from 10 conscious, nonimmunized SHR, into 8% sodium citrate (1:10, vol/vol) at 5, 7, 10, 13, and 15 wk of age, and plasma was assayed for

APir by an ELISA developed in our laboratory (22). Systolic blood pressure, measured by the indirect tail cuff method, and body weight were determined weekly. Rats were placed into individual metabolic cages at 5, 11, and 16 wk of age and given free access to food (0.4% NaCl rat chow, Ralston-Purina Co.) and water for determination of 24-h sodium excretion.

Mineralocorticoid escape. 10 immunized rats with titers ranging from 1:2,000 to 1:20,000 and 10 nonimmunized rats were kept for 10 d in individual metabolic cages for measurement of daily urine volume and sodium excretion. Rats were fed once daily with 24 g of standard Purina rat chow (0.4% NaCl), an amount that provided \sim 1 meq of sodium/d. Rats were given free access to tap water. On day 7 after the urinary sodium excretion ($U_{Na}V$) had stabilized, rats were given 10 mg of desoxycorticosterone acetate (DOCA) (Sigma Chemical Co.) in sesame oil subcutaneously by the method of Ballerman (12).

Results

Autoimmunity validation. Initially, to validate that our immunized rats were indeed devoid of the effects of circulating AP, we measured the amount of urine produced in response to the exogenous administration of rat AP24. As seen in Fig. 1, infusion of AP24 (100 or 300 ng/kg per min) to nonimmunized, anesthetized rats produced a 600% increase in urine flow rate. Immunized rats produced only a slight increase in urine output in response to 300 ng/kg per min AP, a dose that effectively raises plasma AP to 1,500 pg/ml as determined by enzyme immunoassay. Furthermore, the hypotensive response to infusions of AP24 was completely blocked at 300 ng/kg per min, and blood pressure decreased by 5 mmHg at an infusion rate of 1,000 ng/kg per min. In nonimmune rats, blood pressure decreased by 8 and 20 mmHg in response to AP24 infusions of 300 and 1,000 ng/kg per min, respectively. Thus, these data indicate that the endogenous circulating antibodies generated against AP in these rats do indeed physiologically inactivate AP28 and that these animals would serve as a valuable tool in probing the physiology of AP in the following experiments.

Intravenous volume expansion. Anesthetized rats respond to an acute intravenous volume expansion with a rapid natriuresis and an elevation of RAP which is thought to be the stimulus for AP secretion (5). To determine whether AP release and natriuresis are functionally or merely temporally linked, natriuresis was measured after expanding intravascular volume (30%) with an infusion of 0.9% saline and 4% BSA at a rate of 1.2 ml/min per kg in anesthetized rats. Plasma AP could not be measured in our autoimmune rats because the circulating antibodies to AP interfered with our immunoassays. In these acute volume loading experiments, the mean

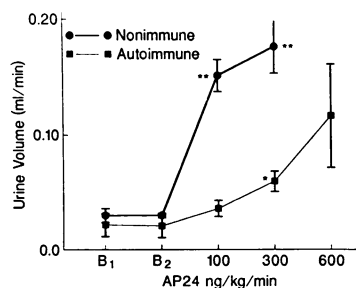


Figure 1. AP24 infusion in the autoimmune and non-immune Sprague-Dawley rat. Each point represents the mean \pm SEM of six animals. B_1 and B_2 represent two basal, 15-min urine collections. AP24 was infused at the specified doses for 15 min and urine was collected during the entire infusion. * P < 0.05, ** P < 0.01.

blood pressure in the nonimmune rats before volume expansion was 85 mmHg, and in the autoimmune rats the blood pressure was slightly but not significantly lower at 75 mmHg. Neither group of rats demonstrated a change in blood pressure during the acute volume expansion or throughout the 75 min of the experimental protocol. As seen in Fig. 2 B, atrial AP secretion in response to this volume loading protocol, raised plasma AP threefold in the nonimmunized rats. We assume that similar amounts of AP were secreted from the atria of autoimmune rats because RAP increased comparably in both groups of animals (Fig. 2 A). Remarkably, stimulated natriuresis was abolished in autoimmune rats, while $U_{Na}V$ increased 30-fold in the nonimmune rats in response to identical fluid infusions (Fig. 2 C). Unlike the short-lived effects of passive immunization on sodium excretion (19), we were unable to detect any stimulation of natriuresis during the entire time course of the study (i.e., 75 min). These data suggest that AP is indeed an endogenous natriuretic agent that responds to acute volume expansion.

Oral salt loading. We next evaluated whether a deficiency of circulating AP would affect the cardiovascular system exposed to a prolonged intravascular volume load. Oral salt loading increases intravascular volume and results in a natriuresis, diuresis, and moderate elevation of circulating AP (6). Autoimmune rats provided us with a means of evaluating the role of AP during chronic intravascular volume expansion, because antibody titers to AP do not appreciably decline for at least 3 mo after inoculation with the AP-thyroglobulin complex. Immune and nonimmune rats were subjected to a stepped salt loading protocol by incrementally adding NaCl to their food and drinking water while daily monitoring fluid intake, $U_{Na}V$, blood pressure, and body weight. As salt intake progressively increased from a diet of tap water and normal rat chow (0.4% NaCl) to 1.5% NaCl drinking water and 8.0% NaCl rat chow, natriuresis increased from 2 to 50 meq/d, respectively. We were, however, unable to detect a difference between the immune and nonimmune rats' ability to excrete sodium (Fig. 3). Similarly, body weight, a reflection of sodium and water balance, was unchanged by diet in both groups of

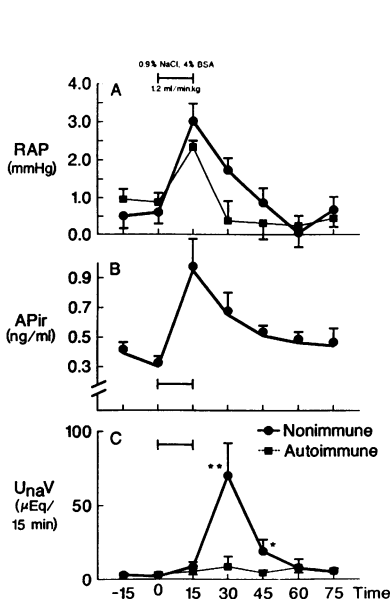


Figure 2. Acute volume expansion in the autoimmune and nonimmune Sprague-Dawley rat. Each point represents the mean \pm SEM of eight animals. Rats received a 15-min intravenous volume expansion with 0.9% NaCl and 4% BSA at 1.2 ml/min per kg. All measurements and fluids were collected every 15 min. RAP measurements in the autoimmune and nonimmune rat (A), plasma APIr in the nonimmune rat (B), $U_{Na}V$ in the autoimmune and nonimmune rat (C). * $P < 0.05$, ** $P < 0.01$.

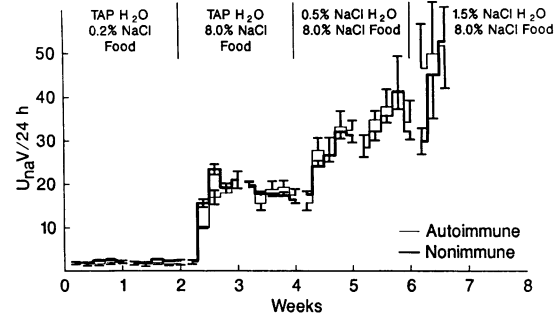


Figure 3. Effect of sodium intake on $U_{Na}V$ in the autoimmune and nonimmune Sprague-Dawley rat. Urine was collected daily for the determination of $U_{Na}V$ and each point represents the mean \pm SEM of 16 autoimmune rats and 8 nonimmune rats. Rats were given free access to the specified diets for 2 wk, and on the following day were placed on the next highest salt loading protocol.

animals (Table I). These data demonstrate that AP is not intimately involved in the day to day maintenance of salt or water homeostasis.

Because positive sodium balance is attained by prolonged administration of a mineralocorticoid and eventually results in the development of hypertension (23), we hypothesized that if autoimmunity to AP results in an antinatriuretic state (i.e., positive sodium balance), then hypertension should develop in immunized rats in response to chronic oral salt loading. Therefore, indirect blood pressures were measured weekly in autoimmune and nonimmune rats during the 8 wk of increasing sodium intake. All rats, autoimmune and nonimmune, remained normotensive throughout the 8 wk of the study (Table I), suggesting that a deficiency of AP does not contribute to the production of hypertension in a genetically normotensive rat.

Table I. Effect of Sodium Intake on Blood Pressure and Weight in Autoimmune and Nonimmune Sprague-Dawley Rats

Conditions	Blood pressure		Relative body weight	
	Autoimmune	Nonimmune	Autoimmune	Nonimmune
	mmHg		g	
Tap H ₂ O				
0.2% NaCl food	115 \pm 4	108 \pm 7	1.0	1.0
Tap H ₂ O				
8% NaCl food	122 \pm 3	128 \pm 12	1.05 \pm 0.01	1.07 \pm 0.02
0.5% NaCl H ₂ O				
8% NaCl food	127 \pm 3	119 \pm 7	1.07 \pm 0.02	1.08 \pm 0.02
1.5% NaCl H ₂ O	123 \pm 8	128 \pm 10	1.03 \pm 0.02	1.04 \pm 0.02

Body weight and blood pressure were measured weekly in 16 autoimmune and 8 nonimmune rats. No differences were detected between weight or blood pressure determined either in the first or second week of each diet protocol. Therefore the data presented is the mean \pm SEM accumulated during week 2 of each salt loading protocol. Body weight data is normalized to the individual rat's weight that was determined during the normal salt diet because the starting weight of the nonimmune rats was 248 \pm 5 g and the weight of the autoimmune rats was 274 \pm 5 g. No significance at $P < 0.05$ was demonstrated between autoimmune and nonimmune rats within each treatment group.

Autoimmunity in the SHR. In the SHR, hypertension begins at 6–8 wk of age and fully develops over the course of 12–14 wk. Inagami (11) demonstrated a progressive increase in AP blood levels and a decrease in atrial AP stores that paralleled the developing hypertension in the SHR. Because the SHR is exquisitely sensitive to the antihypertensive effect of AP (10, 24), and plasma AP levels rise in response to hypertension in these animals, it is intriguing to hypothesize that the rate or degree of development of hypertension would be accelerated in the autoimmune, AP-deficient rat. To address this hypothesis we developed a population of autoimmune SHR. Animals were initially immunized at 4 wk of age to begin titer development before the onset of hypertension and were boosted at 8 wk of age to develop maximal titers before full expression of hypertension. Our data, like that of Inagami (11), demonstrated a threefold increase in plasma AP (Fig. 4 B) that paralleled the time-dependent rise in blood pressure (Fig. 4 A). After measuring indirect tail cuff blood pressure weekly during the 16 wk of hypertension development, no difference was detected between the rate or degree of the hypertension that developed in either the autoimmune or nonimmune SHR (Fig. 4 A). These data confirm that AP is indeed incrementally released in response to hypertension but suggest that AP is not critically involved in the expression of hypertension in these animals.

We have demonstrated that the natriuresis that occurs in response to acute volume expansion is dependent on AP but natriuresis produced by chronic salt loading is not. Because plasma AP is incrementally increased in the SHR with time, the kidney would be presented with increasingly higher amounts of AP during the course of developing hypertension. Therefore, we measured the daily $U_{Na}V$ in the autoimmune and nonimmune rat at 5, 11, and 16 wk of age, to address whether natriuresis or diuresis in the SHR correlates with the animals plasma AP concentration. Nonimmune rats excreted ~ 1 meq of sodium over 24 h (a value that did not change even though plasma AP increased from 80 to 310 pg/ml as hypertension developed) (Fig. 4 C). The pattern and degree of sodium excretion in the autoimmune rats were identical to that of the nonimmune animals. Therefore, we were unable to detect a correlation between plasma AP and natriuresis in either group of animals. Equally important, no difference in sodium excretion was detected when autoimmune rats were compared with nonimmune rats, further demonstrating that AP does not regulate renal function in the SHR.

Mineralocorticoid escape. DOCA, a mineralocorticoid, acts predominantly at the renal distal tubule causing a reabsorption of tubular sodium. Upon continued administration of DOCA, an initial period of sodium retention is followed by a natriuresis referred to as “mineralocorticoid escape,” a phenomenon signifying escape of the renal tubules from the sodium-retaining effects of the mineralocorticoid. The mechanism of this escape phenomenon has eluded explanation. It has been hypothesized that the natriuretic effect of AP is the cause of tubular sodium escape, because plasma AP increases coincident with the escape phenomenon (12). To test this hypothesis, autoimmune and nonimmune rats were administered DOCA subcutaneously (10 mg in sesame oil) on day 7 according to the protocol previously used (12), after daily $U_{Na}V$ had stabilized. 24 h after DOCA administration, $U_{Na}V$ decreased by 50% in the nonimmune rat. 48 h after DOCA administration, $U_{Na}V$ escaped to pre-DOCA levels in the same

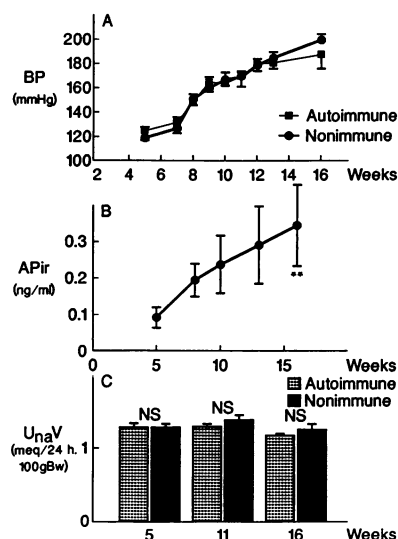


Figure 4. Autoimmunity in the SHR. All values represent the mean \pm SEM of 15 autoimmune SHR and 10 nonimmune SHR. Tail cuff blood pressures were measured at 5, 7, 8, 9, 10, 11, 12, 13, and 16 wk of age in the autoimmune and nonimmune SHR (A), APir was measured at 5, 8, 10, 13, and 16 wk of age in the nonimmune and autoimmune SHR (B), and $U_{Na}V$ was measured at 5, 11, and 16 wk of age in the nonimmune and autoimmune SHR that were given free access to food and water. * $P < 0.05$. ** $P < 0.05$.

group of animals (Fig. 5). Sodium retention and the resumption of natriuresis, in response to DOCA, was identical in timing and degree in the autoimmune rat as compared with the nonimmune rat (Fig. 5). Therefore, the rise in plasma AP that occurs at a time when the renal tubules escape from the sodium-retaining effects of DOCA, may not be the mechanism of mineralocorticoid escape.

Discussion

Once a new biologic factor is discovered, an initial body of knowledge is generated which attempts to describe its role in physiology and pathophysiology. Since the structure of AP was elucidated (1, 2), volumes of data have been generated describing AP as an important hormone that is intimately involved in intravascular volume homeostasis. This description has been approached in two ways. First, the natriuresis, diuresis, and vasodilation that is produced by exogenous adminis-

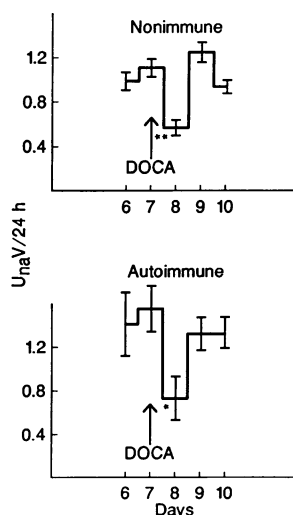


Figure 5. DOCA escape in the autoimmune and nonimmune Sprague-Dawley rat. Each value represents the mean \pm SE of 10 autoimmune and nonimmune rats. $U_{Na}V$ was determined daily. DOCA was administered on day 7 of the study. * $P < 0.05$, ** $P < 0.01$ as compared with pre-DOCA levels.

tration of AP demonstrates its pharmacological effects. However, such experiments can only suggest its true physiological role. A second approach would be to deprive the animal of AP and observe any secondary deleterious effects. Deprivation of circulating AP by exogenous administration of anti-AP antiserum (i.e., passive immunization) demonstrated the importance of AP as an endogenous natriuretic agent that responds to acute volume expansion (18, 19). Passive immunization is limited to studying the effects of AP on acute cardiovascular changes. It cannot be used in chronic experiments where there are major shifts in sodium or water balance, or to evaluate the role of AP on blood pressure regulation. We hypothesized that prolonged AP deprivation could be attained by immunizing animals against their own AP, effectively producing autoimmune animals. Autoimmunity is a disease process in which the immune system is activated and antibodies are generated that react against one's own endogenous proteins, often producing deleterious effects. We hoped that by using autoimmunity as a research tool, we could further delineate the role of endogenously secreted AP on renal and cardiovascular physiology and pathophysiology.

Our initial studies were designed to evaluate whether rats who developed antibodies to their own AP were resistant to the effects of the peptide. Because of the possibility that antibodies would bind but not inactivate the AP, our definition of autoimmunity was dependent on the observation that immunized rats would minimally or not at all respond to the exogenous infusion of AP with a resultant diuresis and a decrease in blood pressure. Because of the terminal nature of these experiments, rats with the smallest titers (500–2,000) were evaluated. Diuresis and a fall in blood pressure were completely inhibited in response to doses of exogenous AP that generate plasma levels observed during the severest states of volume overload in rats. Since rats with the lowest titers were so effective at blocking the effects of exogenously administered AP, and since immunized rats that were entered into further studies all attained serum titers of 2,000–20,000, we were confident that antibody would be in excess of any circulating AP that would be generated. Furthermore, antibody titers were maintained for at least 3 mo after the last immunization, thus providing us with the ability to evaluate AP's effects on acute, as well as chronic, cardiovascular alterations.

Similar to the results elicited during passive immunization (18, 19), we confirm that the natriuresis that occurs in response to acute intravenous volume expansion is dependent on the heart's ability to secrete AP. However, the blunted natriuresis measured during volume expansion in the autoimmune rats may have been a result of the animal's inability to secrete similar amounts of AP to an identical volume load. It is possible that AP autoimmunity alters hemodynamics such that for a given amount of fluid administration, there is no equivalent rise in RAP, a stimulus for AP release, when compared with nonimmune rats. This was not the case since RAP increased equally in both groups of animals (Fig. 2). Unlike the short-lived effects of passive immunization (19), autoimmunity confers a more prolonged deprivation of AP because the natriuresis that occurred in response to intravenous fluids remained totally inhibited during the 75 min of urine collections.

Studies done with rats (6) demonstrate an increase in plasma AP that temporally correlates with natriuresis in response to oral salt loading. We couldn't detect a difference in

the daily $U_{Na}V$ of the autoimmune and nonimmune rats exposed to identical oral salt loading protocols. Even during extremely high sodium intake, an amount that increased daily $U_{Na}V$ by 50-fold, no difference in sodium excretion was detected. Since we did not assess total daily sodium consumption, we cannot disregard the possibility that autoimmune rats consumed more daily sodium than their matched controls. If so, equivalent $U_{Na}V$ would actually represent an inhibition of natriuresis if the data were expressed as a function of salt intake in the autoimmune animals. This is highly unlikely because daily fluid intake, whether it was tap water or water supplemented with sodium chloride, was identical in the autoimmune and nonimmune rats. Likewise, we detected no weight gain in either group of animals during the 8 wk of oral salt loading, again demonstrating that autoimmune rats did not retain sodium or water. Because we did not measure plasma AP in these rats, we cannot be sure that AP release was stimulated by our method of oral salt loading. However, we would still expect autoimmune rats to at least acutely develop positive sodium balance because a deprivation of circulating AP should induce an antinatriuretic and antidiuretic state if indeed AP is an important endogenous regulator of volume and salt homeostasis. From these data, we concluded that AP is not an important modulator of intravascular volume homeostasis during prolonged volume loading.

The SHR is genetically programmed to develop hypertension that is maximally expressed at ~ 16 wk of age. One would hypothesize that a deficiency of AP may result in a more rapid and maximal expression of the hypertension since these rats are exquisitely sensitive to the vasodilatory effects of this peptide and because AP secretion increases with the degree of hypertension. In this study, the autoimmune SHR developed hypertension at the same rate and to the same degree as their nonimmunized counterparts. Therefore, we conclude that circulating AP is not involved in the development of hypertension in the SHR. However, we cannot exclude the possibility that AP may play a role in the central regulation of blood pressure in the SHR as evidenced by the fact that AP levels are elevated in the hypothalamus and pons of these animals (11). If so, we would not expect autoimmunity to alter the course of developing hypertension in these animals because autoantibodies to AP should not cross the blood brain barrier.

AP plasma levels increase as a function of blood pressure in the SHR. This property enabled us to evaluate the relationship between plasma AP and sodium excretion. If endogenous AP stimulates natriuresis in a manner similar to the dose-dependency observed by exogenous administration of the peptide, one would anticipate that daily $U_{Na}V$ would increase with increasing plasma AP concentrations and daily $U_{Na}V$ would be lower in the autoimmune vs. the nonimmune rats. No correlation was detected when natriuresis was evaluated as a function of plasma AP in the autoimmune or nonimmune SHR. Likewise, daily $U_{Na}V$ in the autoimmune rat was indistinguishable when compared with the nonimmune rat.

The mechanism of mineralocorticoid escape has not yet been resolved. Because plasma AP increases at the time of increasing tubular sodium excretion (12), it was not unexpected that AP would be the hypothesized etiologic factor responsible for this escape phenomenon. However, this is not the case since the profile of mineralocorticoid-induced sodium retention, then escape, was unaltered in the autoimmune rat.

Experimentally induced autoimmunity to AP has allowed

us to examine, for the first time, the physiological role of AP on chronic volume homeostasis and chronic blood pressure regulation. It is clear from our studies and from those that employed passive immunization, that AP is indeed an important hormone that can acutely unload the cardiovascular system in response to acute volume changes. We determined that AP, alone, is not an important physiological substance that regulates, on a daily or chronic basis, intravascular volume in the unperturbed or volume expanded animal. It is not surprising that animals, in positive fluid or salt balance, do not retain salt and/or water when deprived of AP because of the myriad of other hormones and neural influences that can modulate renal and cardiovascular function. However, we would have expected that animals in positive fluid or sodium balance, who demonstrate elevated circulating levels of AP, would be compromised by deprivation of this hormone. Since no deleterious effects were observed in our autoimmune rats, we suggest that AP secretion in response to chronic volume changes, which we studied, is not absolutely necessary for maintenance of intravascular homeostasis. Alternatively, the inability of chronically elevated AP to stimulate natriuresis and diuresis, in some circumstances, may be a result of AP tachyphylaxis at the level of the renal AP receptor. Tachyphylaxis to AP is supported by studies demonstrating a down-regulation of renal AP receptors in response to chronic infusions of AP (25). Finally, it is intriguing to hypothesize that some types of hypertension could be a result of alterations in AP, especially since the aim of antihypertensive therapy is to produce a natriuresis and vasodilation, which are two pharmacologic properties attributed to AP. We found no evidence suggesting that AP is intimately related to blood pressure regulation in the SHR rat. In conclusion, until a specific AP receptor antagonist is developed, the use of AP autoimmunity will remain a critical tool to further evaluate the physiology and pathophysiology of this intriguing hormone.

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