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Effects of Rat Atrial Extract on Sodium Transport and Blood Pressure in the Rat[1,2] (41851)

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Abstract. Atrial cardiocytes contain specific atrial granules (SAGs) which are the storage site of atrial natriuretic factor (ANF). The purpose of the present study was to determine whether ANF produces natriuresis by inhibiting Na^+-K^+ pump activity and whether this factor is similar to the humoral sodium transport inhibiting factor (HSTIF) previously demonstrated in acutely volume expanded animals and humans as well as in experimental and human essential hypertension. Our results indicate that, in contrast to the HSTIF, ANF does not inhibit membrane Na^+,K^+-ATPase, vascular smooth muscle cell Na^+-K^+ pump activity, or sodium transport in the toad bladder. Intravenous infusion of ANF in the bilaterally nephrectomized, hexamethonium-treated rat produces only a small transient pressor response, probably due to potentiation of endogenous norepinephrine. These findings strongly suggest that the ANF is not the same as the HSTIF detected on acute volume expansion and in some forms of hypertension. They also suggest that the diuretic and natriuretic effects of ANF are due to mechanism(s) other than blood pressure elevation and inhibition of Na^+-K^+ pump activity.

Mammalian atrial myocytes contain specific atrial granules (SAGs) (1–3) and the number of these SAGs is affected by water and electrolyte balance (4–6). SAGs are absent in ventricular myocytes and intravenous infusion of rat atrial, but not ventricular, extract into rats produces a rapid and potent diuretic and natriuretic response (7, 8). Tissue fractionation studies suggest that SAGs are the storage site of ANF (9, 10). Similar diuretic and natriuretic effects of atrial extracts from various other mammalian species have also been demonstrated (11, 12). The mechanism of action of ANF is unknown.

Plasma from either acutely volume expanded animals or animals with low-renin, volume-dependent hypertension has been shown to contain HSTIF which inhibits vascular Na^+-K^+ pump activity (13, 14), short circuit current in the toad bladder (15–17), and renal Na^+,K^+-ATPase activity (18–20). This factor(s) also has vasopressor activity and sensitizes small vessels to vasoactive agents (21, 22). It has, therefore, been proposed that inhibition of Na^+-K^+ pump activity by humoral sodium transport inhibiting factor (HSTIF) may play a role in the elevation of total peripheral resistance associated with hypertension (14, 23) and may also be responsible for the natriuresis and diuresis associated with acute volume expansion (24, 25). The chemical structure of HSTIF is unknown.

The question, therefore, arises as to whether the ANF is similar or identical to the HSTIF. If these factors are one and the same, then the ANF should have transport and hemodynamic effects similar to those of HSTIF. We, therefore, examined the effects of rat atrial extracts having natriuretic and diuretic activity on: (i) vascular Na^+-K^+ pump activity, (ii) renal and myocardial Na^+,K^+-ATPase activities, and (iii) Na transport in the toad bladder. We also examined the effect of these extracts on blood pressure in rats.

Methods. Atrial and ventricular extracts were used in four types of experiments to determine their effects on: (i) urine flow and sodium excretion in the rat, (ii) vascular Na^+-K^+ pump activity and renal and myocardial Na^+,K^+-ATPase activities, (iii) sodium

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and water transport in the toad bladder, and (iv) blood pressure in the rat.

Preparation of atrial and ventricular extracts. Male Wistar rats weighing between 250 and 300 g with documented normotension were used in this study. Rats were maintained on normal rat chow (Agway, Inc., 0.42% sodium) and drank tap water *ad libitum* until sacrificed, for the study, by decapitation. Each atrial and each ventricular extract was prepared from pooled atria and pooled ventricles obtained from 5 to 10 rats (atrial tissue from each rat weighed 80–100 mg). The hearts were excised and immediately placed in cold phosphate-buffered saline (PBS, 10 m*M* sodium phosphate in 0.9% NaCl, pH 7.4). Atria (with atrial appendages) and ventricular apices were excised and pooled separately in PBS (100 mg myocardial tissue/ml of PBS). For ATPase studies the tissue extracts were prepared in distilled water (diuretic and natriuretic activities of atrial extracts prepared in PBS and distilled water were the same). The tissues were homogenized with a polytron homogenizer (PT-10 ST Probe, Brinkman Instruments) at the number 8 setting for 30 sec. The homogenates were boiled at 100°C for 5 min and then centrifuged at 36,000*g* (Sorval RC-5 Refrigerated Centrifuge) for 90 min and supernates of boiled atrial extract (SA) and supernates of boiled ventricular extract (SV) were removed and stored at −70°C until assayed.

I. Diuretic and natriuretic activity. Diuretic and natriuretic activities were assayed using the method of deBold *et al.* (7). Male Wistar rats (250–300 g) with documented normotension were used for the assay. The animal was anesthetized with sodium pentobarbital, 50 mg/kg. The external jugular vein was cannulated for intravenous infusions and the urinary bladder was cannulated for collection of urine. Normal saline at 37°C was infused iv at the rate of 0.15 to 0.21 ml/min for 20 min and then at the slower rate of 0.02 ml/min for 60 min. Control urine output was calculated from the urine collected during the last 30 min of the slow infusion period. At the end of the slow infusion period, SA was infused at the rate of 0.5 ml for 4 min. Saline infusion at the slow rate of 0.02 ml/min was then restarted. Urine was collected during SA infusion and for an additional 36 min following completion of SA infusion. A control Wistar rat similarly prepared underwent exactly the same assay procedure except that the animal was infused with SV rather than SA.

II. Effects on Na^+-K^+ pump and membrane Na^+,K^+-ATPase activities. (a) Effect on vascular Na^+-K^+ pump activity. We used our standard technique to study the effect of SA on ouabain-sensitive radioactive rubidium (^{86}Rb) uptake, a measure of Na^+-K^+ pump activity, by rat tail arteries (14). Briefly, tail arteries were excised from pentobarbital anesthetized normotensive rats. The arteries were quickly cleaned of adventitia and placed in Krebs–Henseleit solution. Each artery was divided in half. One-half was preincubated at room temperature in SA and the other half in SV. After 2 hr of preincubation, ouabain-sensitive and insensitive ^{86}Rb uptakes were measured as previously described for boiled plasma supernates, i.e., without sodium preloading in order to examine steady state pump activity (14).

(b) Effects on myocardial and renal Na^+, K^+-ATPase activities. Male Wistar rats weighing between 200 and 250 g were anesthetized with Na pentobarbital (75 mg/kg, ip) and their hearts and both kidneys were excised and rinsed in a cold solution (0°C on ice) containing 5 m*M* ethylenediaminetetraacetic acid (EDTA), 0.25 *M* sucrose, and 5 m*M* histidine-HCl (pH 7.4). The atria, right ventricles and large arteries and veins were removed from the heart and microsomes were prepared from the left ventricle including the septum. Renal arteries, veins, papillae, and capsules were removed from the kidneys, and after midsagittal bisection, the cortex was dissected from the medulla and microsomes were prepared from the cortex by the same method used for the left ventricles. The tissues were generally used immediately but were occasionally frozen in the rinsing solution (−40°C) for short periods of time (less than 1 week).

Microsomal fractions were prepared from the left ventricle (and kidney cortex) as described previously (26). Briefly, the left ventricle or both cortexes were homogenized (motor-driven Teflon pestle) in approximately 10 vol of a cold buffered solution containing 0.15% deoxycholate. The homogenate was centrifuged (Sorvall RC 5 centrifuge, SM-24 rotor) at 1100*g* for 10 min and 12,300*g* for 20 min to remove the nuclear and mitochon-

drial fractions and the supernate was centrifuged at 49,000*g* for 1 hr to obtain the microsomal fraction. The microsomal fraction was resuspended in a cold buffered solution containing 1 *M* NaI and was stirred repeatedly for 1 hr on ice. It was then recovered by a final centrifugation at 49,000*g* for 1 hr, resuspended in 10 m*M* imidazole-HCl (pH 7.4), and frozen until used (not more than 2 weeks).

Total ATPase activity was assayed in duplicate by measuring the amount of inorganic phosphate (P_i) liberated from ATP (Boehringer Mannheim Biochemicals) during incubation of microsomes for 1 hr at 37°C in 1 ml of a medium containing (m*M*): 120 NaCl, 10 KCl, 2.5 $MgCl_2$, 2 Na_2ATP, 0.5 ethylene glycol bis(aminoethyl ether) *N,N'*-tetraacetic acid (EGTA), 40 Tris–HCl (pH 7.5), and 0, 0.05, 0.15, or 0.35 ml SA or SV. Mg^{2+}-ATPase activity was assayed under similar conditions except that KCl was replaced by 1 m*M* ouabain. Na^+,K^+-ATPase activity was calculated, in the presence or absence of SA or SV by subtracting Mg^{2+}-ATPase from total ATPase activity. Any contaminant P_i contributed by the supernates was therefore subtracted along with Mg^{2+}-ATPase activity. In preliminary studies we verified that the supernates did not affect the linearity of the P_i assay. P_i was assayed by the method of Fiske and SubbaRow (27) and protein was assayed by the method of Lowry *et al.* (28). Na^+,K^+-ATPase activities were expressed as fractional activities (FA) and were obtained by dividing the activity in the presence of supernate by the control activity in the absence of supernate.

III. Effects on Na^+ and water transport in the toad bladder. Hemibladders of the toad were prepared and mounted as sacs by the methods previously described by Chen and Walser (29). In each experiment, both control and experimental bladders were bathed on both sides with phosphate buffered sulfate Ringer's solution (PR) containing (m*M*): 55 Na_2SO_4, 1 $CaSO_4$, 1 $MgSO_4$, 3 K_2SO_4, 2.4 Na_2HPO_4, 0.6 NaH_2PO_4. The initial fluid volumes in the mucosal and serosal baths were 15 and 50 ml, respectively. Both bathing media were vigorously stirred. All experiments were performed at room temperature (22–25°C).

After equilibration for 60–90 min under open circuit conditions, the bladders were continuously short-circuited throughout the experiment. When short circuit current (SCC) in both bladders became almost steady, electrical parameters in each bladder were measured as previously described (30). SA was then added to both sides of the experimental bladder, with a bath volume ratio of SA to PR equal to 0.045, and SV was added to both sides of the control bladder in the same manner. Electrical parameters in each bladder were again measured when a maximal change in SCC was observed approximately 5–10 min after the addition of either supernate. In order to assess the effect of SA or SV on passive membrane permeability of the toad bladder, SCC was eliminated by ouabain (2 m*M*).

In some experiments, the effects of SA and SV on unidirectional water fluxes across the toad bladder were also studied under short circuit conditions. The method for measuring unidirectional water flux using a tracer was slightly modified from one previously described for measuring nonelectrolyte flux (31). When SCC in the toad bladder became almost steady, about 0.1 μCi/ml of $[^3H]H_2O$ was added to either the mucosal or serosal bathing medium. Measurement of unidirectional water flux began after about 10 min of mixing. Because the bladder is highly permeable to water, three 1-ml samples were withdrawn simultaneously from both bathing media at 8- to 12-min intervals. SA or SV was then added to both sides of the bladder as described above. When the inhibitory action of the supernate was complete and a SCC was again observed, three 1-ml samples were again withdrawn from both bathing media. Each 1-ml sample was then counted in 10 ml of Aquasol-2 (New England Nuclear) using a liquid scintillation counter. The cumulative counts obtained at each interval were expressed as the ratio of the counts per minute of the sample obtained from the "cold bath" to that of the sample simultaneously withdrawn from the "hot bath" to which the isotope had been added. Unidirectional water flux was obtained with a computer program ("FLUX") which calculated linear regression of cumulative counts crossing the membrane (corrected for diminishing bath volume at each interval) against time. Flux was expressed as ml/hr/mg of wet weight. The wet weight of the toad bladder was obtained after blotting on filter paper at the conclusion of each experiment.

IV. Effect on blood pressure. Normal Wistar rats (250–350 g) with documented normotension were bilaterally nephrectomized (2NX) and then used for bioassay 24 hr later. Under pentobarbital anesthesia (50 mg/kg, ip), the common carotid artery was cannulated (PE 50) for the measurement of arterial pressure. The external jugular vein was cannulated for injection of drugs and infusion of SA or SV. After a stabilization period of 20 min, ganglion blockade was produced by injection of hexamethonium chloride (HEX); doses of 1 mg, usually three or four doses, were given until there was no further fall in the blood pressure. When the blood pressure was steady, the pressor effects of 4, 5, 10, and 50 ng of norepinephrine (NE) were recorded. SA or SV was then infused intravenously at the rate of 0.2 ml/min for 10 min (same volume of SA which produced a pronounced diuretic and natriuretic effect) and its effect on blood pressure recorded. At the end of the infusion period, the NE dose–response relationship was again determined.

In another series of experiments, 10 ng NE were mixed with 0.05, 0.1, and 0.15 ml of SA or SV. The mixtures were then injected intravenously in 2NX, HEX-treated rats.

Since the results of these experiments suggested that SA potentiates the pressor effect of NE, we repeated the infusions of SA and SV in autonomically blocked (phentolamine 1 mg in 50 μl saline, propranolol 2 mg in 50 μl saline, and atropine 1 mg in 50 μl saline given separately at 3-min intervals) (AB) rats.

Student's t test was used to compare the diuretic, natriuretic, and the pressor activities of SA and SV. The paired t test was used to compare the effects of SA and SV on ^{86}Rb uptakes, myocardial and renal Na^+,K^+-ATPase, SCC, and other parameters measured in the toad bladder. Analysis of variance and Duncan's multiple range test were used to determine the significance of differences in the dose–response studies. In all these comparisons, $P < 0.05$ was considered to be a significant difference.

Results. SA produced a prompt (within 2 to 4 min) and brisk diuresis and natriuresis (Fig. 1). Urine excretion increased 10-fold and sodium excretion increased 25-fold (from 0.28 to 7.17 μeq/min). The diuretic and natriuretic responses were complete in about 40 min. SV was essentially without effect.

Ouabain-sensitive ^{86}Rb uptake (which reflects Na^+-K^+ pump activity) by normal tail arteries incubated in SA was not different from that by arteries incubated in SV (Fig. 2). This was also the case for ouabain-insensitive ^{86}Rb uptake (which in part reflects passive penetration of ^{86}Rb into cells which depends upon permeability, surface area and concentration gradient).

Figure 3a shows cardiac Na^+,K^+-ATPase (FAs) as a function of the volume of SA and SV in 1 ml of assay medium. The values were not different at 0.05 or 0.15 ml. However, at 0.35 ml the FA with SV was significantly less than that with SA. Figure 3b shows that the renal Na^+,K^+-ATPase FAs observed in the presence of SA and SV were not significantly different at any of the three volumes tested.

Addition of SA or SV to the toad bladder produced an immediate decrease in SCC, reaching a minimum in about 6 min, but the changes were not significantly different (Fig. 4a). This was also the case for membrane potential (ψ_{sp}), measured under open circuit conditions, and total tissue conductance (g_t), calculated as SCC/ψ_{sp} (Figs. 4b, c). Passive

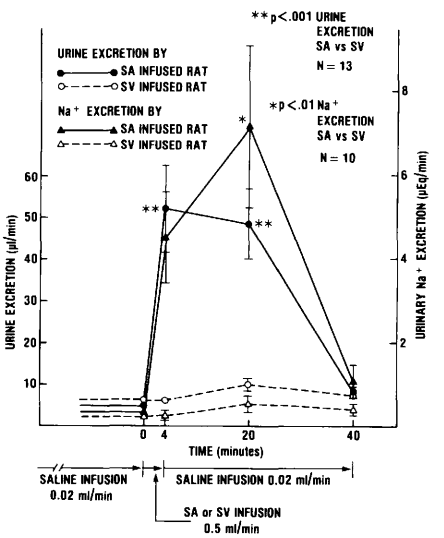

FIG. 1. Urine (circles) and urinary sodium (triangles) excretion rates by assay rats before, during and following intravenous infusion of atrial supernate (SA, closed symbols) and ventricular supernate (SV, open symbols).

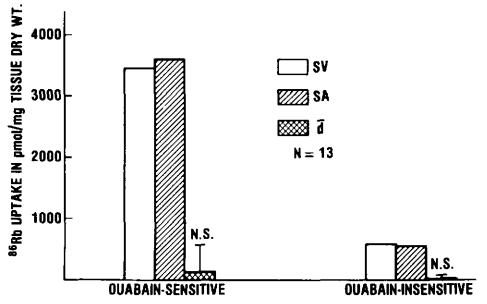

FIG. 2. Ouabain-sensitive and insensitive ^{86}Rb uptakes by tail arteries from normal untouched rats incubated in atrial supernate (SA) and ventricular supernate (SV). $\bar{d}$ Represents mean and SE of the differences.

membrane conductance (g_p), i.e., the ouabain-insensitive conductance measured after elimination of SCC with 2 mM ouabain, was not different in SA- and SV-treated bladders (not shown) and not different from the value previously found in bladders bathed in the same Ringer's solution. Ouabain-sensitive conductance (g_a), calculated as the difference between g_t and g_p, decreased on addition of SA or SV but again the changes were not different (Fig. 4d). The E_{Na} of the sodium pump, calculated as SCC/g_a, was essentially unaffected by treatment with SA or SV (Fig. 4e). Both SA and SV reduced serosal-to-mucosal water flux by about 32% and increased mucosal-to-serosal water flux by about 20 to 24% (Table I).

Relative to infusion of SV, infusion of SA into 2NX, HEX-treated rats increased mean arterial pressure by about 15% ($P < 0.005$) (Fig. 5). However, the effect was transient, disappearing by the fourth minute of infusion. Pressor responses to various doses of NE were essentially the same before and following SA or SV infusion (Fig. 6) except that 50 ng of NE following SV infusion produced significantly less pressor response than 50 ng of NE before SV infusion. However, when the NE–extract mixtures were injected, the mixtures containing SA produced greater responses than the mixtures containing SV (Fig. 7). The pressor responses to the SA–NE mixtures appeared to be dose dependent (Fig. 7). Infusion of SA into 2NX rats with AB (Fig. 5) failed to produce the transient pressor effect observed in 2NX rats treated with HEX (Fig. 5).

Discussion. These studies show that, although SA has potent natriuretic and diuretic activities and SV does not, the effects of SA on cardiac and renal Na^+,K^+-ATPase activities, rat tail artery Na^+-K^+ pump activity, and toad bladder sodium transport do not differ from those of SV. SA does not raise blood pressure even in animals whose buffering mechanisms have been blocked. These findings indicate that ANF is different from the HSTIF seen on acute volume expansion and in low-renin hypertension and that it probably causes natriuresis and diuresis by mechanisms other than pressure elevation and Na^+,K^+-ATPase inhibition.

deBold *et al.* (7) first reported the potent natriuretic and diuretic activities of atrial extract. Sonnenberg *et al.* (32) showed that administration of probenicid reduces but does not prevent the diuretic responses to atrial extract. Since probenicid blocks the organic acid transport system in the proximal tubule, the authors concluded that probenicid reduces the delivery of ANF to the distal tubules where ANF probably acts (8, 33). Its mechanism of action at this site is unknown.

In our experiments, SA relative to SV failed to inhibit Na^+,K^+-ATPase activity in microsomes prepared from myocardium and kidney cortex. Thibault *et al.* (34) also found that

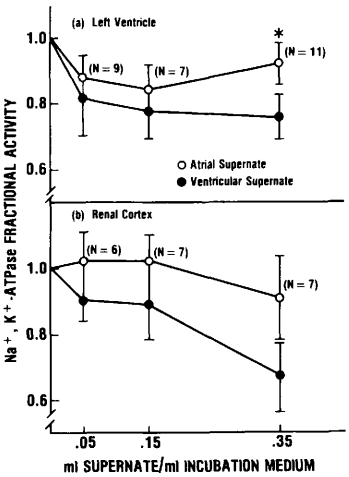

FIG. 3. Effects of atrial and ventricular supernates on Na^+,K^+-ATPase activities of microsomes from left ventricle (a) and renal cortex (b). Vertical bars represent SE. * Indicates significant difference ($P < 0.05$) between the effects of atrial (0.35 ml) and ventricular (0.35 ml) extracts.

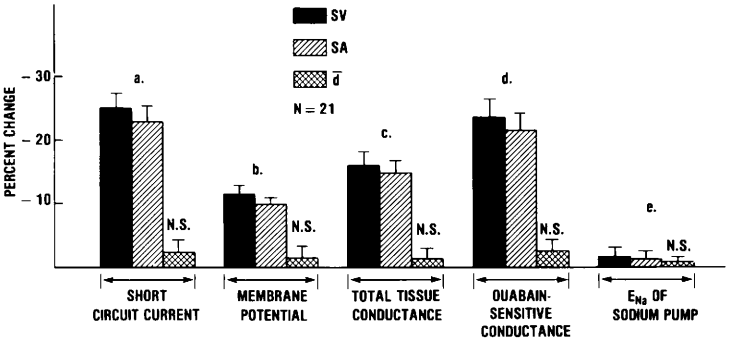

FIG. 4. Effects of atrial supernate (SA) and ventricular supernate (SV) on various parameters measured in the toad bladder. $\bar{d}$ Represents mean and SE of the differences.

atrial extract does not inhibit renal Na^+,K^+-ATPase activity. Furthermore, SA did not inhibit ouabain-sensitive ^{86}Rb uptake by rat tail arteries relative to SV and had no effect on the driving force of the sodium pump (E_{Na}) in toad bladder. Since these findings indicate that SA does not effect Na^+,K^+-ATPase and Na^+-K^+ pump activities, its potent natriuretic and diuretic activities must be due to some other mechanism(s). Possibilities include increased permeability of the tubular cell to water and/or sodium. Our data fail to support these possibilities since SA, relative to SV, had no effect on any of the relevant parameters measured in the toad bladder nor did it effect ouabain-insensitive ^{86}Rb uptake by the rat tail artery; the similar changes produced by SA and SV in the toad bladder must have resulted from nonspecific effects of the supernates. It should be pointed out, however, that the fluxes of water across the toad bladder were measured in the absence of an osmotic gradient. It is possible that SA and SV would produce different effects on water flux if they were measured in the presence of an osmotic gradient.

Another possible mechanism by which SA could induce potent diuresis is through an increase in the glomerular filtration rate (GFR). In our studies, infusion of SA in an amount sufficient to cause potent diuretic and natriuretic effects produced only a small transient increase in blood pressure in the 2NX, HEX-treated rats (possibly due to potentiation of endogenous NE). This pressor effect appears to be insufficient to produce the potent diuresis and natriuresis observed by increasing GFR. Additionally, deBold *et al.* (7) and Briggs *et al.* (8) have shown that atrial extract does not alter GFR except in high doses. Kleinert *et al.* (35) showed that, in the isolated rat kidney perfused at constant pressure, atrial extract significantly increases renal vascular resistance, GFR, filtration fraction, and sodium excretion. These authors also showed (36) that these effects were calcium dependent since they were abolished by perfusion with low

TABLE I. EFFECTS OF ATRIAL AND VENTRICULAR SUPERNATE ON UNIDIRECTIONAL WATER FLUXES IN THE SHORT-CIRCUITED TOAD BLADDER

Serosal-to-mucosal water flux (ml/hr-mg)				Mucosal-to-serosal water flux (ml/hr-mg)			
Experimental		Control		Experimental		Control	
PR	PR + SA	PR	PR + SV	PR	PR + SA	PR	PR + SV
0.060 ± 0.011 (7)	0.041 ± 0.006 (7)	0.061 ± 0.008 (7)	0.042 ± 0.006 (7)	0.064 ± 0.006 (7)	0.077 ± 0.008 (7)	0.054 ± 0.005 (7)	0.067 ± 0.006 (7)

Note. PR, phosphate-buffered sulfate Ringer's solution; SA, atrial supernate; SV, ventricular supernate. The ratio of added volume of SA or SV to initial bath fluid volume is 0.045.

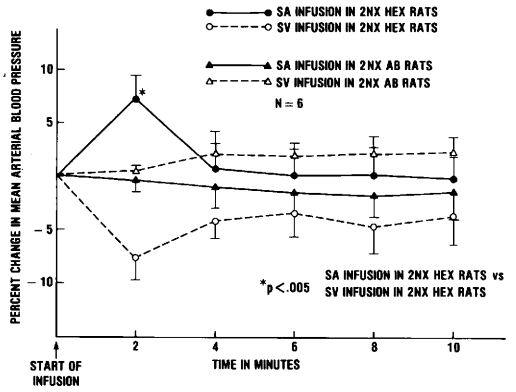

FIG. 5. Effects of 10-min intravenous infusions of atrial supernate (SA, closed symbols) and ventricular supernate (SV, open symbols) on mean arterial pressure in bilaterally nephrectomized (2NX), hexamethonium (HEX)-treated rats (circles) and in bilaterally nephrectomized (2NX), automically blocked (AB) rats (triangles). AB achieved with phentolamine, propanolol, and atropine. Vertical bars represent SE. * Indicates significant difference ($P < 0.005$) between the pressor effect of SA versus SV in 2NX, HEX-treated rats.

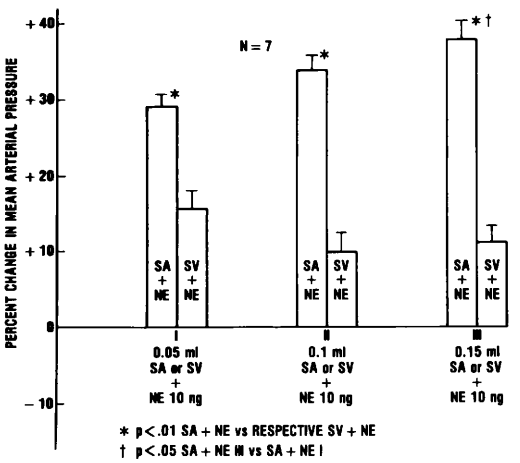

FIG. 7. Percentage change in mean arterial blood pressure on intravenous injection of mixtures of atrial supernates (SA) or ventricular supernate (SV) with norepinephrine (NE) in bilaterally nephrectomized (2NX), hexamethonium (HEX)-treated rats.

[Ca^{2+}] and were decreased by verapamil. In intact animals, however, atrial extract decreased both peripheral and renal vascular resistance (36). More recently this laboratory reported (37) that atrial extract contains a factor which acts as a functional competitive inhibitor of angiotensin II in isolated blood vessels. This could explain diuresis and natriuresis if, through this action, ANF alters renal vascular hemodynamics. More studies are needed in this area.

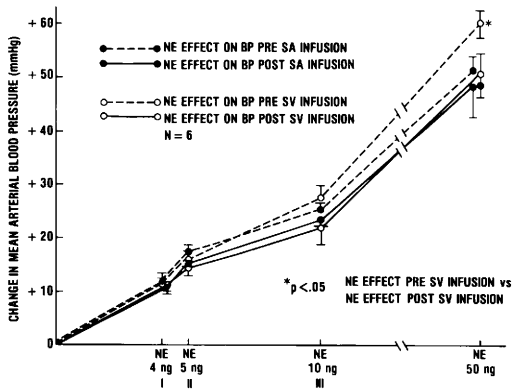

FIG. 6. Effects of intravenous bolus injection of norepinephrine (NE) before (dashed lines) and after (solid lines) infusion of atrial supernate (SA, closed circles) and ventricular supernate (SV, open circles) on mean arterial blood pressure in bilaterally nephrectomized (2NX), hexamethonium (HEX)-treated rats. Vertical bars represent SE. * Indicates significant differences ($P < 0.05$) between the pressor effects of 50 ng NE before and after SV infusion.

Our finding of a small transient pressor response to SA seems to differ from those of Camargo *et al.* (36) who reported decreases in peripheral and renal resistances and deBold *et al.* (38) who reported a fall in blood pressure lasting about 20 min following intravenous infusion of atrial extracts in rats. Trippodo *et al.* (12) also reported a transient fall in mean arterial pressure following intravenous infusion of crude atrial extract; partially purified extracts, however, had no effect on the blood pressure. The difference between our results and those of other investigators may be related to our use of 2NX, HEX-treated rats. We saw no effect on the blood pressure in 2NX, AB rats, suggesting that it does not have an overall direct effect on cardiovascular muscle.

Our findings strongly suggest that the ANF present in SA is different from the HSTIF present in acutely volume-expanded animals (13, 15, 16, 18, 19, 21, 24, 25, 39) and humans (40) as well as in some forms of low-renin

experimental hypertension (13, 14, 17, 41, 45) and in human essential hypertension (20, 23, 43, 44). The HSTIF *in vitro* inhibits renal Na^+,K^+-ATPase (18–20, 24) and Na^+-K^+ pump activity in vascular smooth muscle cell (13, 14, 41, 42) and SCC in the toad bladder (17). It also depolarizes vascular smooth muscle cells (45) and causes increased vascular reactivity (21). It seems to come from or be influenced by the anteroventral third ventricular (AV3V) area of the brain (14). In contrast, the results of our present study indicate that the ANF *in vitro* does not inhibit renal or myocardial Na^+,K^+-ATPase. Furthermore, ANF does not inhibit vascular smooth muscle cell Na^+-K^+ pump activity or pump activity in the toad bladder. Finally, it has only negligible effects on blood pressure. These findings, indicate that the actions of ANF and the HSTIF are different and that the two factors probably are not the same. However, since ANF is harvested directly from atria and not from plasma, a note of caution is in order; it is possible, that, if released into the circulation, structural alterations due to the action of circulating hydrolytic enzymes may cause the factor to behave differently, possibly like a HSTIF.

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