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Research Article

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A Natriuretic Principle Derived from Kidney Tissue of Volume-Expanded Rats

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ABSTRACT Homogenates of kidneys from hydropenic and volume-expanded rats were subjected to gel filtration with Sephadex G-25. A fraction of the eluate coincident with the fourth UV peak was injected into the aorta of rats with one kidney excluded. A fraction eluting before the albumin peak was utilized as a control. Significant natriuresis and diuresis were observed after infusion of the fraction obtained from volume-expanded kidneys but not after infusion of the fraction from hydropenic kidneys or the control fraction. The natriuresis occurred in the absence of changes in mean blood pressure, hematocrit, plasma sodium and potassium, glomerular filtration rate, and potassium excretion. The response was apparent immediately after infusion and persisted for up to 150 min. These results verify the existence of a low molecular weight natriuretic substance which may be preferentially bound to the kidney after its volume-stimulated release into the circulation.

INTRODUCTION

The existence of a natriuretic hormone remains controversial. Many reports have suggested the presence of a natriuretic substance or transport inhibitor in plasma and urine in various experimental and clinical conditions, principally volume expansion and uremia (1–23). However, the nature of the substance, its molecular weight, structure, site of production, and site and mode of action in the nephron have not been elucidated. Major difficulties inherent in the investigation of these questions include problems in isolating a sufficient quantity of the material for purification and identification, lack of a standardized assay technique to permit comparison of results from different investigators, and the possible presence of a carrier protein in plasma.

Based on previous observations that there are specific protein-binding sites in target organs for many circulating hormones (24), we have reasoned that in an animal secreting maximal amounts of a natriuretic hormone, the kidney, as a major target organ, should be a rich source of free and bound hormone. By extracting whole kidneys from volume-expanded rats we have been successful in obtaining a substantial yield of a low molecular weight substance which produces a consistent natriuretic response in assay animals. The bioassay procedure was adapted from that described by Sealey and Laragh (13) in which one kidney of a normal rat is ligated and the test substance is injected into the aorta immediately proximal to the remaining renal artery.

METHODS

Fractionation procedures for isolation of humoral material and preparation of samples for assay. 12 conscious rats of the Sprague-Dawley strain, weighing approximately 300 g, were volume expanded with isotonic saline by 10% of initial body weight over a 1-h period. Volume expansion was maintained by a sustaining infusion of saline at a rate of approximately 0.15 ml/min for an additional 1 h, and the rats were then sacrificed after verifying that they were in a diuretic phase. Both kidneys were removed and immediately placed in 10 ml of boiling deionized water acidified to pH 3.4 with acetic acid. After 20 min of boiling, the kidneys were cooled, blotted gently to remove excess water, decapsulated, cut into small pieces, and then weighed. Deionized water was added to make a 10% solution (wt/vol). The tissue was homogenized with a Tri R tissue homogenizer (Tri R Instrument Corp., Rockville, N. Y.) at maximum speed for a minimum of 10 strokes. The resultant solution was centrifuged at 2,300 rpm for 15 min at 4°C to remove cell debris. 3 ml of the supernate was then applied to a Sephadex G-25 Fine column (Pharmacia Fine Chemicals Inc., Piscataway, N. J.) (2.5 × 40 cm, void volume

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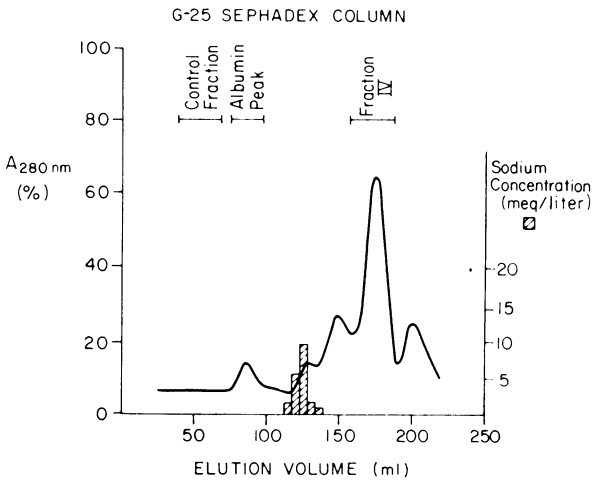

FIGURE 1 Boiled whole rat kidney homogenate applied to a Sephadex G-25 column (2.5 × 40 cm) 0.1 M acetic acid buffer. Plot indicates location of control fraction and fraction IV with relationship to UV absorption peaks at 280 nm and to salt peak.

70 ml) and eluted from above with 0.1 N acetic acid at 4°C. Eluate speed was 1 ml/min. Fractions were monitored with an automated ultraviolet absorptiometer (Uvicon-540, Toyo Co., Japan) at 280 nm (Fig. 1). Eluate contained within the fourth UV peak, approximately 35 ml, (partition coefficient, K_{av}, 0.72–0.94[1]) was pooled and lyophilized after addition of 12 mg of bovine serum albumin (Cohn Fraction V, Sigma Chemical Co., St. Louis, Mo.) and 0.05 ml of 1 N mercaptoacetic acid. This fraction, termed expanded fraction IV, was thought to contain the natriuretic principle on the basis of earlier work in this laboratory (25) (*vide infra*). An identical volume of eluate appearing before the albumin peak (i.e., the first UV peak) was treated similarly and considered as a control fraction. The lyophilized material was accurately weighed and stored at −40°C. Immediately before use in the assay animal exactly one-third of the material (average wt 5 mg) was dissolved in 0.4 ml of isotonic saline.

A similar number of rats were dehydrated overnight, then sacrificed, and the kidneys prepared as above. The material found in the fourth UV peak has been termed hydropenic fraction IV.

Bioassay procedure for detection of natriuretic activity. The assays were performed on female Sprague-Dawley rats, weighing from 277 to 317 g, maintained on a standard Purina laboratory rat chow diet and ad lib water intake. The animals were anesthetized with Inactin®, 100 mg/kg body weight (Promonta, Hamburg, West Germany). A rectal probe for temperature control was inserted, and the temperature was kept at 38±0.5°C with the help of a heat lamp. After tracheostomy with a polyethylene catheter (PE 240), the bladder was entered with a PE 50 through a midline lower abdominal incision. A PE 50 was also inserted into the carotid artery and connected to a mercury manometer for monitoring of blood pressure. The left renal vein and artery were ligated through an abdominal midline incision and a PE 10 catheter was introduced through the right femoral artery to a length of 7.0±0.5 cm from the point of insertion. The tip of the catheter was thus immediately cephalad to the remaining renal artery. The jugular vein was next catheterized with a PE 10 and a priming dose of [^{125}I]iothalamate (Glofil, Abbott Laboratories, Chicago, Ill.), 0.8–0.9 μCi in a volume of 0.5 ml of isotonic saline given over a 30-s period, followed by a sustaining infusion of the same solution at a rate of 1.2 ml/h. This concentration of [^{125}I]iothalamate provided a plasma count which was at least 10 times background.

After a recovery period of 90–120 min, several control or equilibration clearance periods 15 min in duration were obtained. Urine flow (V)[2] was determined by weighing tared centrifuge tubes at the conclusion of a collection period. The equilibration phase was terminated when three consecutive clearance periods were obtained with less than 10% variation in V. The test fractions, obtained from individual rat kidney homogenates (either hydropenic or volume expanded), were then dissolved in a volume of 0.4 ml of isotonic saline and 0.3 ml was infused into the aorta at a rate of 150 μl/min. This volume corresponded to the amount of fraction IV extracted from 5% of the original kidney tissue.

The control fraction was infused first, followed by either the hydropenic fraction IV or the expanded fraction IV in a randomized sequence. In nine experiments, all three fractions were infused. In three experiments, only control and hydropenic fractions IV were infused. In one of these experiments, infusion of the standard quantity of hydropenic fraction IV was followed by infusion of a fivefold concentrate of the same fraction. In two experiments, only control and expanded fractions IV were infused, while in one experiment, only the expanded fraction IV was infused immediately after the equilibration phase. Three or more clearance periods of 15-min duration were obtained after the infusion of each fraction until two consecutive clearance periods showed return of V to base-line values. Blood samples (0.2 ml) were collected from the aortic or carotid catheters at the beginning and end of the equilibration period, before the injection of each fraction, and at the end of the experiment. The blood was allowed to flow directly into heparinized microhematocrit tubes which were immediately centrifuged, the hematocrit determined, and the plasma separated. Sodium and potassium concentrations were measured in all the samples. The mean values of the two samples collected at the beginning and end of each injection were used for calculation of the filtered load employing a Donnan correction factor of 0.95.

For the determination of radioactive iothalamate in urine and plasma, 20-μl samples of either undiluted urine or serum were pipetted into plastic counting vials. The samples were counted in a Packard Dual-Channel Lin-Log Analyzer (Model 271, Packard Instrument Co., Inc., Downers Grove, Ill.). Sodium and potassium were measured with a Perkin-Elmer atomic absorption spectrophotometer (Model 303, Perkin-Elmer Corp., Norwalk, Conn.). Fractional excretion of sodium and potassium (FE_{Na} and FE_K) was calculated as clearance of electrolyte divided by clearance of [^{125}I]iothalamate. All isotopic and chemical determinations were made in duplicate.

[1] Calculated from the formula $K_{av} = (V_e - V_o)/(V_t - V_o)$, where V_e is eluted volume, V_o is void volume, and V_t is total volume of the gel bed.

[2] *Abbreviations used in this paper:* FE_K, fractional excretion of potassium; FE_{Na}, fractional excretion of sodium; GFR, glomerular filtration rate; MBP, mean blood pressure; U_KV, potassium excretion; $U_{Na}V$, sodium excretion; V, urine flow rate.

TABLE I

Representative Experiment

Experimental phase	Clearance period	Time	GFR	V	FL_{Na}	$U_{Na}V$	FE_{Na}	FL_K	U_KV	FE_K	MBP	Temp	Hematocrit
			ml/min	*μl/min*	*μeq/min*	*μeq/min*	%	*μeq/min*	*μeq/min*	%	*mm Hg*	°C	%
Surgery*		-210											
Recovery‡		-105											
Equilibration	1	0-15	1.08	15.6	125	2.21	1.77	4.03	1.61	40.0	124	37.5	39
	2	15-30	1.09	15.8	126	2.23	1.76	4.04	1.61	39.9	124	37.5	
	3	30-45	1.06	15.8	123	2.18	1.77	3.92	1.63	41.6	124	37.5	
		45-47	0.3 ml control fraction infused intra-aortically at 150 μl/min.										
Control fraction	4	47-62	1.01	16.2	117	2.18	1.86	3.84	1.35	35.2	122	37.5	38
	5	62-77	1.00	12.2	113	1.95	1.72	3.89	1.44	37.0	120	37.5	
	6	77-92	1.02	13.0	115	1.96	1.69	3.97	1.17	29.5	120	38.0	
		92-94	0.3 ml hydropenic Fraction IV infused intra-aortically at 150 μl/min.										
Hydropenic fraction IV	7	94-109	1.10	11.0	120	2.01	1.67	4.28	1.65	38.6	118	38.5	36
	8	109-124	1.03	11.4	120	1.39	1.15	4.10	1.79	43.7	118	38.0	
	9	124-139	1.00	11.1	117	1.40	1.19	3.99	1.65	41.4	118	37.5	
		139-141	0.3 ml expanded Fraction IV infused intra-aortically at 150 μl/min.										
Expanded fraction IV	10	141-156	1.01	9.6	125	1.90	1.52	4.12	1.70	41.3	118	37.5	37
	11	156-171	1.12	14.8	144	3.70	2.57	4.57	1.14	24.9	118	37.5	
	12	171-186	1.05	16.2	135	4.52	3.35	4.28	1.10	25.7	118	38.0	
	13	186-201	1.00	13.0	128	2.64	2.05	4.08	0.96	23.5	118	38.0	
	14	201-216	0.92	9.5	122	1.89	1.54	3.67	0.61	16.6	118	38.0	37

* Rat anesthetized with Inactin. Catheters placed in bladder, carotid artery, jugular vein, and femoral artery. Left renal hilus ligated through abdominal incision.

‡ Priming solution containing 0.9 μCi of [^{125}I]iothalamate in 0.5 ml of isotonic saline followed by a sustaining solution containing 18 μCi in 10 ml of isotonic saline at 1.2 ml/h.

Abbreviations: FL_{Na}, filtered load of sodium = GFR × plasma sodium concentration × 0.95; FL_K, filtered load of potassium = GFR × plasma potassium concentration × 0.95.

The data were analyzed by comparing the mean and peak values for V, glomerular filtration rate (GFR), sodium excretion ($U_{Na}V$) potassium excretion (U_KV), and FE_{Na} and FE_K for the three 15-min periods constituting the 45-min interval immediately preceding the injection of the control fraction (equilibration phase) with the mean and peak values obtained during the 45-min interval immediately after injection of each fraction (control, hydropenic fraction IV, and expanded fraction IV).

Statistical evaluation was initially performed by analysis of variance (26). When the F test showed a P of < 0.05, differences between individual groups were determined by the Newman-Keuls multiple-range test (27). The paired Student's t test was also used to ascertain differences between individual experimental periods.

RESULTS

Mean weight of the 15 animals before surgery was 299±3 g and at the end of the experiment was 298±4 g; thus no volume changes were induced throughout the experiment.

Representative experiments. The results of a typical experiment are presented in Table I. Detailed are the sequential changes in mean blood pressure (MBP), hematocrit, and temperature, as well as the effects of the control fraction, hydropenic fraction IV, and expanded fraction IV on GFR, V, and $U_{Na}V$ and U_KV. After the surgical procedure had been completed, a period of 105 min was allowed for the animal to recover and to achieve a relatively constant rate of V. During the three 15-min equilibration clearance periods preceding injection of the control fraction, GFR averaged 1.08 ml/min., V 15.8 μl/min, and $U_{Na}V$ 2.21 μeq/min. No consistent changes occurred in any of the measured parameters after infusion of the control fraction and of the hydropenic fraction IV. However, after infusion of expanded fraction IV there was an immediate natriuretic response reaching a maximum at the end of 45 min. By 60 min, V and $U_{Na}V$ had returned to base-line values. MBP, hematocrit, and body temperature remained constant throughout the experiment.

Three individual experiments are plotted in Figs. 2, 3, and 4. Fig. 2 illustrates the sequential infusions of control fraction, hydropenic fraction IV, and fivefold concentrate of the hydropenic fraction IV. There was no significant change in GFR, $U_{Na}V$, or FE_{Na}, although V increased transiently after infusion of each fraction. In Fig. 3, we illustrate an experiment in which infusion of the control fraction was followed sequentially first by infusion of the expanded fraction IV and then the hydropenic fraction IV. After the infusion of the control fraction, there was no significant change in any of the parameters. Immediately after infusion of the expanded fraction IV there was an increase in V from 10.2 to 33.2 μl/min, in $U_{Na}V$ from 1.27 to 4.98 μeq/min,

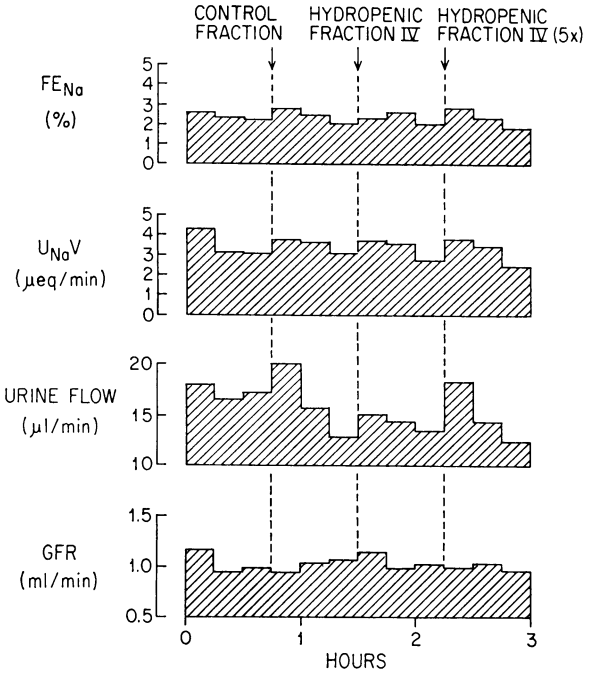

FIGURE 2 Effects of infusion of control fraction, fraction IV from hydropenic rat kidney, and fivefold concentrate of the latter on $U_{Na}V$, V, and GFR.

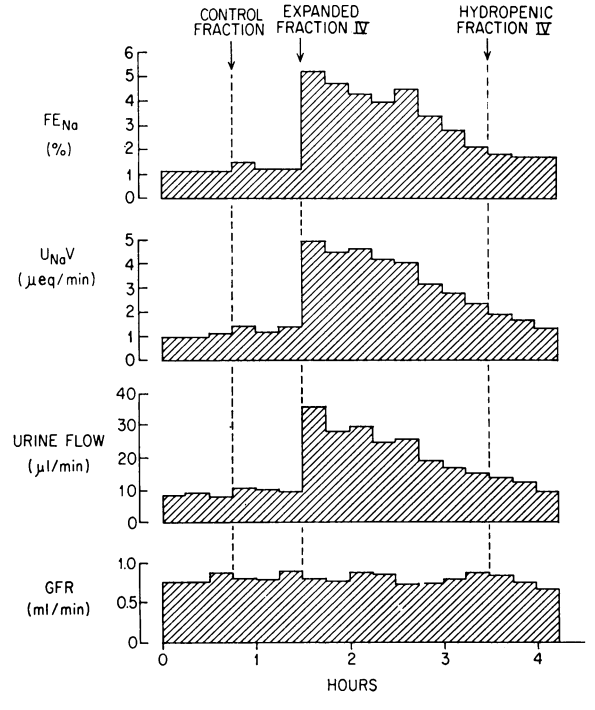

FIGURE 3 Effects of sequential infusions of control fraction, fraction IV from volume-expanded rat kidney, and fraction IV from hydropenic rat kidney on $U_{Na}V$, V, and GFR. Note immediate response to infusion of expanded fraction IV.

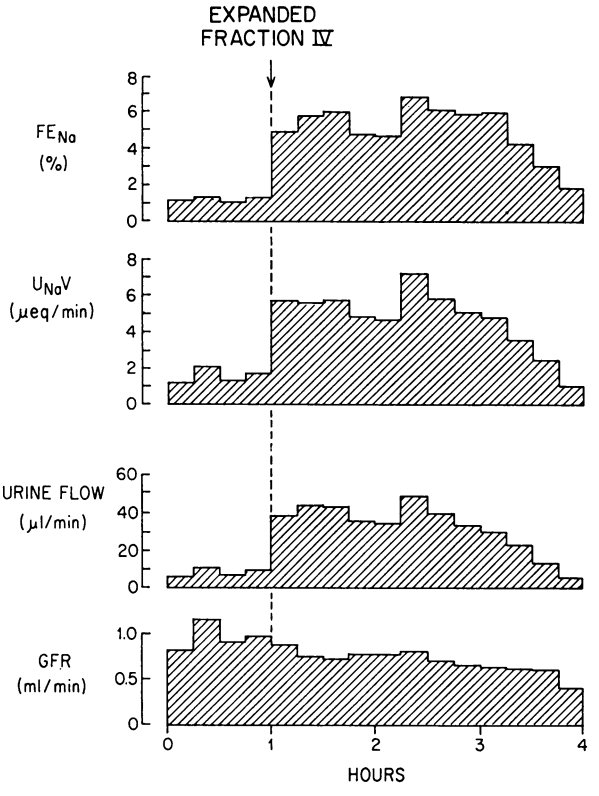

FIGURE 4 Effects of infusion of fraction IV from volume-expanded rat kidney on $U_{Na}V$, V, and GFR. Note both immediate and delayed response and protracted duration of natriuresis.

and in FE_{Na} from 1.29 to 5.22%; GFR remained constant. The increase in $U_{Na}V$ persisted for 120 min before returning to base-line values. When the hydropenic fraction IV was subsequently infused, there was no further significant change in any of these parameters over a period of 45 min. Fig. 4 demonstrates an experiment in which two peaks in sodium excretion were seen after infusion of the expanded fraction IV. In this experiment fraction IV was infused immediately after the equilibration phase. Sodium excretion rose from 1.51 μeq/min during the last three collection periods of the equilibration phase to 5.71 μeq/min immediately after infusion of the expanded fraction IV. There was a subsequent, delayed further rise in sodium excretion to 7.29 μeq/min 75 min after infusion of fraction IV; the natriuresis persisted for 165 min. With the exception of the final collection period, there was no significant change in GFR throughout the experiment.

Composite results. In Table II we present the consolidated results for MBP, hematocrit, plasma Na, and plasma K expressed as mean ±SEM for all of the experiments. No significant differences were noted in any of these parameters when values obtained during the equilibration phase and in the periods after the in-

TABLE II

Effects of Infusion of Control Fraction and Fraction IV on Mean Blood Pressure, Hematocrit, and Plasma Sodium and Potassium

Experimental phase	MBP	Hematocrit	Na_p	K_p
	mm Hg	*%*	*meq/liter*	*meq/liter*
Equilibration, $n = 15$	130 ±3	44 ±1	138 ±4	3.7 ±0.2
Control fraction, $n = 14$	126 ±4	43 ±1	139 ±5	4.0 ±0.2
Hydropenic fraction IV, $n = 12$	123 ±3	42 ±1	136 ±3	4.0 ±0.2
Expanded fraction IV, $n = 12$	125 ±3	42 ±1	142 ±5	3.8 ±0.2

Abbreviations: Na_p, plasma sodium; K_p, plasma potassium. Results are expressed as mean ±SEM.

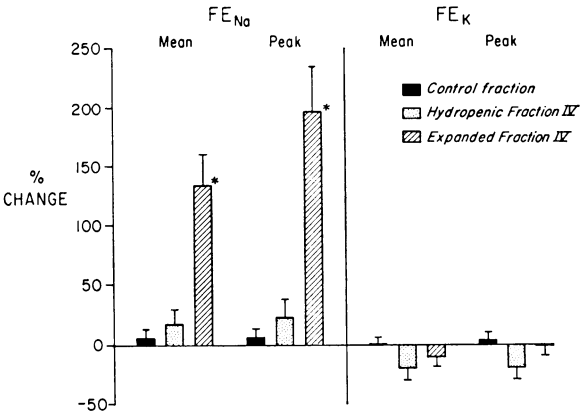

FIGURE 5 Average percent change±SEM in mean and peak fractional excretion of sodium (left panel) and potassium (right panel) obtained after infusion of control fraction and fraction IV from hydropenic and volume-expanded rat kidneys. Changes are expressed with respect to mean values during the equilibration period.

fusion of the control fraction, the hydropenic fraction IV, and the expanded fraction IV were compared.

Table III similarly summarizes the results for V, GFR, $U_{Na}V$, FE_{Na}, U_KV, and FE_K. The mean values represent averages of the three 15-min collections obtained during the equilibrium phase, after infusion of the control fraction, and after infusion of the hydropenic and expanded fractions IV. The peak results express only the highest values obtained during each of the experimental phases. When analysis of variance was applied to the mean values, a significant difference was found only in $U_{Na}V$ and FE_{Na} ($P < 0.001$). Similarly, when the peak values were examined analysis of variance revealed significant differences only with regard to these two parameters ($P < 0.001$). Analysis of the individual experimental phases by the Newman-Keuls multiple-range test revealed that both the mean and peak values for $U_{Na}V$ and FE_{Na} after injection of the expanded fraction IV differed significantly from all other experimental phases ($P < 0.01$). In absolute terms $U_{Na}V$ increased from a mean of 1.83±0.23 μeq/min and a peak of 2.07±0.27 μeq/min during the equilibration phase to a mean of 4.35±0.70 μeq/min and a peak of 5.68±0.67 μeq/min after infusion of the expanded fraction IV. FE_{Na} increased from a mean of 1.34±0.15% and a peak of 1.47±0.15% during the equilibration phase to a mean of 3.16±0.60% and a peak of 4.18±0.63%. No differences in results were observed when either the expanded fraction IV preceded the hydropenic fraction IV or the reverse sequence was used. Only 1 of the 12 animals in which the expanded fraction IV was infused failed to show a natriuretic response.

Fig. 5 illustrates the percent change in the mean and peak values of FE_{Na} and FE_K for all three experimental

TABLE III

Effects of Infusion of Control Fraction and Fraction IV on V, GFR, $U_{Na}V$, FE_{Na}, U_KV, and FE_K

Experimental phase	V		GFR		$U_{Na}V$		FE_{Na}		U_KV		FE_K	
	Mean	Peak	Mean	Peak	Mean	Peak	Mean	Peak	Mean	Peak	Mean	Peak
	μl/min		*ml/min*		*μeq/min*		*%*		*μeq/min*		*%*	
Equilibration, $n = 15$	11.2 ±1.1	12.3 ±1.1	1.06 ±0.07	1.16 ±0.08	1.83 ±0.23	2.07 ±0.27	1.34 ±0.15	1.47 ±0.15	1.51 ±0.13	1.73 ±0.14	41.5 ±2.3	46.0 ±2.9
Control fraction, $n = 14$	11.8 ±1.1	13.5 ±1.2	1.16 ±0.08	1.24 ±0.08	2.01 ±0.25	2.32 ±0.28	1.45 ±0.20	1.60 ±0.22	1.79 ±0.16	1.99 ±0.17	41.2 ±3.1	45.6 ±3.4
Hydropenic fraction IV, $n = 12$	11.4 ±0.9	13.4 ±1.2	1.24 ±0.08	1.18 ±0.08	2.15 ±0.26	2.47 ±0.31	1.49 ±0.17	1.69 ±0.20	1.43 ±0.19	1.52 ±0.20	31.2 ±3.5	34.3 ±3.8
Expanded fraction IV, $n = 12$	20.0* ±3.2	28.8* ±4.5	1.20 ±0.07	1.21 ±0.08	4.35* ±0.70	5.68* ±0.67	3.16* ±0.60	4.18* ±0.63	1.56 ±0.17	1.76 ±0.17	37.3 ±3.8	45.1 ±4.4

* Significant at $P < 0.001$ when compared with values obtained during all other experimental phases.
Results are expressed as mean ±SEM.

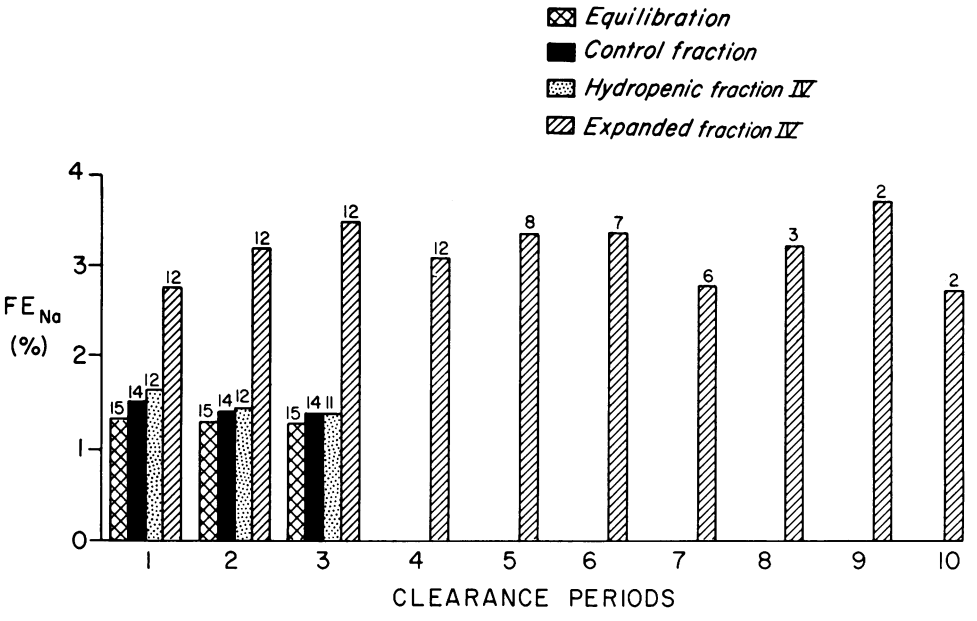

FIGURE 6 Mean fractional excretion of sodium during each 15-min clearance period in the equilibration phase and after infusion of the control fractions, hydropenic fraction IV, and expanded fraction IV. The numbers above each column refer to the number of animals on which the mean data are based. Thus, for example, only two animals demonstrated a natriuretic effect extending more than eight clearance periods.

phases, in comparison to the equilibration phase. When values obtained after infusion of the hydropenic fractions IV and the control fractions were compared, no significant differences were found. In contrast, after infusion of the expanded fractions IV the increase in mean FE_{Na} was 136±31% and the increase in peak FE_{Na} was 197±39%, as compared to 5±7% and 5±9%, respectively, after infusion of the control fractions ($P < 0.001$). Changes in FE_K and GFR (not plotted) were not significantly different from the equilibration phase.

In Fig. 6 we have plotted the mean FE_{Na} for each clearance period during the four experimental phases. There was no significant variation in FE_{Na} in each of the first three clearance periods during the equilibration phase or after infusion of control or hydropenic fraction IV. In contrast, there was an immediate increase in Fe_{Na} after infusion of the expanded fraction IV (2.78±0.56%, expanded vs. 1.54±0.21% control, $P < 0.05$). The natriuresis persisted for at least 60 min (four clearance periods). In two of the experiments, natriuresis persisted for as long as 150 min (10 clearance periods). Although the figure shows an apparent maximum response at 45 min (third collection period), when this was compared to the first period the differences were not statistically significant (3.50±0.59% third period vs. 2.78±0.56% first period, $P < 0.05$).

DISCUSSION

Those factors that control active transport of sodium by the kidney have long been the focus of investigative effort. In 1957, when Homer Smith (28) addressed himself to a theoretical consideration of salt and water receptors, the possible existence of a natriuretic hormone was considered but rejected in favor of an antinatriuretic system responsive to changes in circulating volume. The first experimental evidence that a natriuretic hormone might exist came from de Wardener, Mills, Clapham, and Hayter (1), who showed that infusion of saline into the donor dog of a cross-circulated pair caused a large natriuresis in the donor dog and a lesser, but still significant, natriuresis in the recipient animal. Since that time many investigators have attempted to show that the natriuresis which follows volume expansion (with saline, whole blood, or plasma expanders) is related to either changes in peritubular physical factors (hydrostatic pressure, oncotic pressure, hematocrit, or sodium concentration) or to the presence of a circulating humoral substance capable of inhibiting proximal tubular reabsorption of sodium (29). It now appears clear that both physical and humoral factors are important in regulating proximal sodium reabsorption.

Several procedures have been developed for assay of humoral activity after volume expansion. Based on the assumption that the hormone probably acts as an inhibitor of active transport, in vitro assays have included: (*a*) inhibition of short-circuit current across an anuran membrane (frog skin or toad bladder) (7, 14, 15, 21); (*b*) inhibition of PAH uptake by rabbit kidney cortex slices

(11); (*c*) inhibition of sodium and potassium transport and PAH uptake in isolated renal tubules (17, 18); and (*d*) inhibition of kidney Na-K-ATPase (25). Final verification, however, requires that the humoral substance possesses natriuretic activity when injected into a test animal in vivo. Early studies employing normal hydrated rats or cats yielded only modest natriuretic responses (7 , 19–21). Sealey, Kirshman, and Laragh (12) improved the accuracy of the assay by using rats with incomplete congenital diabetes insipidus. Even more striking results were obtained when the assay animals were normal rats in which one kidney was ligated and the test substance was injected into the aorta at the level of the remaining renal artery (Table IV Assay) (13). Bourgoignie, Hwang, Espinel, Klahr, and Bricker (23) used partially nephrectomized rats as assay animals for a natriuretic substance extracted from the serum of uremic patients on the assumption that the residual nephrons are more sensitive to the action of a natriuretic hormone than normal nephrons.

The majority of studies of volume expansion-induced natriuretic hormones have employed either plasma or urine as the source of hormone activity. Plasma has been employed intact (17), after dialysis or ultrafiltration (14, 16), after deproteinization with trichloroacetic acid (7), or after fractionation with gel chromatography (11–13, 15, 25). Urine has usually been concentrated and extracted with veronal (19, 21) or fractionated with gel chromatography (12, 13, 18, 22). Cort, Sedlakova, and Lichardus (8, 10) first suggested that the hormone might be a polypeptide, mol wt about 1,000, as it eluted from a Sephadex G-25 column shortly after exogenously-added vasopressin, and its activity was reduced by incubation with proteolytic enzymes. Subsequently, Sealey et al. (12) isolated a natriuretic substance from urine and blood which they estimated from column chromatography separation to have a mol wt between 5,000 and 70,000. The majority of other investigations, however, tend to support Cort's original contention that the mol wt of the active compound is closer to 1,000 (14, 15, 25), suggesting that perhaps Sealey et al had isolated either a carrier-bound hormone or a precursor hormonogen.

In previous preliminary studies from this laboratory, we have demonstrated a small molecular weight compound in a specific chromatographic fraction of plasma from volume-expanded rats and dogs which appears to act as an inhibitor of Na-K-ATPase (25). This inhibitor was eluted reproducibly from a Sephadex G-25 column immediately after the salt fraction and after exogenously added vasopressin and was coincident with the fourth UV Peak. We have presumed that the Na-K-ATPase inhibitor may be the same substance as that shown by others to possess natriuretic properties since both substances appear in approximately the same locus on column chromatography under similar conditions (10). Furthermore, subsequent experiments have shown that the same plasma fraction also inhibits active transport in the isolated frog skin (30). In designing the present experiment in which kidney tissue rather than plasma or urine was employed, we have, therefore, chosen to pool and concentrate all of the eluate contained within the fourth UV peak and have designated this as either hydropenic or expanded fraction IV depending on the condition of the donor animal before sacrifice. A similar quantity of eluate appearing before the albumin peak was utilized as a control fraction. The Type IV assay described by Sealey and Laragh (13) was chosen as the standardized assay procedure as it was felt that maximum delivery of natriuretic material to the target organ would be achieved in this way. The only modification from the original procedure was that the lower aorta was not ligated.

With this approach it was possible to demonstrate the presence of a natriuretic substance in kidney tissue of acutely volume-expanded rats but not in kidney tissue of hydropenic rats. This substance was heat and acid stable and of relatively low molecular weight. The onset of natriuretic effect was seen immediately after injection, reached a peak within 45 to 60 min, and had a duration of action ranging from 90 to 150 min. The increase in Na excretion was paralleled by a similar increase in V but not by significant changes in K excretion. These findings were obtained in the absence of alterations in GFR, MBP, hematocrit, and plasma Na and K concentrations. The lack of change in K excretion suggests that the humoral substance does not affect the distal Na-K exchange site. The parallel change in Na excretion and urine volume is noteworthy but a precise definition of the site of action of the hormone within the nephron is not possible under the conditions of this study.

In a previous study, Mills, Wilson, and deBono (31) have also successfully extracted a natriuretic substance from kidney tissue. Because T_6H_2O was unchanged during natriuresis when the extract was administered into the left renal artery of test dogs, these investigators concluded that the substance acted on the proximal tubule. Mills et al. favored the concept that the renal humoral substance is of large molecular weight and originates within the kidney with release triggered by an intrarenal pressure-sensitive mechanism. To further explore this concept, Sadowski, Morrison, and Selkurt (32) employed an ingenious cross-circulation preparation in which renal venous blood from volume-expanded dogs was pumped directly into the system perfusing the right kidney of oliguric recipient animals. As virtually

no natriuretic response was obtained, these authors concluded that natriuretic hormone was not of renal origin. Others have suggested that the hormone is produced within the brain, probably in the posterior hypothalamus (5–8, 33–35), perhaps stored in the posterior pituitary (36), and then released into the circulation in response to the stimuli of volume expansion or carotid occlusion (1–18, 25, 33, 34). The results of the present study are compatible with either a renal source of the hormone or renal trapping by receptor proteins of a hormone secreted into the circulation from another site. As it was not possible to extract a natriuretic substance from kidneys of hydropenic animals, we prefer the latter alternative.

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