Renal

MEDICAL GRAND ROUNDS

Atrial Natriuretic Factor

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Table of Contents

I. Introduction

The ability of the kidney to excrete sodium in response to a volume challenge has been a fascinating subject to renal physiologists for decades. At the center of this interest has been a debate over whether the kidney was able to regulate sodium excretion independently, or whether the organ simply responds to signals it receives from other sources. In support of the former view, Starling wrote in 1909: "The kidney presents in the highest degree the phenomenon of 'sensibility', the power of reacting to various stimuli in a direction which is appropriate for the survival of the organism; a power of adaptation which almost gives one the idea that its component parts must be endowed with intelligence" (1). The later view was succinctly expressed by Homer Smith who noted that the kidney was "only a passive agent operating blindly and automatically according to the dictates of receptor-effector systems located elsewhere in the body; the integration of these receptor-effector systems constitutes the wisdom behind salt and water balance" (2).

The present review focuses primarily on atrial natriuretic factor (ANF), a peptide synthesized in mammalian cardiac atria with potent effects on renal sodium handling and smooth muscle contractility. Few scientific discoveries have stimulated the intense flurry of interest and number of publications as ANF. Preceding the discussion of ANF, a section on several general considerations in renal sodium excretion and a section on other natriuretic hormones are presented as background.

II. Renal Sodium Excretion: Basic Considerations

Several recent excellent overviews of renal sodium excretion are available (3,4); the present discussion is limited to those factors most pertinent to the physiologic effects of ANF.

The regulation of the extracellular fluid volume (ECV) is integrally related to renal sodium excretion. This is so because sodium is the major extracellular cation and because sodium ions are actively extruded from the intracellular space. In fact, the sodium ion and its major anions (chloride and bicarbonate) constitute more than 90% of the solute in the ECV. Because sodium is primarily relegated to the ECV, any increase in ECV sodium produces an osmotic gradient which then causes water to relocate from cells to the ECV. The combination of these two occurences causes expansion of the ECV. Net sodium balance thus depends on the relationship between sodium intake, extrarenal sodium loss, and renal sodium excretion. In support of their critical role as regulators of the ECV, the kidneys are

able to excrete urine which is sodium-rich or sodium-free very rapidly. It is important to distinguish between the terms "plasma osmolality" and "total body sodium": changes in osmolality are not a reliable index of the ECV whereas a change in total body sodium is the determinant of ECV overexpansion or underexpansion. This relationship between sodium intake and the adjustment of sodium excretion is illustrated in **Figure 1.** As shown in the Figure, a new steady state is established after 3-5 days of either increased or decreased sodium intake.

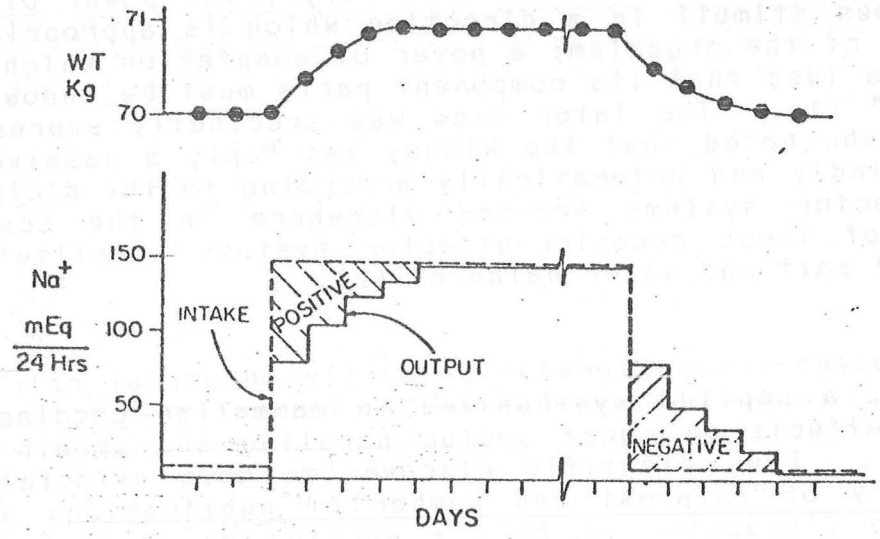


Figure 1: Adjustments in renal sodium excretion to changes in dietary sodium intake. (From Ref 3)

What factors are involved in "sensing" the changes in sodium intake and ECV? Most physiologists agree that sensing receptors are located in both low pressure capacitance areas (eg., intrathoracic great veins and atria) and high pressure resistance areas (eg., carotid sinus and juxtaglomerular apparatus in afferent arterioles of the glomerulus). Sensors may also exist intracranially and in the liver. The concept of low pressure sensors is particularly attractive since up to 85% of the total blood volume resides in the more compliant venous circulation at any one time. Maneuvers which decrease venous return such as standing (5), lower extremity tourniquets (6,7), and positive pressure breathing (8) are associated with decresed renal sodium excretion. Conversely, maneuvers which augment venous filling such as recumbancy (9), head-out water immersion (10, see **Figure 2**), and negative pressure breathing (11) all increase sodium excretion.

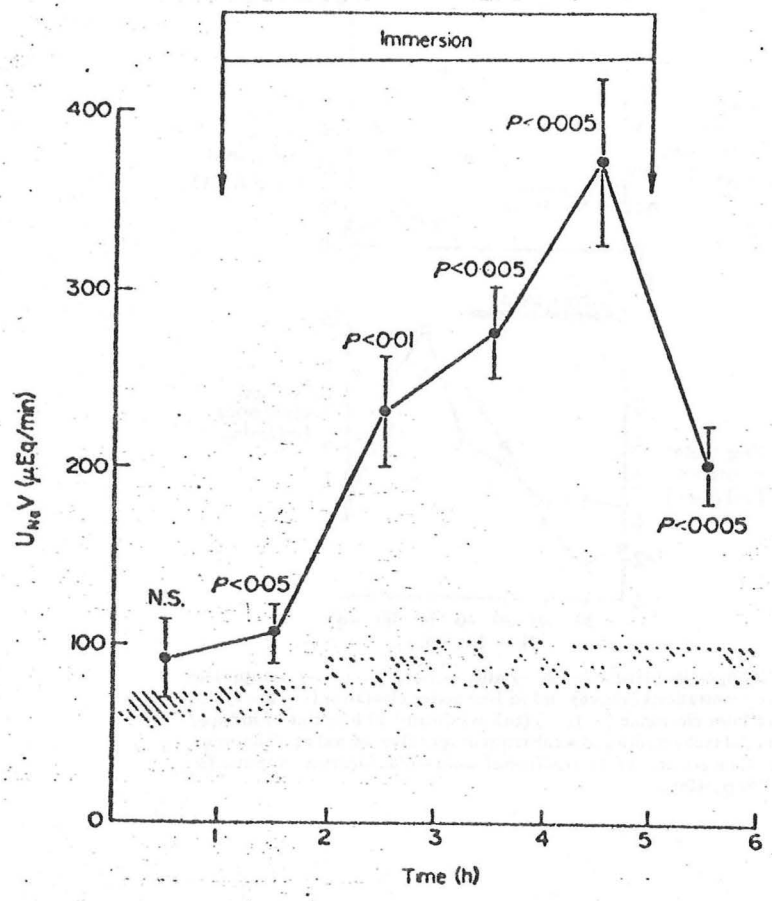
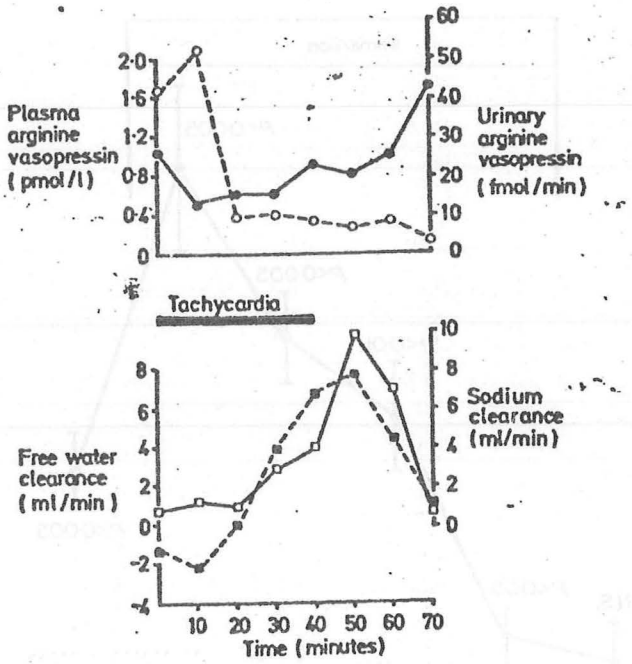


Figure 2: Response of urinary sodium excretion to head-out water immersion. (From Ref. 10)

A direct relationship between left atrial pressure (LAP) and renal sodium excretion has been shown experimentally, lending further credence to the notion that the atria are important intrathoracic sensors (12). Compelling indirect support for this concept is also available in humans; in one instance atrial tachycardia (which caused an increase in (LAP) was shown to suppress plasma AVP levels leading to an increase renal water and sodium excretion (13, see **Figure 3**).



Changes in plasma (—●—) and urinary (- - ○ - -) vasopressin concentrations (above) and in free water clearance (- - ■ - -) and sodium clearance (— □ —) (below) during 40 minutes of induced nodal tachycardia and a subsequent recovery period of 30 minutes.
Conversion: SI to traditional units—Vasopressin: 1 pmol/l ≈ 108 pg/100 ml.

Figure 3: Period of tachycardia resulted in suppression of AVP and increases in sodium and water clearance (From Ref 13)

Evidence that high pressure sensors exist is derived from observations that closure of traumatic A-V fistulae have been associated with a prompt increase in sodium excretion (14, See **Figure 4**). In addition, denervation of efferent sympathetic neural pathways which emanate from high pressure areas results in inhibition of the natriuretic response to volume expansion (15,16).

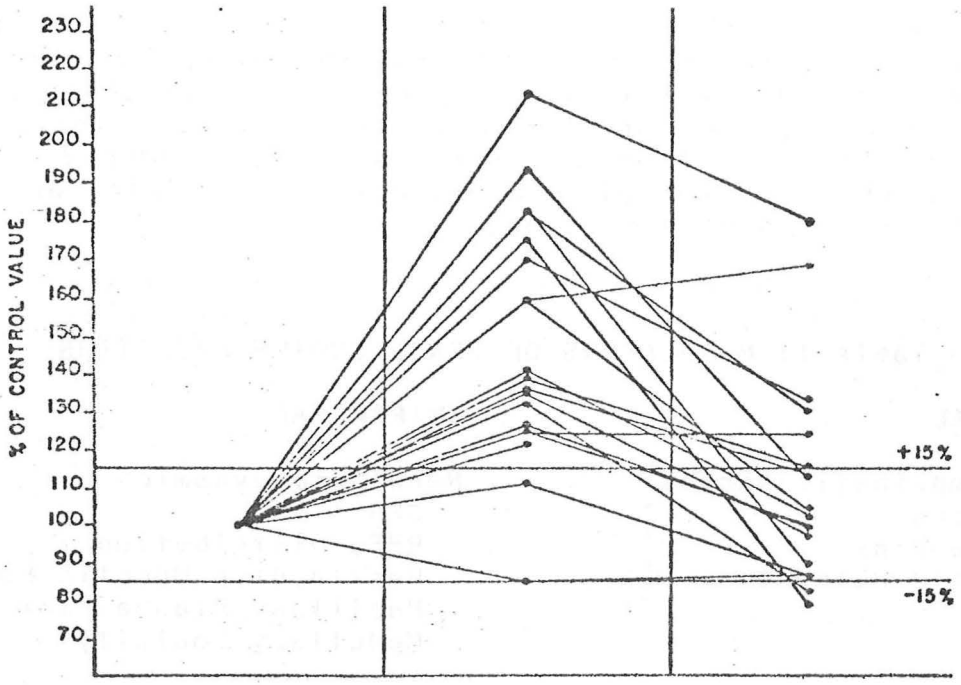


Figure 4: Percentage change in urinary sodium excretion in response to closure of traumatic A-V fistulae (From Ref. 14).

An integrated scheme for these responses is presented in **Figure 5** below.

FIGURE 5

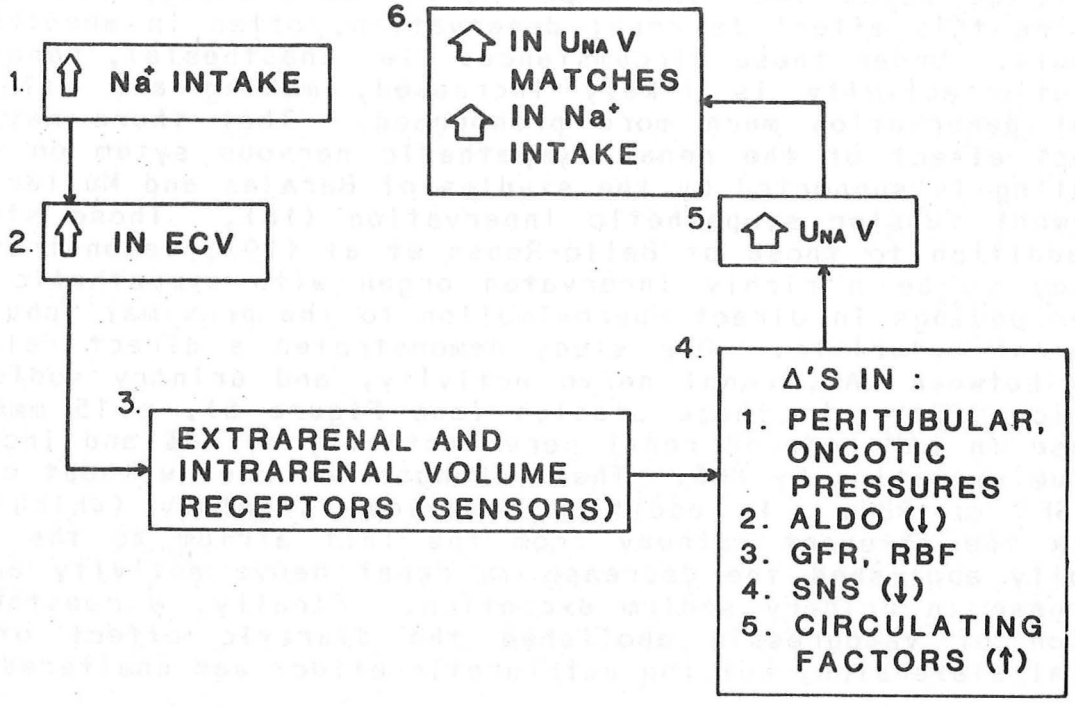


Figure 5: Overview of factors involved in renal sodium regulation.

Given the presence of a "sensing" mechanism for an ECV change, what are the known means by which the kidney responds to ECV expansion? Stated another way, what factors serve as messengers or effectors from the sensors to the kidney? **Table 1** provides a list of several key extrarenal and intrarenal modulators of renal sodium excretion.

Table 1: MODULATORS OF RENAL SODIUM EXCRETION

EXTRARENAL		INTRARENAL	
Renal Sympathetic Nerves	*	Renal Hemodynamics	
Aldosterone	+	GFR	
Prostaglandins	+	RBF, Distribution	*
Natriuretic Hormone	*+	Peritubular Oncotic Forces	*
ANF@	*+	Papillary Plasma Flow	*
		Medullary Tonicity	*

*Proximal Effects
+Distal Effects
@Proximal Effects Mediated by RBF Changes

Among the most profound effectors of renal sodium excretion is the renal **sympathetic nervous system.** Sympathetic efferent neural stimulation attenuates the natriuretic response to volume expansion (15,16,17). Conversely, maneuvers which decrease renal sympathetic activity result in a natriuresis. It should be noted that the experimental methodology utilized to examine this effect is renal denervation, often in anesthetized animals. Under these circumstances (ie, anesthesia), renal sympathetic activity is likely increased, making any effect of renal denervation much more pronounced. That there may be a direct effect of the renal sympathetic nervous sytem on sodium handling is supported by the studies of Barajas and Muller which document tubular sympathetic innervation (18). These studies, in addition to those of Bello-Reuss et al (19), demonstrate the kidney to be a richly innervated organ with sympathetic nerve fiber endings in direct approximation to the proximal tubule and efferent arteriole. One study demonstrated a direct relationship between LAP, renal nerve activity, and urinary sodium excretion (20). In these studies (see **Figure 6**), a 15 mmHg increase in LAP reduced renal nerve activity by 40% and increased sodium excretion by 80%. These changes occurred without changes in GFR or RBF. In addition, cervical vagotomy (which would block the afferent pathway from the left atrium to the brain) totally abolished the decrease in renal nerve activity and the increase in urinary sodium excretion. Finally, a constant infusion of vasopressin abolished the diuretic effect of left atrial distension, but the natriuretic effect was unaltered.

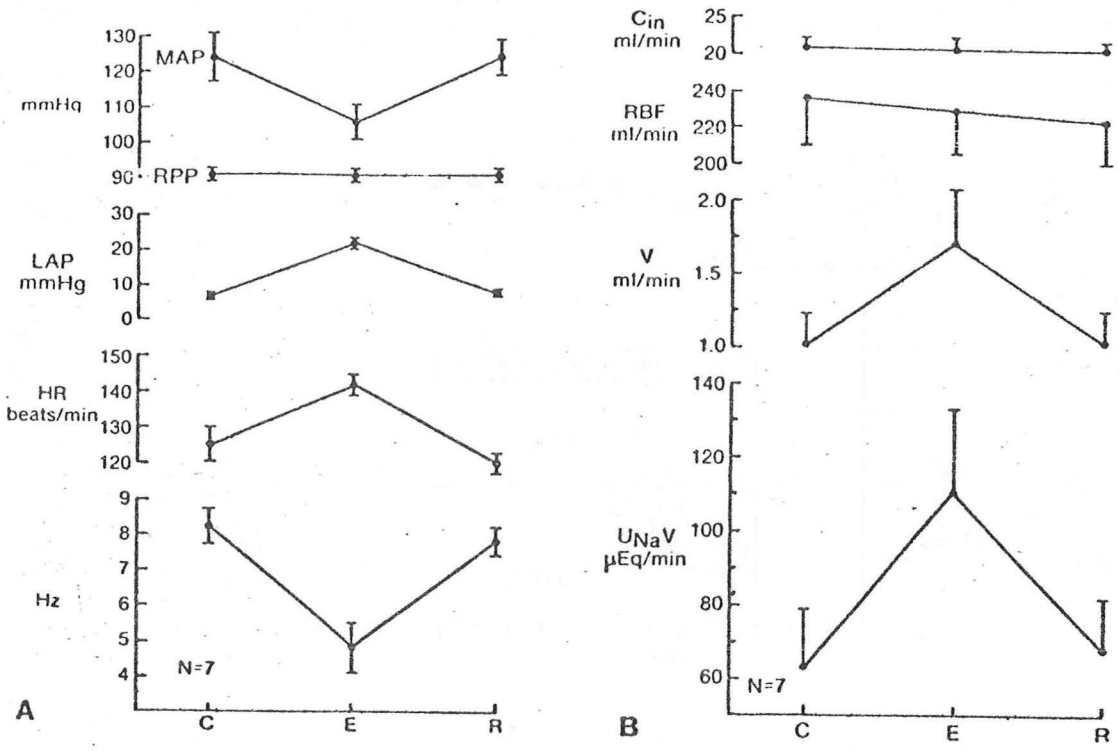


Figure 6: Effects of elevated LAP on renal nerve activity and urinary sodium excretion. (From Ref. 20).

Other studies have documented that increases in carotid sinus pressure can also reduce renal nerve activity (21); conversely, others have shown that carotid ligation increases renal nerve activity and decreases urinary sodium excretion, even when renal perfusion pressure is controlled (22,23). The predominant site for these sympathetically-mediated effects on renal sodium handling is the proximal tubule.

Aldosterone acts in the distal nephron (in the cortical collecting tubule) to enhance sodium reabsorption and potassium excretion (24). Aldosterone is secreted in response to volume depletion via renin-angiotensin system activation and suppressed in volume-expansion states. Aldosterone deficiency impairs maximal renal sodium conservation (25). However, several lines of evidence indicate that aldosterone is not the predominant factor in the regulation of renal sodium excretion particularly acutely. The onset of any sodium-retaining effect of aldosterone is delayed for 60 minutes as new protein is synthesized. Further, decreases in renal sodium excretion may be observed without an increase in aldosterone secretion. Chronic administration of aldosterone results in only a transient period of sodium retention, after which "escape" occurs and sodium balance is restored

(see **Figure 7**). Thus, while there is little doubt aldosterone is capable of modifying renal sodium excretion, there is also ample evidence that other factors are equally or more influential.

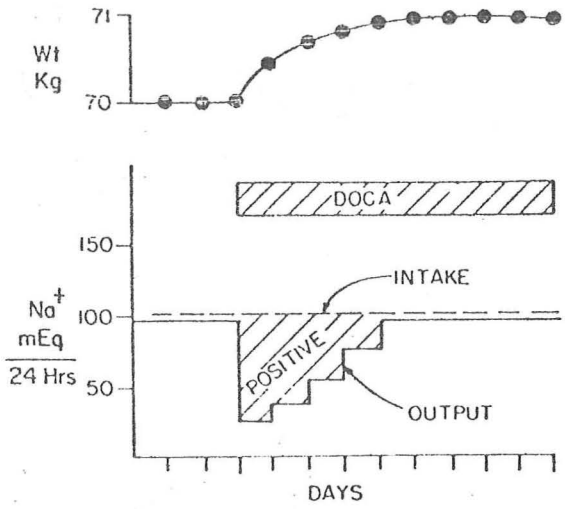


Figure 7: The DOCA escape phenomenon. (From Ref 3).

Prostaglandins may also exert a moderating influence on sodium excretion in some circumstances, although the consequences of PG inhibition are usually mild. Several studies have noted a direct ability of PGE_2 to inhibit sodium reabsorption in cortical collecting tubule (26,27), a finding of some controversy (28). Most investigators have noted a small antinatriuresis (at least transiently) when therapeutic doses of a nonsteroidal antiinflammatory drug are given. This antinatriuresis may be due in part to transient changes in RBF as much as any direct effect on renal tubular sodium handling. Hence, any regulatory role for prostaglandins in renal sodium handling appears to be modest at best.

Natriuretic Hormones and **ANF** are discussed in detail separately.

The status of **renal hemodynamics** is an important determinant of renal sodium excretion. In **Figure 8**, several of the local Starling forces which influence proximal tubular sodium reabsorption are schematically depicted.

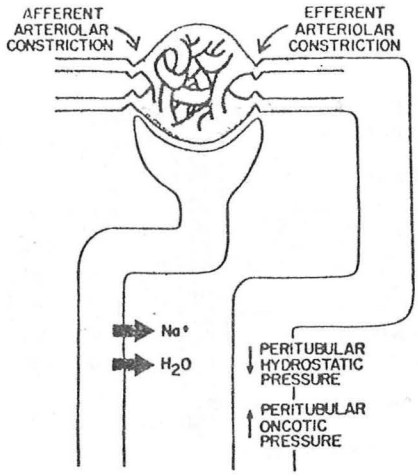


Figure 8: Decreases in peritubular hydrostatic oncotic pressure favor sodium (and water) reabsorption in the proximal tubule. (From Ref 4).

Factors which lower plasma protein concentration would decrease peritubular oncotic pressure and fascilitate a natriuresis. A decline in the filtration fraction (GFR/renal plasma flow) results in a lower percentage of protein-free ultrafiltrate formation at the glomerulus, so that the normal rise in postglomerular protein concentration is less and postglomerular oncotic pressure falls. This fall in peritubular oncotic pressure is associated with a reduction in tubular sodium reabsorption. The mechanism(s) responsible for these alterations are not known, but several tenable theories exist. As depicted below in **Figures 9 and 10,** Knox et al (29) have provided a working construct demonstrating the importance of peritubular factors in sodium reabsorption proximally. In this model, active transport of sodium into the intercellular spaces occurs, accompanied by osmotic water flow (**Figure 9**). The flow of fluid into paracellular spaces increases hydrostatic pressure and fluid flow toward the capillary. The uptake of fluid by the capillary is influenced by the balance of hydrostatic and oncotic pressure across the basement membrane of the tubule and capillary. If the balance of Starling forces across the peritubular capillary wall does not favor reabsorption, then the pressure in the interspace tends to rise. This would occur after saline loading (**Figure 10**) in which hydrostatic pressure in the peritubular capillaries is increased and oncotic pressure is decreased, both of which oppose the uptake of fluid by the capillary. The result of the increased interstitial pressure is an increased backleak into the tubule lumen. The so-called tight junction becomes leaky and active transport processes are not inhibited--but net

fluid reabsorption is decreased because of the markedly enhanced backleak.

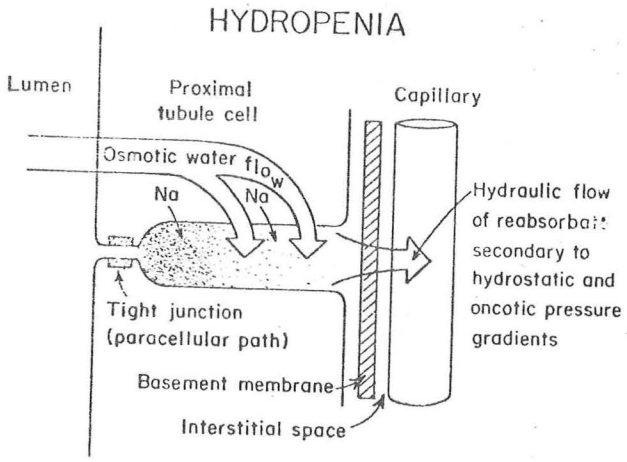


Figure 9: Model of reabsorption from proximal tubule during hydropenia. (From Ref 29)

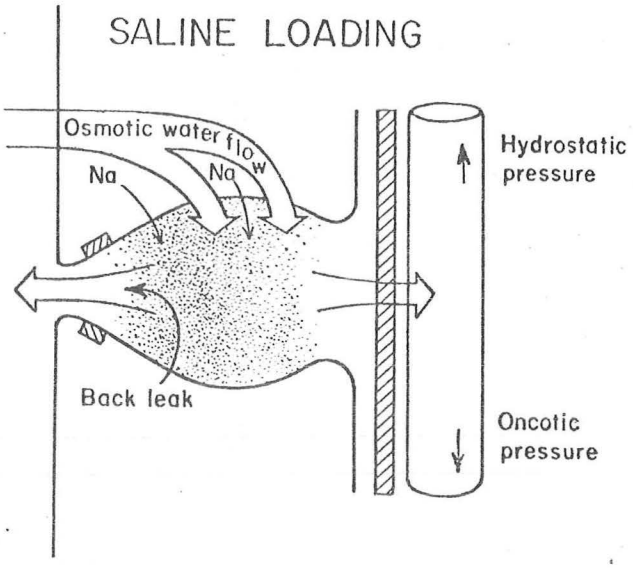


Figure 10: Model of sodium handling during saline loading (From Ref 29).

Knox and associates extended this construct to ask if changes in peritubular Starling forces were the principal regulators of proximal tubular sodium reabsorption (30). Intrarenal albumin infusions were performed in Ringer's expanded and hydropenic animals (**Figure 11**). In the Ringer's expanded group of animals, the albumin infusion increased proximal reabsorption markedly; in contrast, no effect was seen in the hydropenic animals.

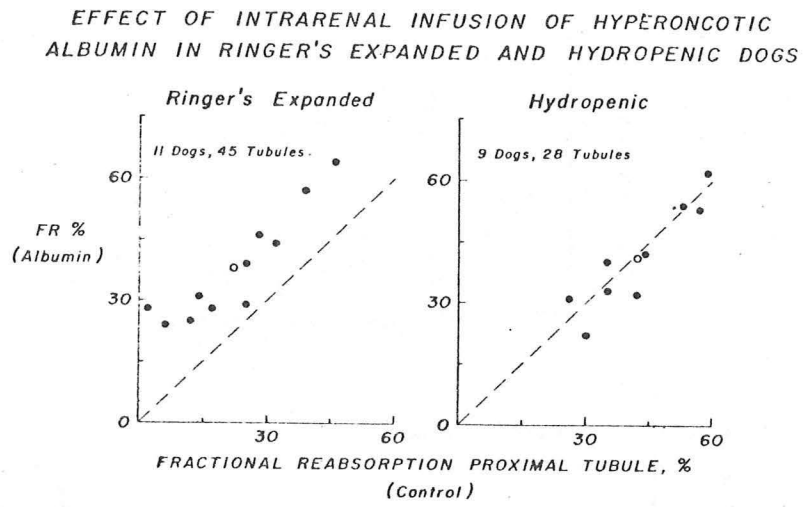


Figure 11: Albumin infusions into the kidney increased reabsorption only in expanded animals. (From Ref 30)

These data indicate that peritubular Starling forces have a marked effect in the presence of volume expansion. **Figure 12** (below) provides another example of the direct relationship between peritubular oncotic forces and proximal tubule reabsorptive rate (31). More recent studies have suggested a decrease in peritubular albumin decreases active sodium chloride reabsorption from the proximal tubule. Thus, the importance of backleak in the process remains unsettled.

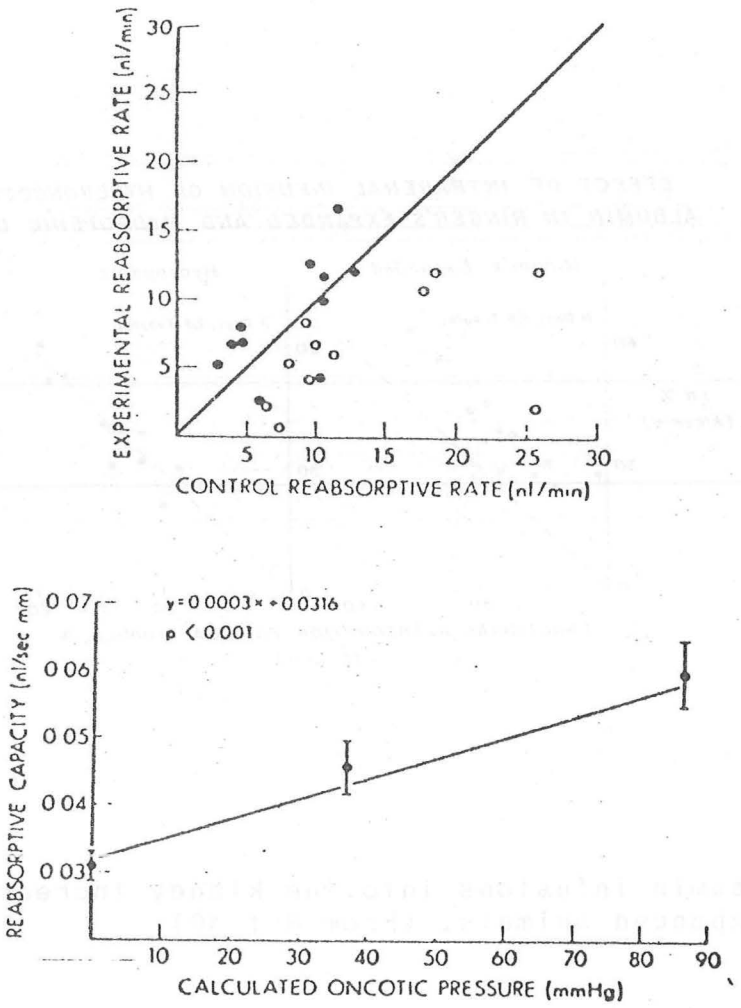


Figure 12: Top Panel: Capillary perfusion with saline (Open circles) or 8% dextran (Closed circles). Reabsorptive rate is greater with the hyperoncotic infusion. Bottom Panel: Relationship between calculated oncotic pressure and reabsorptive capacity. (From Ref 31)

The above described changes in sodium reabsorption affect the proximal tubule, the location in the nephron where the majority of sodium is reabsorbed via active transport and co-transport (ie., coupled to amino acids, glucose, anions, etc) processes. Later in the proximal tubule, a higher lumen chloride concentration develops since glucose, amino acids, and organic substrates have been removed from tubular fluid earlier. The higher lumen chloride in late proximal tubule constitutes a driving force for passive chloride reabsorption and, at the same time, renders the lumen electrically positive by generating a chloride diffusion potential. As a consequence of the slight lumen positive potential, a significant portion of total salt and water reabsorption (20-60%) in late proximal tubule is believed to be passively transported. **Figure 13** schematically depicts these relationships along the proximal tubule.

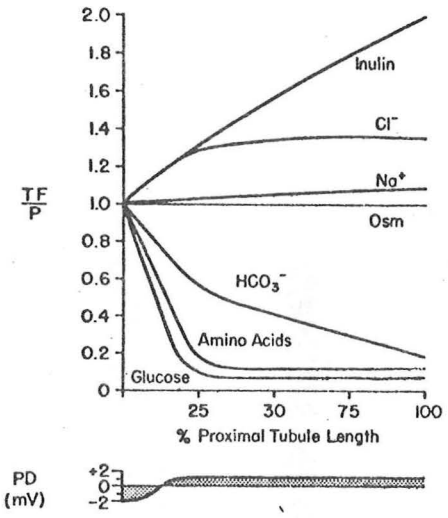


Figure 13: Reabsorption of solutes in the proximal tubule in relation to tubule length. Note proximal lumen potential (PD) changes from negative to positive along the length of the tubule.

Active sodium reabsorption occurs in several other loci in the nephron. Active chloride and passive sodium reabsorption occur in the thick ascending limb of the loop of Henle. This segment of the nephron is also capable of increasing reabsortion in the face of increased proximal delivery of solute, a phenomenon referred to as glomerulotubular balance. With decreased sodium chloride delivery to the loop, sodium chloride reabsorption diminishes. The distal tubule begins at the macula densa and is recognized to actively reabsorb sodium against an electrochemical gradient. The cortical collecting duct appears to be the primary site of mineralocorticoid effect on sodium reabsorption. The medullary and papillary collecting duct regions also have a sodium reabsorptive capacity and have been postulated as sites for natriuretic hormone effect. These segments are able to increase reabsorptive capacity in the context of increased delivery of sodium chloride, thereby participating in glomerulotubular balance.

Two additional factors deserve particular mention as regulators of sodium reabsorption. One recent study examined the importance of **renal papillary blood flow** as a determinant of sodium excretion (32). These investigators sought to resolve the issue of how comparable drug-induced vasodilatation could result in differential amounts of sodium excretion. For example, bradykinin and secretin increase renal blood flow comparably, but only bradykinin induces a natriuresis. As shown in **Figure 14**, the primary difference in the agents is the increment in renal papillary plasma flow. The authors postulated that the increase in papillary plasma flow caused a washout of medullary

interstitium and a resultant increase in delivery of solute and water to the loop and distal structures.

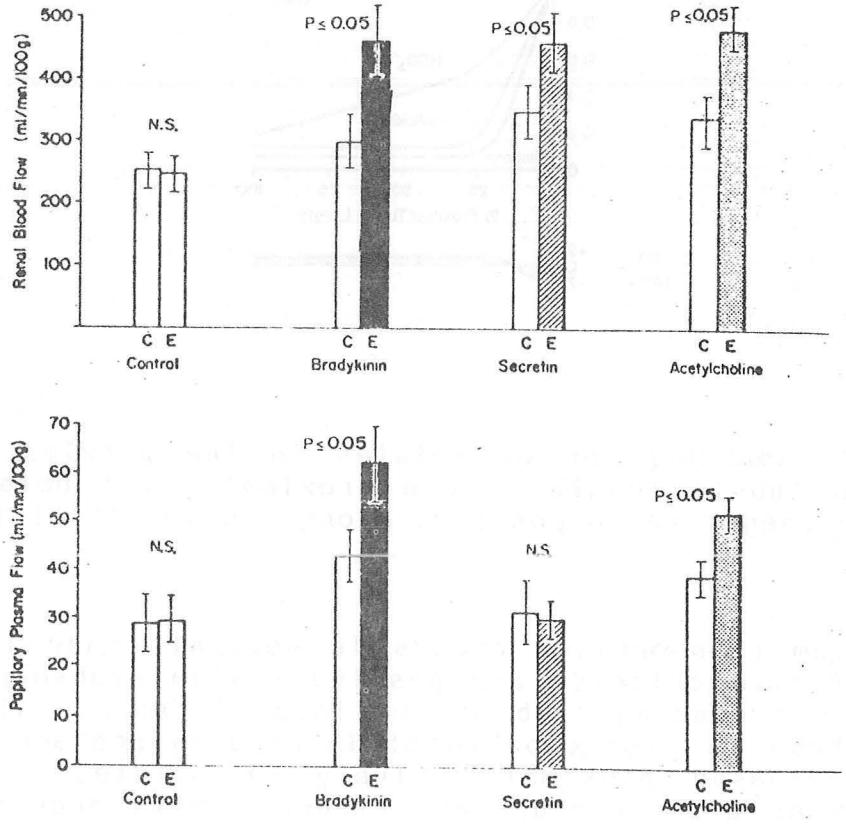


Figure 14: Total RBF (top) and PPF (bottom in control, bradykinin, secretin, and acetylcholine-infused dogs. Note that secretin did not increase PPF. (From Ref 32).

In support of medullary washout being a cofactor in the natriuresis of volume expansion, Reineck and Pama demonstrated that medullary washout accompanied by mild volume expansion dramatically enhanced sodium excretion (33). In these studies, both papillary exposure (by removal of a ureter) and a water diuresis greatly decreased the medullary tonicity and increased sodium excretion with volume loading (**Figures 15 and 16**).

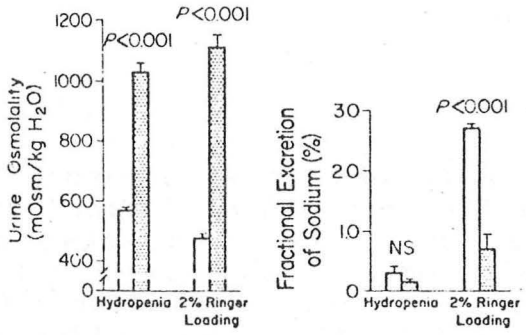


Figure 15: In the right panel note the increase in FE_{Na} in kidneys with the reduced medullary tonicity in response to 2% RL loading (open bars denote kidneys with ureter removed) (From Ref 33).

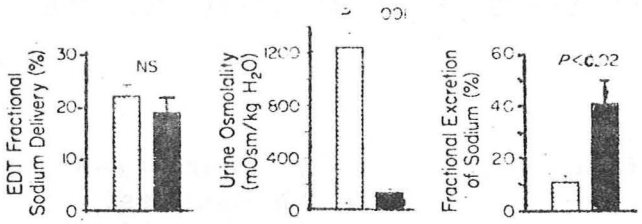


Figure 16: The right panel depicts the increased FE_{Na} in water-loaded, volume-expanded animals (solid bars) in contrast to volume-loaded animals (From Ref 33).

III. Natriuretic Hormone

The classic experiments of de Wardener (34) demonstrated that hormonal influences could affect renal sodium reabsorption independently of changes in GFR or aldosterone. Levinsky and Lalone (35) then coined the term "third factor" to describe this substance, and subsequently it has been known as "natriuretic hormone". While it has become increasingly clear that the third factor probably consists of several factors (such as renal nerves and blood composition), the major evidence marshalled to support its existance has centered on experiments in which plasma or urine from volume-expanded man or animals has caused a natriuresis when infused **in vivo** or tested in **in vitro** systems. An assay for the hormone, determination of its site of origin, and identification and purification have not been accomplished. Of interest is the fact that Homer Smith first suggested the existence of a natriuretic hormone "x" 30 years ago as an explanation for the sodium escape from mineralocorticoid (2). Several characteristics of the natriuretic hormone (NH) are shown in **Table 2** below.

Table 2: CHARACTERISTICS OF NATRIURETIC HORMONE

Time to maximum effect	1 hour (2-3 hours for higher MW species)
Biologic half life	Approx. 30 min
Site of action	Distal nephron (loop onward)
Na-K-ATPase inhibitor?	Probably
Structure	Peptide, MW <1000
Origin	? Hypothalamus
Relevance	? Needed for maximum natriuresis

Many of the prior studies in this area of research have utilized a cross-perfusion system, where blood or urine from a volume-expanded animal is infused into a recipient animal which is not allowed to become volume expanded. Representative results from a urine infusion (**Figure 17**) and a plasma infusion (**Figure 18**) are shown below. Note that in both of these studies a time delay of 1-2 hours occurs before a natriuresis ensues.

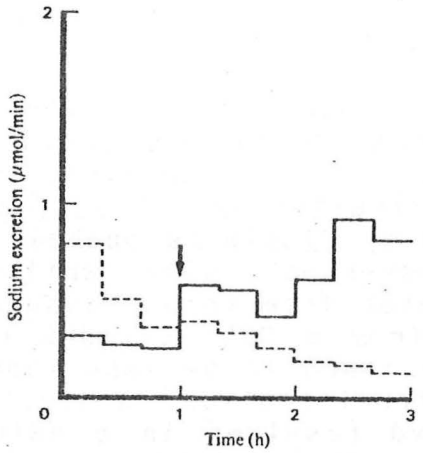


Figure 17: Sodium excretion following I.V. injection of 1 ml of plasma from volume expanded (solid line) rats. (From Ref. 36).

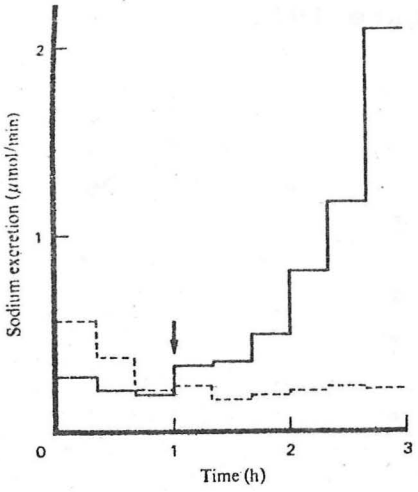


Figure 18: Sodium excretion following I.V. infusion of 1 ml urine from volume expanded rats (solid line) (From Ref 36).

Several **in vitro** experiments have also been performed to demonstrate the existance of a natriuretic factor. The mechanism of action of the natriuretic hormone has been related to direct effects on sodium transport or to inhibition of Na-K-ATPase activity. However, there is weak evidence that the natriuresis of volume expansion is due to a decrease in Na-K-ATPase activity. In fact, ouabain infusion directly into the renal artery is not natriuretic unless it is injected in pharmacologic concentrations (36). Blood or plasma removed from volume-expanded animals have caused a decrease in sodium transport in frog skin (37), isolated renal tubules (38), and white blood cells (39).

Most evidence points toward any natriuretic substance having a peptide structure since acid hydrolysis characteristically inactivates the substance. More specific activity has been obtained by gel ultrafiltration or chromatography. The substance inhibits Na-K-ATPase, displaces ouabain bound to cellular receptors, and may cross-react with antibodies to digoxin (40,41). Extracts of plasma from volume expanded but not volume depleted animals eluted from a G25 Sephadex column suggest the MW of the natriuretic substance to be less than 1,000. Extracts of urine obtained by ultrafiltration, dialysis, or gel filtration on G50 Sephadex have resulted in a natriuretic substance with a MW of over 50,000. The high MW extracts tend to produce a delayed (1-2h) natriuresis, leading to the conclusion that the higher MW substance may be a precursor substance (42). It is possible to obtain a purified natriuretic material from urine; gel filtration with recovery of a post-salt fraction (F4) and then followed by cation-exchange chromatography, organic solvent exchange, and reverse phase HPLC have yielded a substance with a molecular weight of around 350 (43). This material causes a prompt natriuresis (see **Figure 19**).

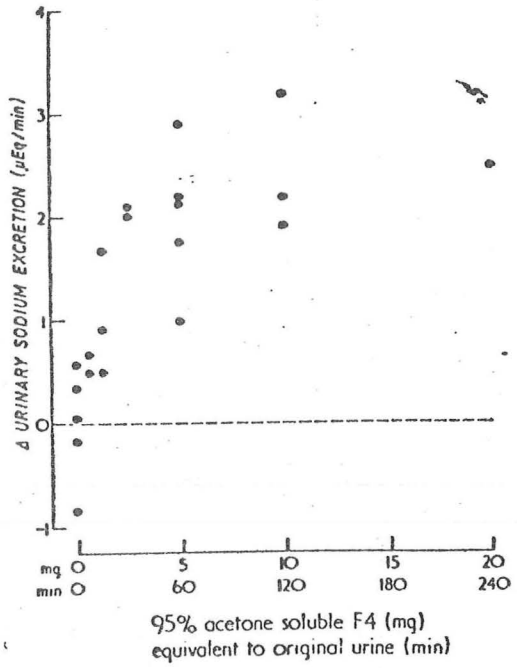


Figure 19: Change in sodium excretion following infusion of the purified NH-like material (From Ref 43).

This purified material is also a potent inhibitor of Na-K-ATPase in intact renal cells--400 pg of the extract produces the same inhibition as 500 to 600 ng (10^{-6}M) of ouabain (44).

Indirect evidence has suggested that the hypothalamus may be the site of origin of natriuretic hormone. However, few reports have supported this contention by demonstrating the isolation of a natriuretic extract from brain tissue. The indirect support for this neural origin of a natriuretic substance derives from the work of Kaloyanides et al (45) who were unable to obtain a natriuresis from an isolated kidney perfused by a blood volume expanded donor dog that had been decapitated with a vise. In addition, Pamnani et al (46) also noted ablation of Na-K-ATPase inhibition when the third ventricles of rats were destroyed. An integrated hypothesis of the relationships involved in the natriuretic substance release is shown below (**Figure 20**).

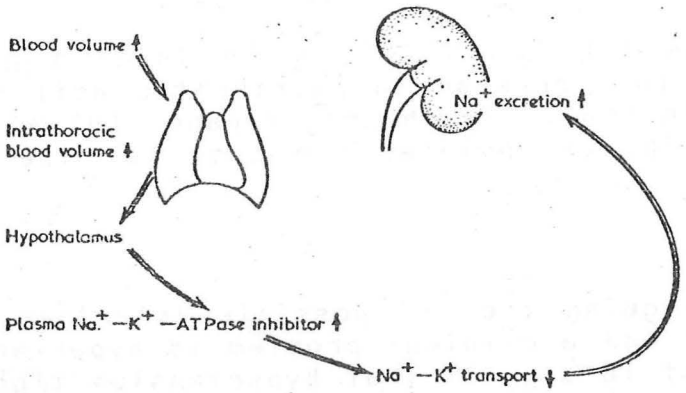


Figure 20: Depiction of the hypothesis for release of natriuretic hormone.

There are at least two clinical circumstances in which this natriuretic substance may play a relevant role. First, in **chronic renal failure,** an adaptive increase in sodium excretion per nephron occurs as renal function declines. This adaptive change is not explained by alterations in cardiac output, mean arterial pressure, peripheral resistance, or filtration fraction. Bricker et al have demonstrated that uremic plasma contains increased amounts of a natriuretic extract (47); Bohan et al have recently shown (48) that urine from uremics competes with labelled ouabain for binding sites. Presumably the relationship between the natriuretic factor and sodium excretion would follow a parallel step-wise increase type of pattern, as depicted in **Figure 21** (below).

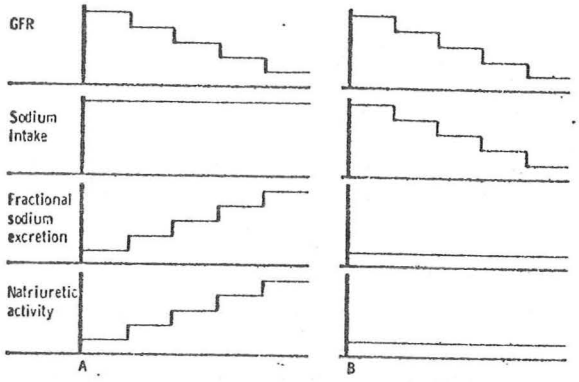


Figure 21: In the left panel (A), a continued high sodium intake necessitates the increase in natriuretic activity and subsequent increase in FE_{Na}. In the right panel (B) a stepwise decrease in sodium intake obviates the need for the increase in natriuretic hormone.

Another intriguing area of possible interface between the natriuretic factor and a clinical problem is hypertension. Dahl was among the first to suggest that hypertension might be linked to a salt-excreting hormone with pressor activity (49). This hypothesis was derived from early experiments on hypertension-sensitive and resistant rats on high salt diets. Hardy and Overbrook (50) found a depressed ouabain-sensitive sodium-potassium pump in blood vessels from renal hypertensive dogs, and later Cort noted plasma extracts containing NH-like material had pressor activity (51). Several investigators have found indirect evidence for elevated circulating levels of a Na-K-ATPase inhibitor in the plasma of hypertensive subjects. For example, MacGregor et al (52) measured th ability of plasma to stimulate guinea-pig renal glucose-6-phosphate dehydrogenase (GGPD); GGPD is alledged to be a marker for Na-K-ATPase inhibition. The GGPD stimulatory activity was elevated in hypertensive patients, correlated with blood pressure, and was inversely correlated with plasma renin activity (see **Figure 22**).

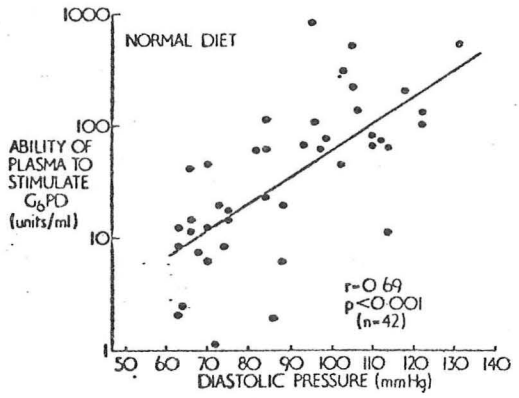


Figure 22: Relationship between GGPD stimulation in guinea-pig plasma and DBP (From Ref. 52).

The clinical problem in which a role of natriuretic hormone has been most suggested is essential hypertension. A primary defect in renal sodium excretion exists. Although an increase in NH activity occurs, the kidney is hyporesponsive. The elevation of NH levels causes (via inhibition of Na-K-ATPase) increased calcium activity in vascular smooth muscle cells with a resultant increase in smooth muscle tone. A major flaw in this theory is that in many forms of experimental hypertension, Na-K-ATPase is increased, not depressed. Buckalew (53) has proposed that NH inhibits neural Na-K-ATPase, thus inhibiting the ouabain-sensitive catecholamine reuptake system; increased vascular tone would then result from increased catecholamine concentration at the site. Catecholamines may also increase sodium influx, raising intracellular sodium and stimulating Na-K-ATPase. **Figure 23** (below) depicts this schema. Direct confirmation of this hypothesis is lacking at present.

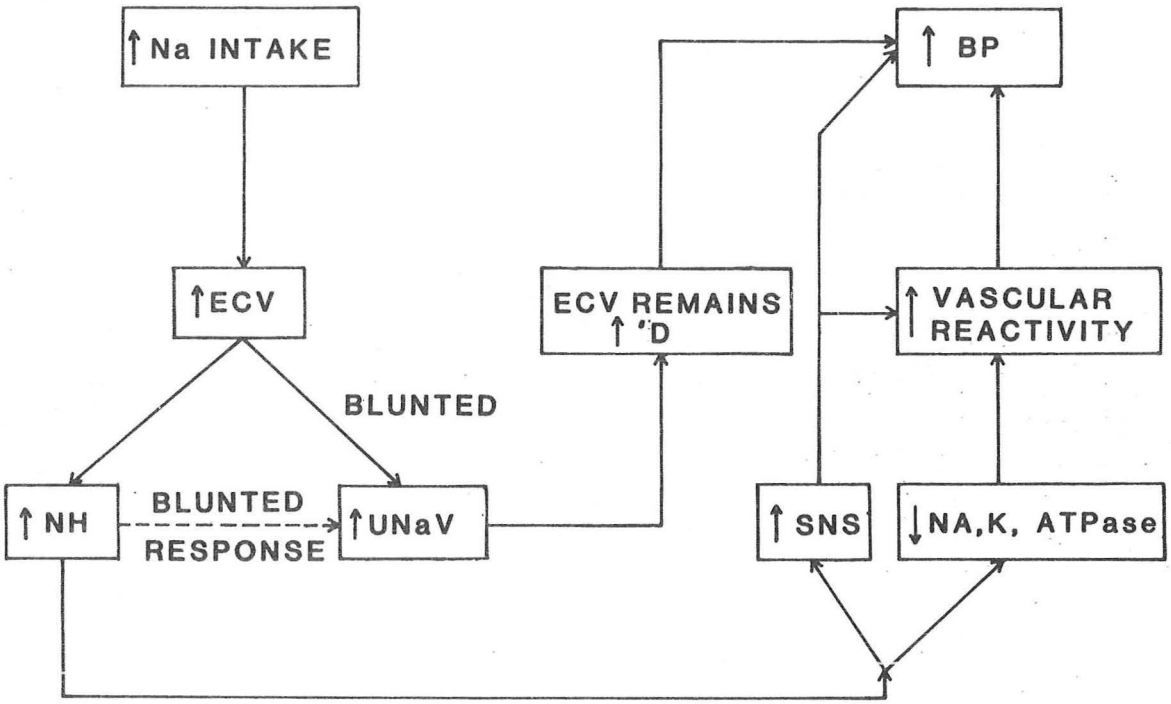


Figure 23: In this schema, the kidney has a decreased ability to excrete sodium. ECV expansion causes the release of NH; the kidney is hyporesponsive to NH, and levels increase. NH then contributes to hypertension by activating the sympathetic nervous system (SNS) or by a direct action on vascular reactivity (perhaps raising intracellular calcium) or both. Peripheral resistance increases, and blood pressure increases (Adapted from Ref 53).

Whether or not abnormal NH levels exist in other disease states characterized by abnormal total body sodium such as cirrhosis and nephrotic syndrome is unknown at present.

IV. Atrial Natriuretic Factor (ANF)

The presence of specific atrial granules likely possessing a secretory function has been known for at least two decades (54). Given the integral importance of the atria as sensors of ECF volume, it is interesting that 15 years elapsed before the key series of experiments were performed by DeBold et al (55). These investigators demonstrated that an intravenous bolus of mammalian atrial extract caused a rapid natriuresis, kaliuresis, and diuresis and also lowered systemic blood pressure. Ventricular extracts were found to be without effect. Moreover, the granularity of the atria changed with salt deprivation and with salt loading. Finally, the crude atrial extract which caused a 40 to 60-fold increase in sodium excretion copurified with the contents of the atrial granules.

The discovery of a hormonal system directly establishing contact between the heart and the kidney has resulted in a striking proliferation in the number of investigations in this area. In part, this interest in the system is due to the attractive simplicity of a system linking low pressure baroreceptors to an immediate change in sodium excretion. It has been logically proposed that an increased ECV stimulates the atrial receptor to release the ANF into the circulation with resultant effects on the kidney, adrenal, and peripheral vasculature. However, a conclusive link between artial distension and release of ANF has not been definitely established to date.

A. Chemical Properties of ANF

A highly purified preparation of ANF has been obtained by several laboratories, and a polypeptide structure established. Purified rat and human atrial peptides have also been obtained, and the amino acid sequences discovered. These amino acid discoveries have allowed for the synthesis of synthetic peptides. Each of these improvements was greatly aided by the ability to test each preparation in a reliable bioassay system: the chick rectum and vascular smooth muscle relaxation preparation. Several of the detailed studies which describe the methodology utilized in purification and sequencing ANF are provided in the references (56-72). **Figure 24** below shows the amino acid sequence of several of the atrial peptides. The biological activity of the peptides is derived from the C-terminal fragment whereas the N-terminal fragment likely serves only a modulating function. Human ANF differs from rat ANF by only one amino acid and (methionine in lieu of isoleucine at position 134). Rat atrial tissue has yielded peptides varying in length from 21 to 126 amino acid residues; human tissue has yielded residues of 28, 56, and 126 amino acids. The B or 56 amino acid human peptide has the structure of a dimer of (28 amino acid units) arranged in an antiparallel fashion joined by 2 sets of intermolecular disulfide bridges (73).

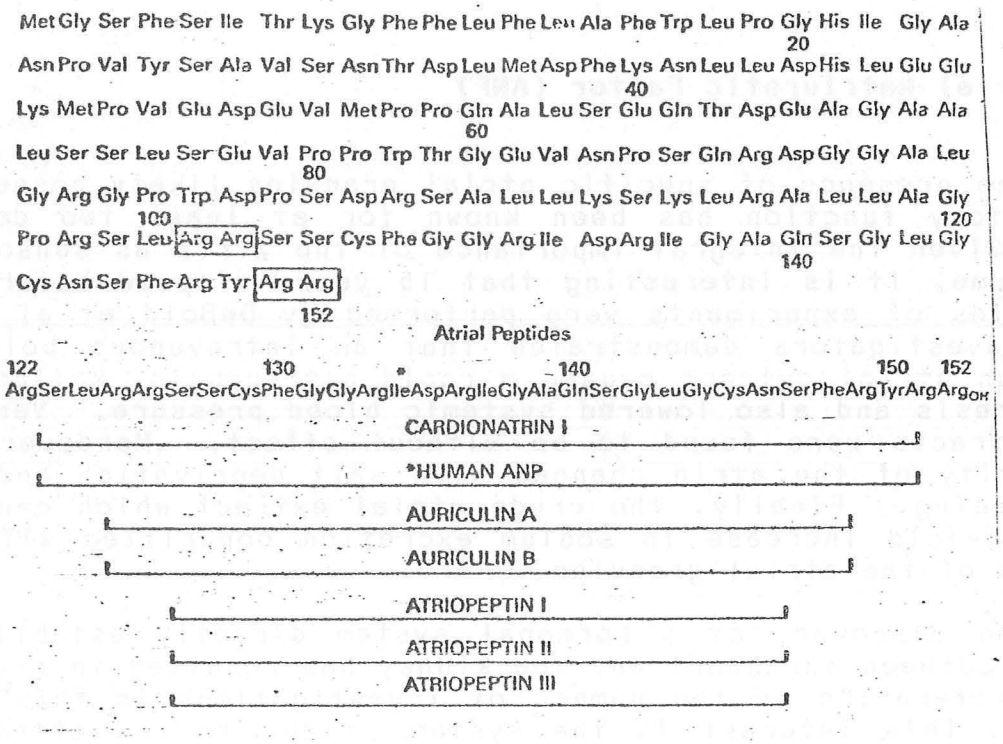


Figure 24: Precursor at top; atrial peptides at bottom. Note different names given to natriuretic peptides isolated from atria. Atriopeptins I-III are synthetic substances (From Ref. 74).

The active ANF peptide is likely derived from the 126 residue peptide precursor, termed **atriopeptigen** with cleavage at ala (residue 24 in the rat or 25 in the human). It is still conjectural as to which of the peptides is naturally occurring. Since paired basic residues such as Arg^{125}-Arg^{126} and Arg^{151}-Arg 152 are common sites of intracellular proteolytic processing (75), two likely candidates for a mature peptide produced **in vivo** would be either the 25-residue peptide auriculin B or the 24-residue peptide termed atriopeptin III (76).

B. Renal Hemodynamic and Natriuretic Effects of ANF

An intrarenal infusion of ANF produces a prompt increase in renal blood flow (RBF), GFR, FE_{Na}, and a decrease in renin secretory rate (RSR) (77). These results are shown below in **Figures 25-27.**

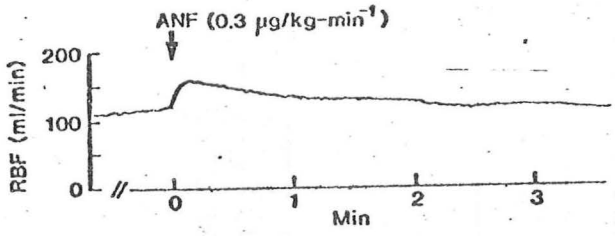


Figure 25: Effects of Intrarenal ANF on RBF (From Ref 77)

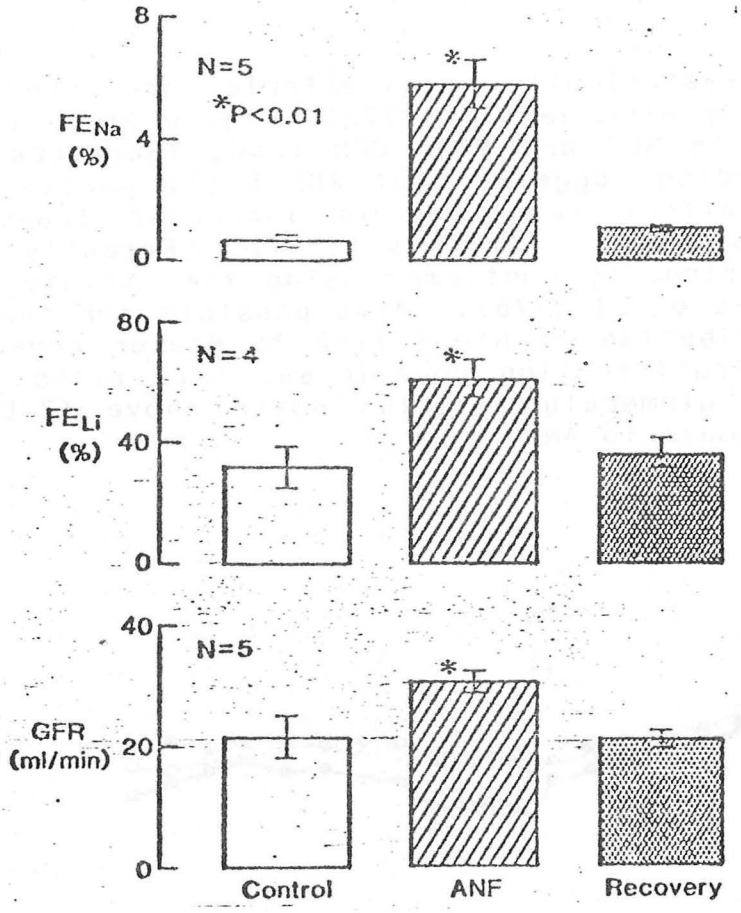


Figure 26: Changes in FE_{Na}, FE_{Li}, and GFR (From Ref 77).

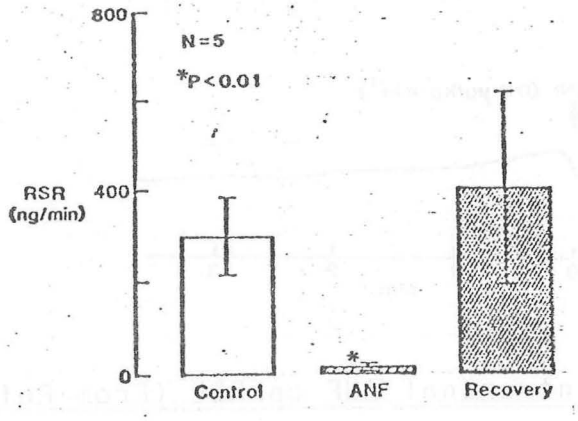


Figure 27: RSR during ANF infusion (From Ref 77).

If infused systemically, mean arterial pressure (MAP) declines, and RBF may also decline (77a) (**Figure 28** below). Despite the decline in RBF and MAP, GFR (and, therefore, FF) increase. This finding suggests that ANF has a partial efferent vasoconstrictive effect resulting in increased transcapillary hydrostatic pressure and an increase in FF. Recently this suggestion has been directly confirmed using the isolated perfused glomerulus by Freid et al (77b). Also possible for the increase in GFR is redistribution of blood flow to deeper nephrons or a change in the ultrafiltration coefficient (K_f) although in the isolated perfused glomerulus studies noted above (77b) K^f did not change in response to ANF.

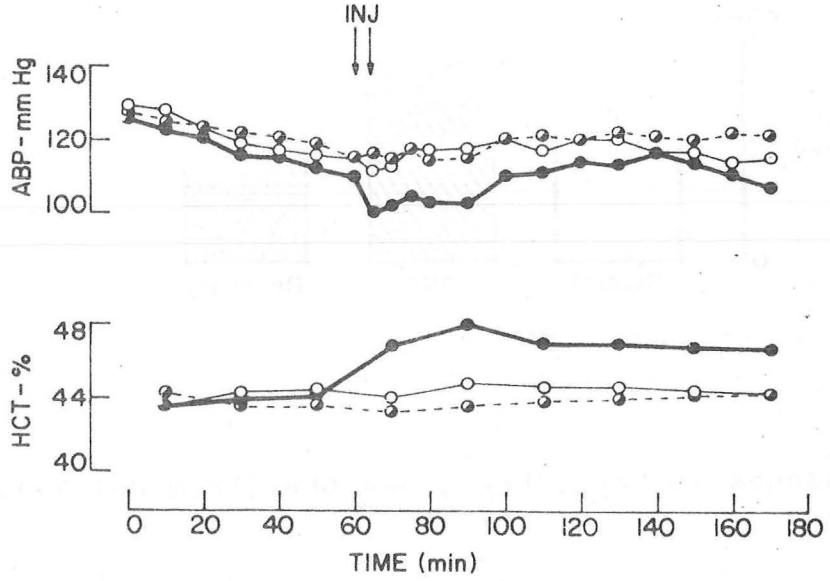


Figure 28: Solid symbols denote MAP after ANF injection (labelled "INJ"); RBF also declined. (From Ref 77).

The direct effects of ANF on renal vasculature appear to depend in large part on the basal conditions of study. The most profound vasodilatation occurs in the presence of other vasoconstrictors, suggesting an antagonistic action for ANF (74). It may be that any efferent vasoconstriction produced by ANF is cancelled by other effects. It seems clear, however, that ANF should not be regarded as a potent renal vasodilator **a priori**. Other effects, such as an increase in papillary blood flow and inner medullary blood flow seem less controversial.

All investigators agree that ANF infusions (IV or IA) produce a prompt and brisk natriuresis, but the mechanism(s) responsible for this remain unclear. Typical results from an infusion of human ANF into a volunteer are shown in **Figure 29** below; $U^{N}aV$ increased in a dose-responsive manner (78).

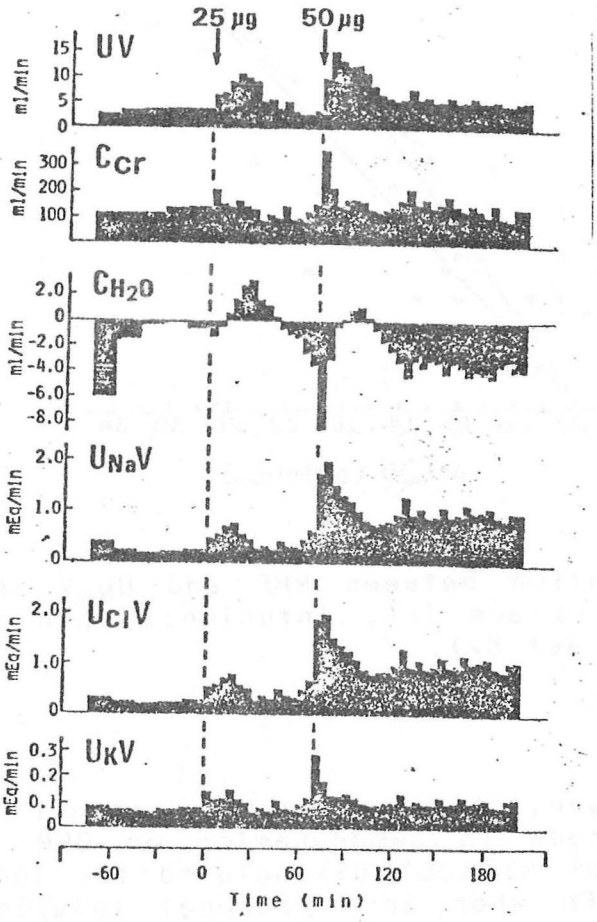


Figure 29: Note increases in C_{cr} and $U_{Na}V$. Serum level of ANF was assayed and increased from 156 pg/ml to 6147 pg/ml 1 minute after the 50 ug injection; the level fell to the control level in 10 minutes (From Ref 78).

Is the natriuresis the result of hemodynamic changes produced by ANF? A direct effect on sodium transport? Both? Most early micropuncture studies found little evidence of a diuretic inhibitory effect of ANF on sodium reabsorption (79-81). Clearly, as shown in **Figure 30** below, both IV and IA (intra-arterial) ANF infusions into anesthetized (and, therefore, vasoconstricted dogs) produces a natriuresis which may be directly correlated to changes in RBF (82). The finding that medullary and papillary blood flow increases with injection of atrial extract (83,84) is consistant with the notion that the peptide-induced natriuresis is secondary to the vascular effect.

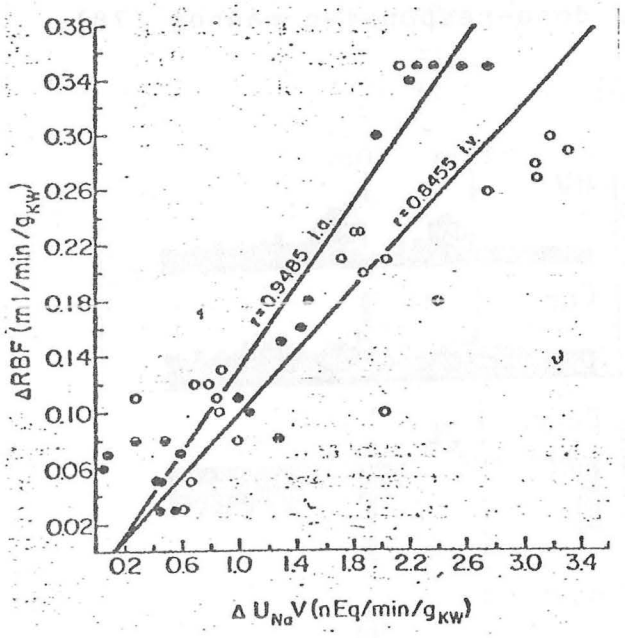


Figure 30: Correlation between RBF and $U_{Na}V$ in anesthetized dogs. Closed symbols are I.A. infusions; open symbols denote I.V. infusions (From Ref 82).

Clearly, however, the natriuresis cannot be fully explained by the changes in hemodynamics or GFR (85). Recent results by Seymour et al (86) dissociated the increase in $U_{Na}V$ from a change in GFR when an intrarenal infusion of ANF was given to anesthetized dogs (**Figure 31**). Thus, the natriuresis could not be explained on the basis of hemodynamics/GFR in this study. Moreover, it has been argued that glomerulotubular balance should be able to adjust to increased sodium reabsorption in cases where GFR increases transiently.

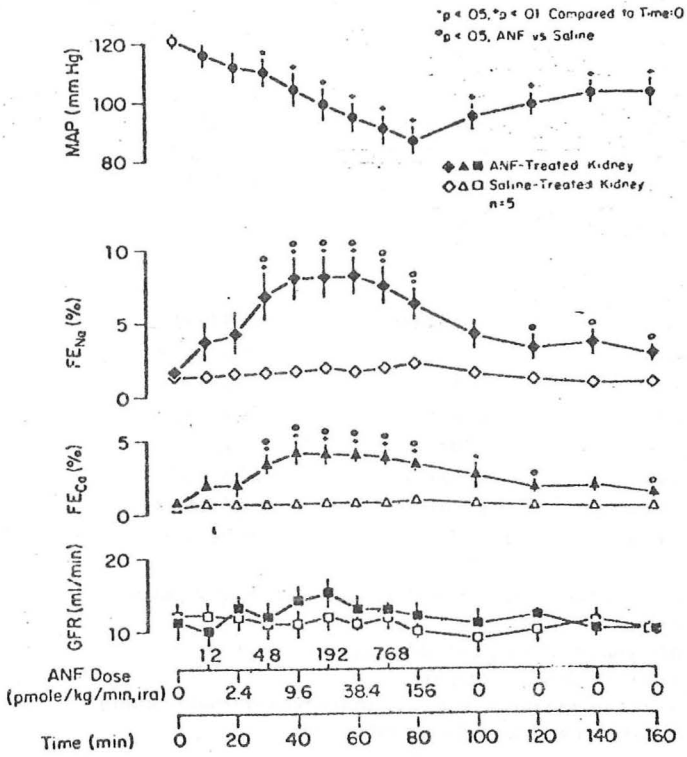


Figure 31: GFR remained constant as $U_{Na}V$ increased (From Ref. 86).

Since ANF does not inhibit Na-K-ATPase (85,87,88), and has no direct effect on sodium transport in amphibian epithelia (87,88), then the issue is how does it inhibit directly and where. Hammond et al (89a) recently addressed this problem using isolated vesicle preparations of proximal tubular brush border membrane (BBM). Several assumptions were made in these studies: 1) filtered phosphate is reabsorbed in proximal tubules via co-transport with sodium; 2) sodium is co-transported with bicarbonate anion (via an electroneutral Na^+-H^+ antiport at the BBM of the proximal tubule. These authors reasoned that if ANF inhibits sodium reabsorption in proximal tubules, one would expect Na^+-P_i symport and Na^+-H^+ antiport across BBM's to be inhibited with a concomitant phosphaturia and bicarbonaturia. As shown below, **Table 3** shows an increase in FEP_i, FE_{Na}, and GFR in thyro-parathyroidectomized rats injected with ANF.

	First period (vehicle)	Second period‖
ANF-infused group (n = 7)*		
FE_{Pi} (%)	3.51±1.49	9.64±1.97‡§
P_{Pi} (*mM*)	2.46±0.19	2.13±0.19
FE_{Na} (%)	1.30±0.26	4.65±0.58‡§
FE_{Ca} (%)	3.20±0.7	3.70±0.5
FE_{K} (%)	37.80±8.6	36.20±5.5
FE_{Mg} (%)	16.30±4.2	20.30±5.4
GFR (*ml/min*)	2.17±0.31	3.63±0.39‡
BP (*mmHg*)	131.00±5.0	131.00±5
Control vehicle-infused group (n = 7)*		
FE_{Pi} (%)	1.61±1.09	1.93±1.02
P_{Pi} (*mM*)	2.21±0.15	2.44±0.18
FE_{Na} (%)	1.01±0.29	2.27±1.58
FE_{Ca} (%)	2.90±0.6	4.10±1.3
FE_{K} (%)	32.20±9.2	26.20±3.5
FE_{Mg} (%)	17.50±4.6	24.10±5.9
GFR (*ml/min*)	3.56±0.48	2.63±0.58
BP (*mmHg*)	130.00±3.0	128.00±3.0

* n denotes number of animals; ANF-infused animals were paired with controls.
‡§ $P < 0.05$ compared with control by paired t test (‡) or ANF with control groups by unpaired t test (§).
‖ ANF-infused group infused with ANF in the second period; control group infused with vehicle in the second period.

Table 3: (Whole animal infusion studies with ANF in TPTX rats (From Ref 89a).

Also, as noted in **Figure 32** below, renal BBM vesicles were demonstrated to have a reduced sodium-dependent uptake of Pi and reduced Na^+-H^+ exchange.

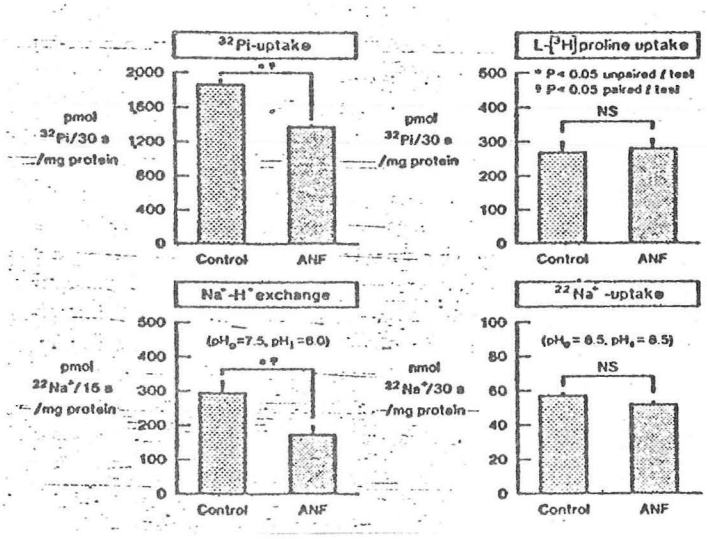


Figure 32: Note the decreased co-transport of sodium with Pi and H^+ (From Ref. 89a).

These authors concluded that ANF in part inhibited sodium reabsorption of solutes transported with sodium in proximal tubules. This conclusion is in argreement with Borenstein et al (83) who, although noting a correlation between inner cortical blood flow and sodium excretion after ANF, reasoned that ANF hemodynamic changes did not fully account for the natriuresis for two reasons: 1) Acetylcholine caused the same degree of change in inner cortical blood flow, but only a 5-fold increase in $U_{Na}V$ (as opposed to a 20 to 90-fold increase with ANF); 2) furosemide produces similar changes in blood flow and $U_{Na}V$ and the natriuresis persists (though blunted) even if the hemodynamic changes are prevented. Sonnenberg et al (89) concluded that proximal sodium reabsorption was blunted following ANF in a series of micropuncture and microcatherization studies. Even more interesting is the fact that these investigators addressed the issue of failure of glomerulotubular feedback and noted reduced reabsorption of sodium in the medullary collecting duct as well-in fact, this failure to reabsorb sodium distally accounted for 80% of the natriuresis (see **Figure 33** below).

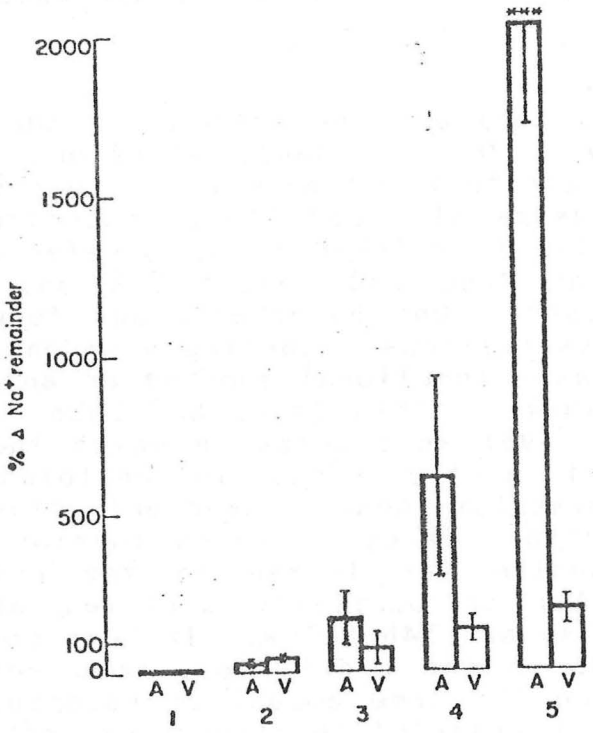


Figure 33: Percent change in sodium delivery after injection of atrial (A) and ventricular (V) extracts at various nephron sites: 1. Glomerulus 2. End proximal tubule. 3. Distal tubule 4. Outer medullary collecting duct. 5. Papilla tip (From Ref 89).

A similar conclusion regarding a distal effect of ANF was also reached by Pollock and Banks in clearance studies in rats (90). Most recently Granger et al (91) argued that ANF had a direct tubular effect on sodium excretion since no change in GFR was noted but FE_{Li} (a marker of proximal reabsorption) and FE_{Na} declined. This same group noted, however, that a decrease in renal hemodynamics produced by acute low output heart failure markedly attenuated the natriuresis of ANF (92). Sosa et al (93) carried this concept even further by placing a snare around the renal artery and preventing any hemodynamic changes from occurring with ANF. This maneuver resulted in a loss of natriuretic action of ANF; if the same procedure is followed with furosemide, a natriuresis still ensues. Finally, in contrast, very recent work performed at this institution did not show a direct effect on proximal tubular sodium reabsorption in the rabbit (94).

C. Effects of ANF on Vascular Smooth Muscle

As mentioned earlier, the effects of ANF on vascular tone are contradictory. On one hand, infusions of ANF into the intact kidney of anesthetized animals decreases vascular resistance and increases renal blood flow; in contrast, infusions of ANF into the isolated perfused kidney preferentially increases efferent arteriolar tone and raises GFR and FF while little change in RBF occurs. One hypothesis put forward to reconcile these disparate observations regarding vasoconstriction has been that ANF behaves as a functional agonist or antagonist to endogenous vasoconstrictors. This issue has been explored in detail by Kleinert et al (95) in studies in which the contractile responses of rabbit aortic rings to angiotensin II, norepinephrine, and potassium-induced depolarization were examined. The results of these studies in which tension from angiotensin II and norepinephrine was tested in the presence of boiled atrial extract (AE) or partially purified atrial factor are shown in **Figures 34a and 34**b below. In both models, ANF shifted the response curve to the right, ie., more vasoconstrictor was required to produce the same amount of vasoconstriction. Thus, ANF blunted the vasoconstrictive effects of both substances.

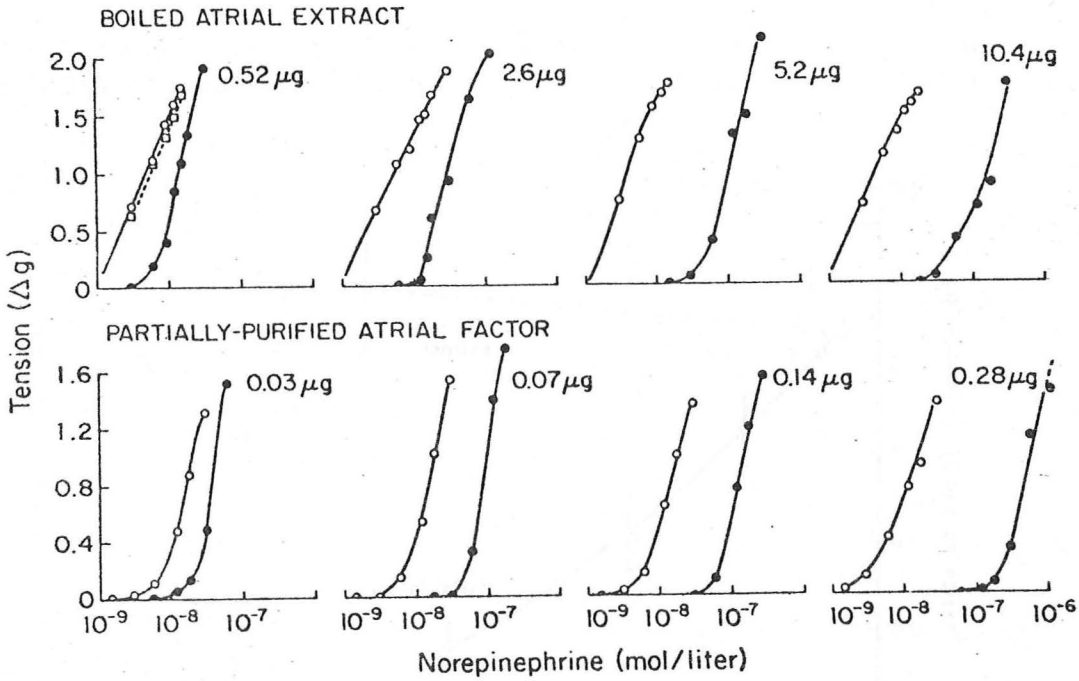


Figure 34a: Open symbols denote control tests, closed symbols denote the presence of AE (top) or PPAF (bottom) (From Ref. 95).

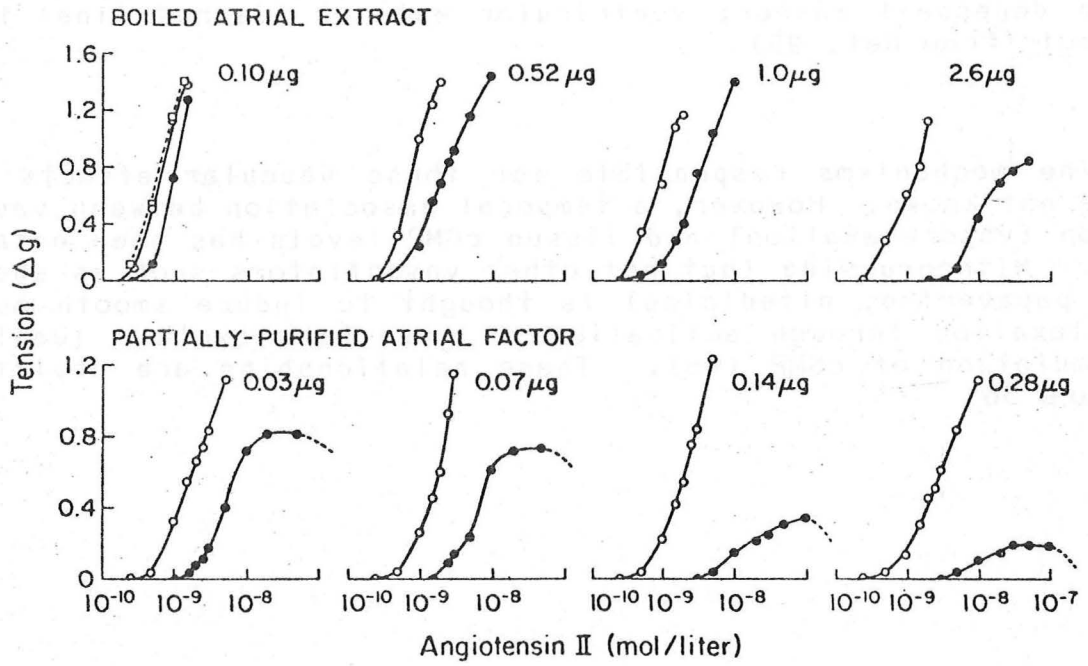


Figure 34b: Open symbols denote control tests, closed symbols denote the presence of AE (top) or PPAF (bottom) (From Ref. 95).

In **Figure 35** below the effects of ANF and ventricular extract on tension induced by a high potassium concentration are shown.

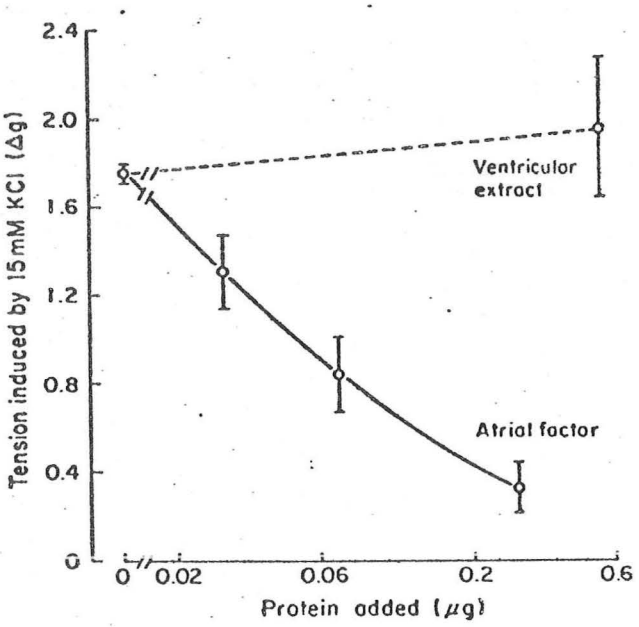


Figure 35: The addition of ANF lowered tension in a concentration dependent manner; ventricular extract (dashed line) had no effect (From Ref. 95).

The mechanisms responsible for these vascular effects of ANF are not known. However, a temporal association between vasodilation (vasorelaxation) and tissue cGMP levels has been established. Nitroprusside (but not other vasodilators such as adenosine, papaverine, nifedipine) is thought to induce smooth muscle relaxation through activation of guanylate cyclase leading to formulation of cGMP (96). These relationships are depicted in **Figure 36.**

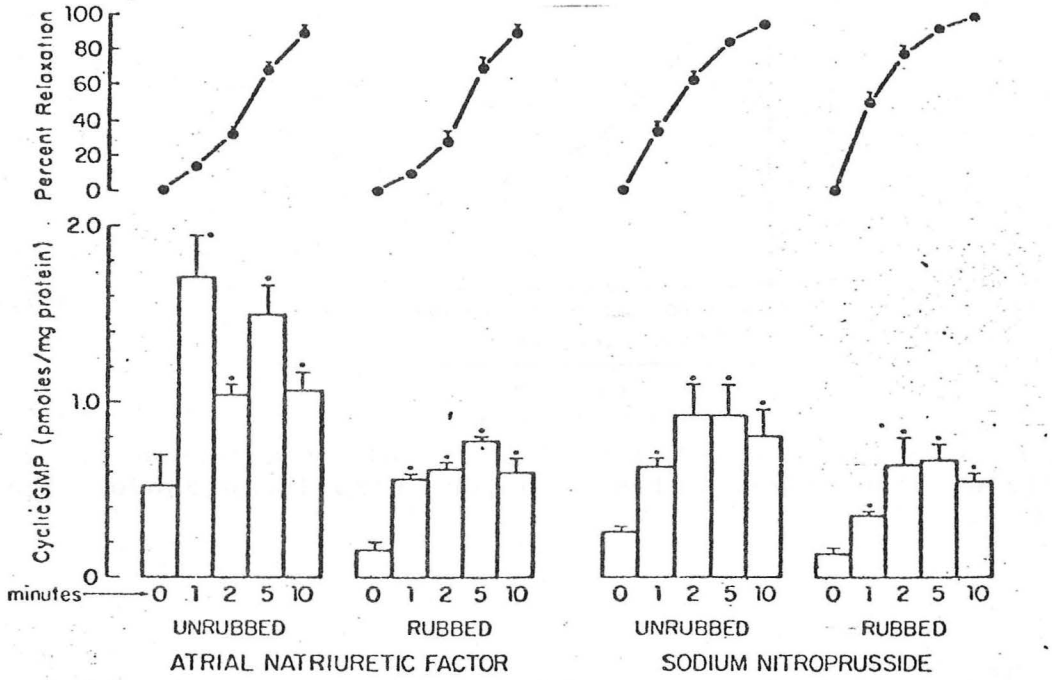


Figure 36: Both ANF and NP induce relaxation of aortic rings which was temporally related to cGMP levels (From Ref. 96).

Interestingly, the above observations on ANF and vascular smooth muscle relaxation appeared to be independent of cAMP or extracellular calcium.

D. Effects of ANF on Aldosterone and Renin

One mechanism by which ANF may exert a natriuretic effect is via inhibition of aldosterone. Clearly such an effect would not influence the early change in $U_{Na}V$ observed in the acute infusion studies, but it could be relevant as a sustaining factor. Several different experimental models have been used to explore these effects of ANF on aldosterone; in one such study in isolated zona glomerulosa cells (97) atrial extract reduced basal release of aldosterone (**Figure 37** below) and ACTH and angiotensin II-stimulated release as well (**Figure 38** below).

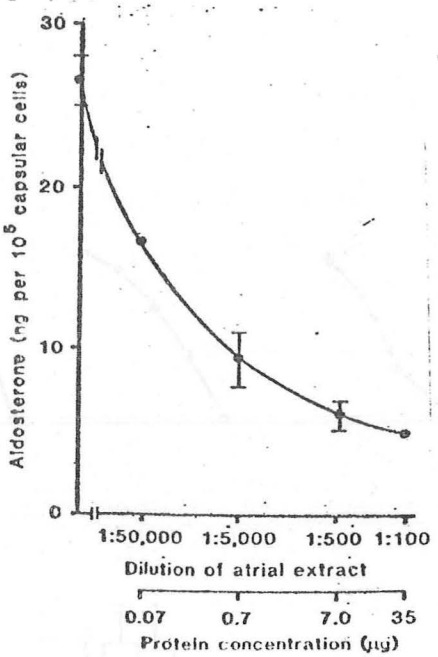


Figure 37: Atrial extract inhibited basal aldosterone release from isolated glomerulosa cells in a concentration-dependent manner (From Ref 97).

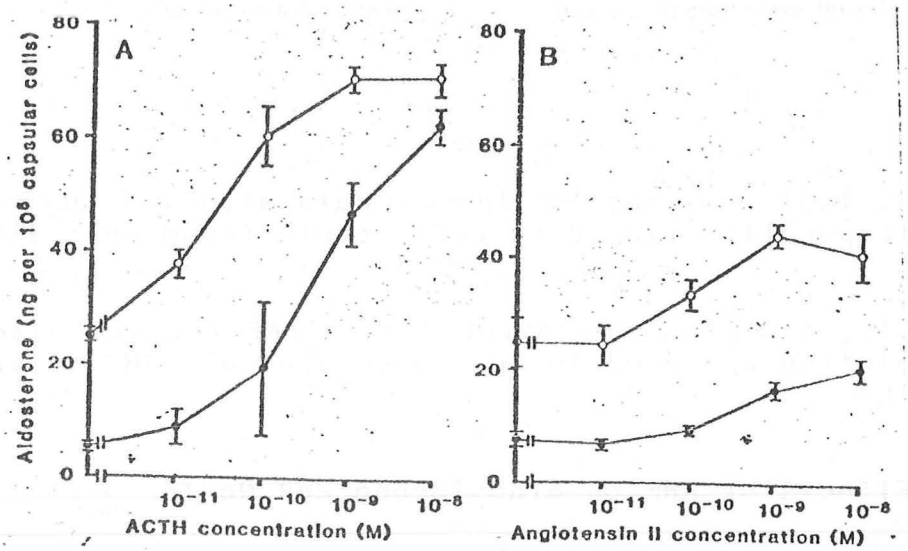


Figure 38: Inclusion of atrial extract (closed symbols) blunted aldosterone release stimulated by both ACTH (left panel) and AII (right panel) (from Ref. 97).

Similar results have been noted in several recent communications (97-100). The study of Campbell et al (100) carefully

documents the ability of atriopeptins to inhibit aldosterone synthesis both early and late in the synthetic pathway. In contrast to the findings of Goodfriend and others (97,98), Campbell et al found that ANF inhibited stimulated but not basal aldosterone release.

An inhibition of renal renin release is another documented effect of ANF infusions both **in vivo** and **in vitro.** This effect also seems logical when viewed in the context of the overall effects of the peptide: inhibition of renin release interrupts a major pathway to aldosterone release and also reduces vasoconstrictive import of angiotensin II on peripheral vasculature. Thus, in addition to the direct effects of ANF on glomerulosa cells and peripheral vasculature, the inhibition of renin release would be expected to fascilitate both peripheral vasodilation and sustained sodium excretion.

One obvious mechanism for a renin-inhibitory effect of ANF is via the macula densa. By increasing proximal rejection of sodium reabsorption, delivery of sodium to the distal nephron is accomplished. This results in a reduction in macula densa-stimulated renin release. In one recent preliminary communication, this inhibition of macula densa-induced renin release was studied in the non-filtering kidney and found to account for the majority of inhibition (101). ANF is capable of inhibiting renin release directly from JG cells as well; for example, isoproterenol and other cAMP-mediated pathways to renin release are blunted by atriopeptin III (102). However, this inhibition of renin release does not seem to occur via a calcium ionophore effect (102).

E. ANF in Pathophysiological States: Measurements

One obvious advance which would greatly enhance progress in ANF research would be the ability to reliably measure the peptide in different physiologic settings. Release of atriopeptins from the heart has been demonstrated by Currie et al (102). These investigators noted the release of a single low molecular weight peptide (<5000 Mr) which was similar to the 1-24 atriopeptin III; there was no evidence that atriopeptigen was released. These findings suggest that atriopeptigen is proteolytically processed in the atria to an atriopeptin which then is released in the form of a short peptide. The authors postulate a proteolytic cleavage of atriopeptigen takes place between the basic arg-arg amino acid residues yielding atriopeptin III. The HPLC retention time of the substance obtained by Currie et al from perfused heart is shown graphically in **Figure 39** below.

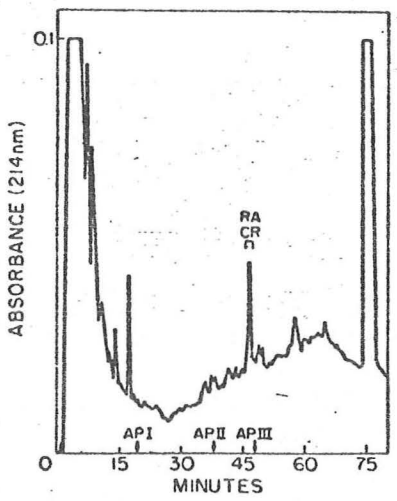


Figure 39: Retention time of the substance released by the isolated perfused heart was 46-47 min which correponded to that of atriopeptin III (47-49 min). RA and CR refer to relaxation of rabbit aorta and chick rectum respectively (from Ref. 102).

A radioimmunoassay for ANF has also been developed (103). Antibodies were prepared in rabbits immunized with the peptide coupled to thyroglobulin. Labelling was performed using Chloramine T as an oxidant. **Figure 40** below shows the standard curve from the assay.

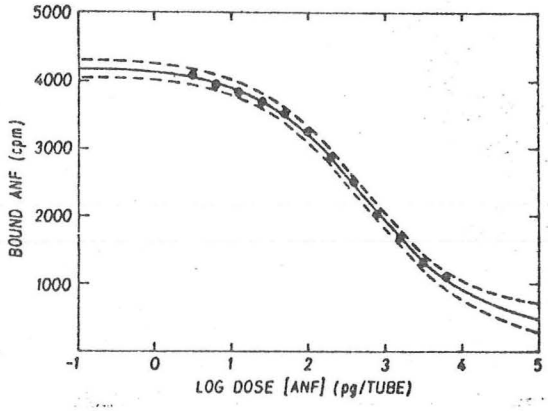


Figure 40: Standard curve for ANF RIA (from Ref. 103).

A number of intriguing experiments have just begun to explore the potential role of ANF in pathophysiologic conditions. The most investigated area to this point has been hypertension. Sonnenberg et al found reduced content of ANF in hypertensive rats compared to normotensive controls (104, **Figure**

41). These authors postulated a reduction in ANF in atrial tissue due to chronic release; it is also possible that defective synthesis accounted for the reduced atrial content. In either case, if ANF is of importance in the model, then chronic sodium retention would result because of either end-organ resistance (in the case of chronic depletion) or synthetic failure.

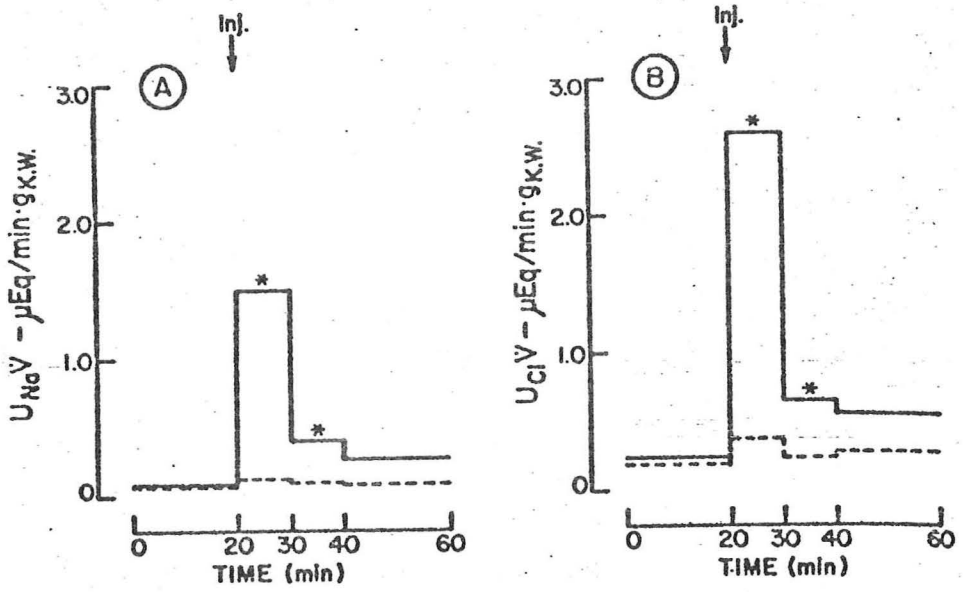


Figure 41: Bioassay of ANF effect on $U_{Na}V$ (left) and $U_{Cl}V$ (right). Dashed bars indicate extracts from atria of hypertensive rats; solid bars from normotensive controls (from Ref 104).

Hirata et al (105) have examined this issue in Dahl salt-sensitive and resistant hypertensive rats. As shown in **Figure 42** below, these workers noted end-organ hyporesponsiveness to ANF in the salt-sensitive (hypertensive) rats as opposed to salt-resistant (normotensive) rats.

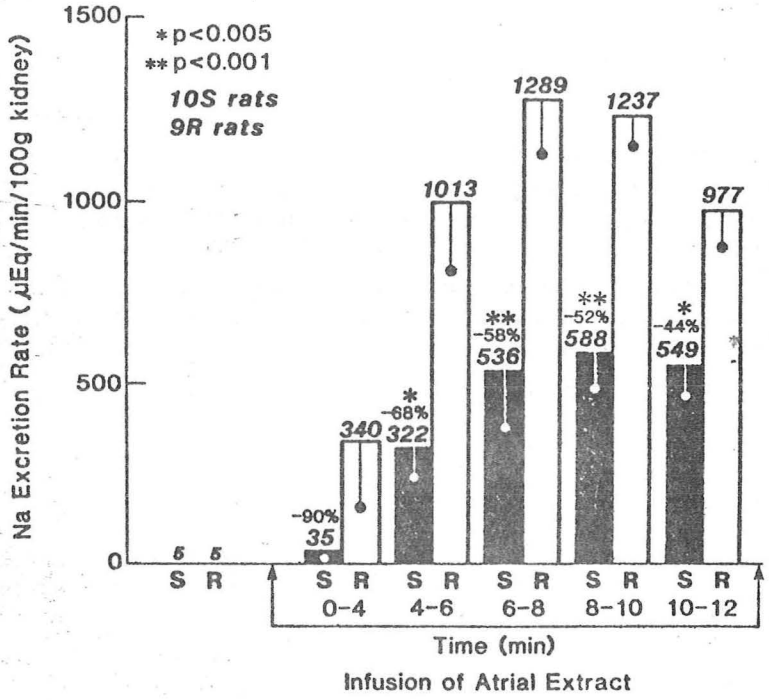


Figure 42: Solid bars represent hypertensive rats; open bars are normotensive rats (from Ref. 105).

Concomittant with the reduced sodium excretion shown above was a diminished renal papillary plasma flow in response to the ANF (**Figure 43**).

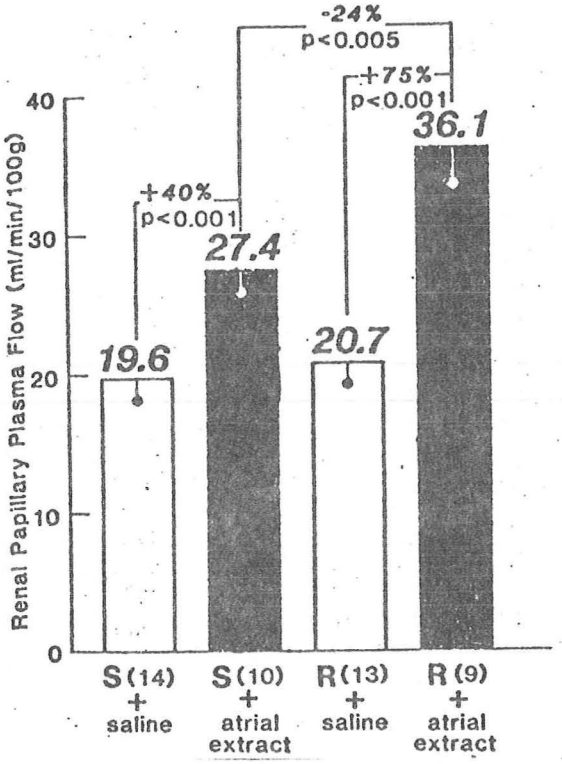


Figure 43: Reduced response of RPPF of hypertensive rats (open bars) compared to normotensive rats (from Ref. 105).

These authors postulate that this observed decrease in renal plasma flow is in part responsible for the decrease in $U_{Na}V$ in "S" rats. Hirata et al also found, in contrast to the work of Sonnenberg (104), an increase in ANF content in the "S" hypertensive animals. Hence, end-organ resistance to ANF accounted for the major findings in this model. Another experimental strategy has been to infuse ANF into hypertensive animals; in one such study (**Figure 44** below), chronic ANF infusion lowered blood pressure in a two kidney, one clip hypertensive model (106). In this study, ANF infusion markedly reduced renin as well, so that a direct effect on peripheral resistance as well as a secondary effect in lowering angiotensin II levels are possible explanations.

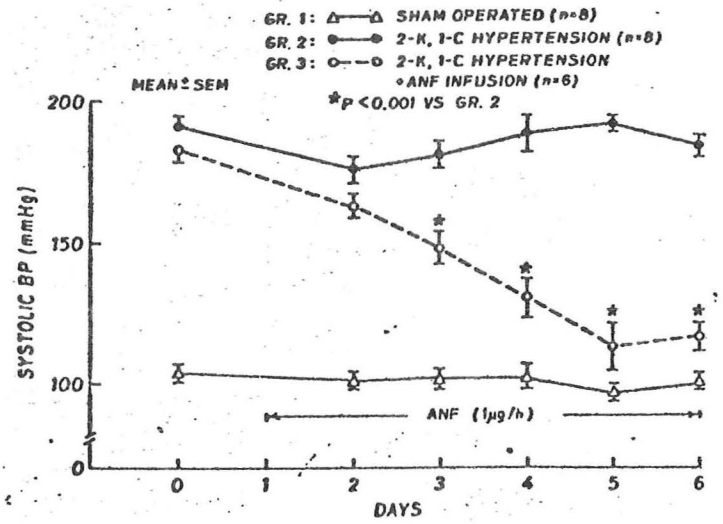


Figure 44: Chronic ANF infusion lowered BP in hypertensive rats (dotted line) compared to non-infused rats (solid line) (from Ref. 106).

A schematic summary of these effects is shown below:

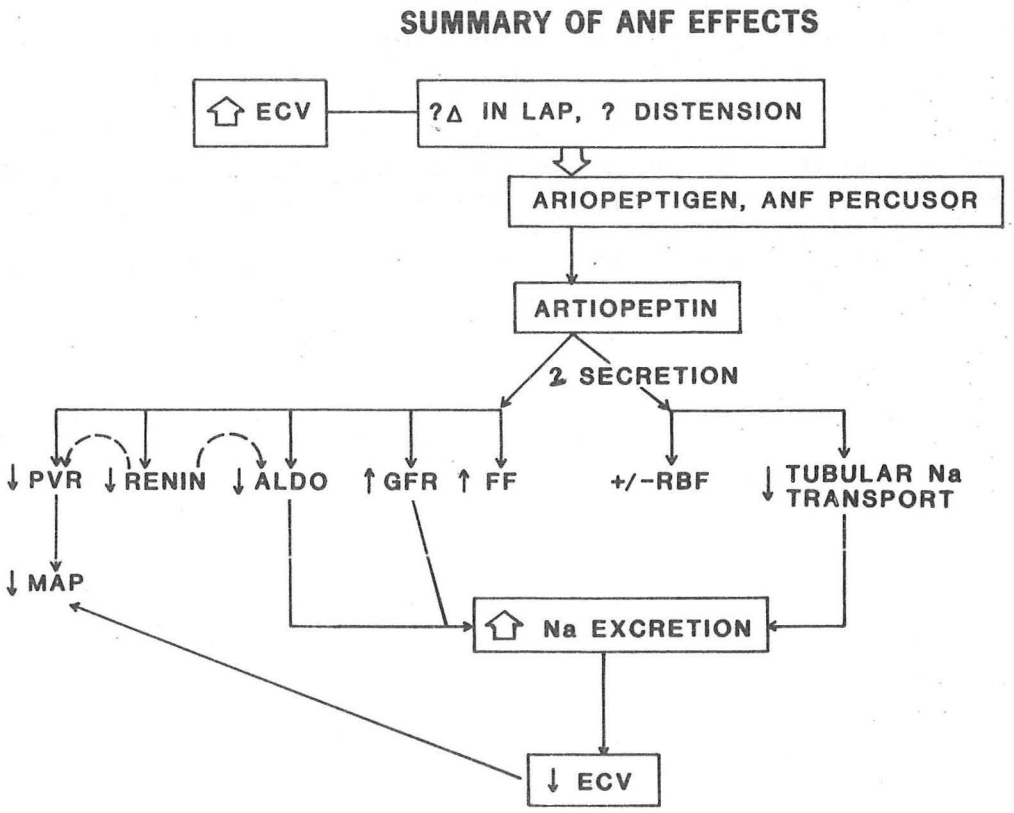


Figure 45: Summary of ANF effects. ANF produces an increase in sodium excretion through a variety of mechanisms and also decreases PVR. Both of these effects lead to a reduction in MAP.

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