Atrial Natriuretic Factor Plays a Significant Role in Body Fluid Homeostasis

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Atrial natriuretic factor (ANF) is a hormone with the physiological characteristics of a regulator of body fluid volume. It is potent, has a short duration of action, and responds to a physiologically relevant stimulus in a negative feedback-controlled system. It can act directly or indirectly (via inhibition of aldosterone biosynthesis) on the kidney to alter sodium transport and may regulate fluid distribution within the extracellular space. The peptide circulates at low (nanomolar) levels, and recent studies with renal inner medullary cells document relevant receptor binding and second messenger activation in this concentration range. In vivo data support a direct action on the kidney to enhance natriuresis, and blockade of a primary catabolic pathway for ANF within the kidney results in augmented natriuresis at concurrent endogenous peptide concentrations. Long-term, low dose infusion directly into the renal artery of conscious dogs supports a physiological action of ANF to promote urinary sodium excretion. Nevertheless, under certain circumstances, natriuresis does not occur even at high circulating levels of ANF. Apparently other factors such as renal perfusion pressure, volume status, and renal nerve activity are important in determining the natriuretic response to a given level of peptide. We hypothesize that the role played by ANF in volume regulation is highly complex, and the kidney responds with increased sodium excretion only when a constellation of variables is appropriately arrayed. That is, ANF is a necessary, but not sufficient, condition to induce natriuresis. (Hypertension 1990;15:2-8)

When the first cell wrapped itself in a membrane, volume regulation became a key to survival. The factors controlling body fluid volume (e.g., the balance between intake and output of salt and water) are many and complex. Intake is mediated through thirst and salt appetite, whereas output is a complex interplay of variables that primarily affect renal function. Historically, the factors considered important for regulation of the renal output of salt and water are glomerular filtration and the facilitated reabsorption of sodium by aldosterone.

The actions of aldosterone in regulating sodium reabsorption from the nephron and changes in glomerular filtration have not proven adequate to explain all of the experimental findings related to extracellular volume control. Based on the compelling experiments of DeWardener et al,1 in which it was shown that the natriuresis associated with saline volume expansion was not prevented by controlling filtration and aldosterone levels, the concept of a salt-excreting reflex pathway was established. Presumably, there are receptors able to distinguish increases in body fluid volume and relay that information to the kidney to increase salt and water excretion.

It has been known from antiquity that water immersion enhances urine formation, and a common treatment for dropsy before discovery of modern drugs was water immersion in therapeutic baths. Such observations suggest that displacement of blood to the cardiothoracic region results in natriuresis.

Given the low pressure and high compliance of the venous side of the circulation compared with the high pressure and low compliance of the arterial tree, it would seem likely that volume sensors are located within the low pressure system. A cogent argument that such receptors are located in the cardiac atria was presented by Gauer and Henry.2 More recent data from Epstein's laboratory,3 among others, has shown clearly that water immersion results in a large increase in urinary sodium excretion. Presumably, the cardiac atria detect the "fullness" of the system and signal the kidneys to increase salt and water excretion. Volume-sensitive receptors regulating vasopressin release4 and water intake5 were known to exist in the cardiac atria, but receptors capable of increasing urinary sodium excretion were less well defined.6 Clearly the system worked, but the mediator was unknown.
The cardiac atria were known to contain specific, secretory-like granules. That the abundance of these granules changes in association with changes in salt and water status was first observed by Marie and colleagues.\(^7\) It fell to DeBold and his coworkers\(^8\) to make the seminal observation that simple saline extracts of atria, when injected into normal test rats, produced a pronounced increase in urinary sodium excretion. Thus, the body appears to harbor its own diuretic, and although not as efficacious as furosemide, atrial natriuretic factor (ANF) is a thousand times more potent.\(^9\)

ANF satisfies all of the conditions to be an endogenous volume-regulating hormone. It is a powerful natriuretic agent as was originally demonstrated with atrial extracts and repeatedly confirmed with synthetic peptides. The peptidic nature of the mediator allows for rapid control because of the short inherent half-life. Release of ANF from the cardiac atria is controlled by a volume-related variable, stretch of the atrial myocytes. Expansion of the extracellular fluid or water immersion is associated with increased circulating levels of ANF in both animals and humans. (See Reference 10 for a recent review to support these contentions.) Blockade of this response with monoclonal antibodies suggests a key role for ANF.\(^{11}\)

Finally, natriuresis occurs at plasma levels of the peptide that are physiologically feasible.\(^{12,13}\)

So what is the question of the debate? There seems irrefutable evidence to support the natriuretic activity of ANF. The debate appeared to hinge on the observation that, under certain circumstances, there is a dissociation of the natriuretic effects of ANF from circulating levels.\(^{14}\) That is, natriuresis is not tightly coupled to the concurrent level of ANF.

One of the most striking observations that we made several years ago was that normal, euvoletic monkeys, when infused with ANF, did not undergo a natriuresis, but when these same animals were anesthetized or very slightly volume expanded, a pronounced increase in urinary sodium excretion was observed with the same dose of ANF.\(^9\) This was the first indication of the dissociation between circulating ANF and natriuresis. These data suggested the lack of a simple relation between plasma level of the hormone and the natriuretic action. Rather, this was a complex interaction and several jointly dependent variables were important, changes in any of which could determine the magnitude of the response. Thus, we would say that for volume regulation, the atrial peptide system is highly complex and the kidney responds with increased sodium excretion only when a constellation of variables is appropriately arrayed. These variables are several, including receptor number, renal perfusion pressure, extracellular volume, renal sympathetic nerve activity, and quite possibly others. It is simplistic to view this system as responding only to changes in plasma levels of ANF.

There are other excellent examples of volume regulatory systems where the mediator and the renal responses are dissociated. A classic is the renal escape from the sodium-retaining actions of mineralocorticoids. As is widely recognized, when mineralocorticoids are administered continuously, sodium is retained for only a few days, after which the individual comes back into balance with an expanded extracellular fluid volume. Indeed, it couldn't be otherwise. If volume-retaining or volume-excreting systems were not feedback controlled, balance would not be possible, nor would life. A similar analogy can be drawn with diuretics. Treat patients with even the most potent and efficacious diuretic and sodium excretion will exceed intake for only a day or two, after which balance is restored despite the continued presence of the diuretic. Were it otherwise, diuretics would be lethal.

One of the key elements to validate the role of ANF as a renally effective hormone is the presence of receptors for the hormone at locations that can result in changes in sodium excretion. Our laboratory was one of the first to identify ANF receptors on renal membranes, but we did not localize these receptors to a specific site in the kidney.\(^{15}\) Later, there was abundant evidence of localization of ANF receptors on the glomeruli\(^{16}\) but binding to renal tubules was scant. Quite recently several papers have appeared documenting a renal tubular receptor that is linked through guanylate cyclase and mediates sodium transport. This receptor is responsive to ANF. Do these receptors respond to ANF at levels that are physiologically feasible? If they do not, then it is immaterial what other conditions must exist simultaneously to support the natriuresis.

Figure 1, from a study by Gunning et al.,\(^{12}\) illustrates the binding of ANF to inner medullary collecting duct cells for the dose range \(10^{-13} \text{ M}\) through \(10^{-7} \text{ M}\) with
specific cation channels were identified in inner medullary collecting duct cells that were inhibited by ANF at $10^{-11}$ M. Cyclic GMP at 0.01 mM had a similar inhibitory action on this cation channel (Figure 2).

These data are important in establishing a renal tubular site of action for ANF and documenting that a fundamental receptor-mediated mechanism exists and can operate in the physiological range of ANF concentrations.

Amiloride, an acylguanidine diuretic with potassium-sparing characteristics, inhibits sodium reabsorption in the distal nephron including the inner medullary collecting duct. Zeidel et al. observed a similarity between amiloride and ANF with regard to inhibition of sodium transport. Although ANF exhibits similar action to amiloride (Figure 3) it is three to four orders of magnitude more potent. Amiloride blocks the sodium entry step into transporting epithelia that are generally described as tight (having high resistance to paracellular shunt pathways). These data would suggest that the action of ANF is similar to amiloride and has its effect from the luminal side of the nephron. If this interpretation is correct, it would make even more remote the relation between plasma levels of ANF and its renal action. An interesting difference between amiloride and ANF is that the latter is not potassium sparing, which suggests a fundamental difference between the two compounds, particularly with reference to the tubular locus of their predominant influence.

Tubular receptors may not be the only way in which ANF modifies renal sodium handling. There is abundant data showing that ANF is a potent inhibitor of aldosterone biosynthesis (see Reference 10 for recent review), and this mechanism may contribute substantially to the renal actions of the peptide.

There may be yet other mechanisms by which ANF plays a role in regulating renal function. The author's personal bias is that there is an important vascular action of ANF that is key to its natriuretic action. In fact, the dependence of the natriuresis on renal perfusion pressure, or some pressure-related variable.
supports this contention. An especially appealing hypothesis is that ANF alters medullary resistance and vasa recta flow. Recent data from Roman’s laboratory (Takezawa et al22) support this contention (Figure 4). It is possible that changes in pressure or concentration gradients play a role in ANF-induced natriuresis.

The in vitro data are certainly important to understanding the action of ANF, but in any physiological discussion the overriding consideration is what happens in vivo.

The in vivo data are confusing for several reasons. The relatively high doses of exogenous peptide that have been administered have actions in addition to enhancement of urinary sodium excretion. These additional actions may greatly influence the net effect that ANF has on urinary sodium excretion. The effects of ANF on blood pressure have been particularly confounding to our understanding of the physiological action of the peptide. It would appear from the published data that at least two conditions must occur simultaneously. These two conditions are adequate renal perfusion pressure and adequate levels of ANF. In other words, adequate plasma levels of ANF are necessary but not sufficient to produce natriuresis; a jointly necessary condition, adequate renal perfusion pressure, is also required.

This point is illustrated with some of our early data from monkeys.9 Figure 5 shows the dose–response relation for synthetic ANF and sodium excretion in anesthetized monkeys. The complex-
In the upper panel of Figure 6 is illustrated the effects of a 3-hour infusion of ANF on conscious euvoletic monkeys. Surprising to us at that time was the total lack of natriuresis in this physiologically relevant setting (conscious, trained, euvoletic monkeys). Notice, however, that arterial blood pressure fell and that the blood pressure reduction was maintained for the duration of the infusion. When these same monkeys were tested in an identical setting, except that they received a modest volume expansion with isotonic saline (1 ml/kg as a bolus injection followed by 0.25 ml/kg/min sustaining infusion), natriuresis was fully apparent (Figure 6, lower panel). This was despite a fall in arterial blood pressure, although it was not as large as when these monkeys were tested during euvoleticism. We also tested whether increasing arterial pressure with intravenous angiotensin II would restore the natriuresis, and that, too, was successful (data not shown). Likewise, if you restore pressure with angiotensin II or metoxamine in rats receiving an ANF infusion, there is a marked potentiation of the natriuretic response.

Renal perfusion pressure is not the only factor determining the renal response to a given dose of ANF, however. We have observed in rats and in dogs that nonpressor doses of vasopressin greatly potentiate the natriuretic response when administered intravenously but not when administered into the renal artery.24 Figure 7 shows the potentiation of the natriuretic response to intravenous vasopressin in dogs with innervated and denervated kidneys. In the innervated kidney, vasopressin potentiates the natriuretic response, but after denervation, ANF alone has a markedly greater effect that is not further potentiated by vasopressin. Based on work from Bishop’s laboratory, these data suggested a major role for the level of renal sympathetic nerve activity in determining ANF natriuretic action.

We have seen how renal perfusion pressure and the renal nerves can dictate the level of natriuresis induced by ANF, but other factors are also at work. These factors, important particularly for altering endogenous levels of ANF in the kidney to regulate the level of activity, are the rate of hydrolysis by endopeptidase 24.11, especially in the renal tubular brush border, and binding of ANF to the non-guanylate cyclase-linked binding site.19 Blocking endopeptidase alone with the inhibitor thiorphan has no effect on the natriuretic actions of ANF. Likewise, blocking the non-guanylate cyclase–linked site alone with a specific inhibitor has no effect. But, blocking both of these pathways reveals the capacity for a pronounced natriuresis based on the endogenous level of the peptide (Figure 8). These data strongly support the idea that endogenous levels of ANF can produce a marked natriuresis when other variables, in this case those determining metabolic clearance, are appropriately arrayed.

Despite these compelling arguments, physiologists demand documentation that exogenous administration of the hormone, at physiologically relevant levels, produce the appropriate response. Such experiments are extraordinarily difficult. They require studies in conscious animals in normal correspondence with their environment, and usually, continued administration for days or weeks. Fortunately, such
experiments have been conducted with ANF; and the results support a role for ANF in regulating urinary sodium excretion. Mizelle et al.12 have shown recently that ANF infused continuously into the renal artery of conscious dogs at levels that produce physiologically relevant plasma concentrations induces a sustained natriuresis. In this study, when the effects of the peptide were restricted to the kidney (e.g., blood pressure remained constant) natriuresis was maintained for the duration of the 5-day infusion. Undoubtedly, the natriuresis would wane in time as significant volume reduction ensued and arterial pressure was reduced. In fact, in a similar study where ANF was infused at physiologically relevant levels intravenously, natriuresis was limited by the fall in arterial blood pressure.27

In a very general sense, the renal response to ANF supports the body fluid–pressure feedback control system originally described by Guyton et al.28 as the controller for arterial blood pressure. In that context, ANF can be viewed as a substance that alters the kidney’s ability to excrete sodium and, in concert with the other factors that alter renal excretory ability, contributes to the overall conditions that determine arterial blood pressure.

In conclusion, I have shown that 1) ANF has the physiological characteristics to be a mediator of body fluid regulation, 2) ANF satisfies the conditions to be a component of a physiological feedback–controlled system, 3) ANF is part of a highly complex and integrated system and is one of several jointly necessary factors that must occur simultaneously to effect natriuresis, and 4) ANF at physiologically relevant plasma concentrations can elevate urinary sodium excretion for long time periods. That natriuresis does not always occur indicates that other limiting conditions dominate.

As a final comment, it is interesting to note the many parallels that have been drawn between the atrial peptide system and the renin-angiotensin system. It is informative to recall also that the same arguments regarding the relevance of the renin-angiotensin system to blood pressure homeostasis were raised. It was only when effective chemical inhibitors of the renin-angiotensin system were discovered, in that case the angiotensin converting enzyme inhibitors, that the true nature of the renin-angiotensin system was appreciated. I suggest a similar circumstance for the ANF system. Until there is an effective inhibitor of the system that allows for clear definition of the actions of ANF, we will not be able to conclude what the “real” function of ANF may be.

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References


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