UNDERFILL AND OVERFLOW REVISITED: MECHANISMS OF NEPHROTIC EDEMA* **

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The management of nephrotic edema continues to be a vexing problem for the clinician. Anasarca often becomes the nephrotic patient's most insistent complaint, and yet many such patients are resistant to conventional diuretic therapy, or develop further reduction in already compromised renal function as diuresis is attempted. The pathophysiology of nephrotic edema formation has traditionally been viewed as occurring through two separate pathways, which, conceptually at least, are mutually exclusive (1). These two pathways result in underfill or nephrotic edema, on the one hand, and overflow or nephritic edema on the other. This article will review the principle features of each, and present data suggesting that a common underlying mechanism could contribute to the abnormal sodium metabolism and renal sodium handling that occur in each form.

DEFENSES AGAINST EDEMA FORMATION

In normal circulatory physiology, large quantities of plasma water and electrolytes leave the vascular compartment via capillary filtration in a manner determined by the Starling equation:

$$J_v = L_p \, \Delta P - \sigma \, \Delta \, \Pi$$

where J_v is the fluid flux across a capillary membrane and ΔP and $\Delta \, \Pi$ represent the hydrostatic and oncotic pressure differences, respectively, between the vascular and interstitial compartments. L_p and σ are constants describing the characteristics of the membrane with respect to its hydraulic conductivity and oncotic integrity. Fluid does not accumulate

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** Supported by grants DK 31623 and HL 35753 from the National Institutes of Health, and grants-in-aid 891124 and 901314 from the American Heart Association. Dr. Gardner was an Established Investigator of the American Heart Association during the conduct of this research.

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in the interstitium despite rather large rates of filtration because it is efficiently returned to the circulation via the lymphatics: increasing rates of infiltration lead to increases in lymph flow. The increased lymph flow also serves to "wash out" interstitial albumin, the chief contributor of Π; as $\Pi_{interstitium}$ falls, $\Delta\Pi$ increases, tending to retard further capillary filtration. Changes in interstitial hydrostatic pressure may also occur in the face of increased capillary filtration. These mechanisms therefore are normally capable of returning large volumes of filtered fluid back to the circulation, and limiting the rate of fluid filtration, so that edema does not normally occur. It follows then that any edematous condition must arise at the local level when the rate of capillary filtration exceeds the rate of return via the lymphatics, ie, the defenses against edema formation have been overcome.

MECHANISMS AND CHARACTERISTICS OF UNDERFILL EDEMA

The basic abnormality in this form of nephrotic edema rests with a reduction in serum albumin concentration and attendant plasma oncotic pressure. This occurs from the increased filtration and urinary excretion of albumin, coupled with increased albumin catabolism (2), and results in increased J_v by decreasing the $\Delta\Pi$ term in the Starling equation. The defenses are overcome, and edema ensues. Since this increased interstitial fluid comes initially at the expense of the plasma compartment, some degree of plasma volume contraction must occur which then triggers secondary renal sodium-retaining mechanisms as from any other hypovolemic stimulus. Renal sodium retention takes place, and if sodium intake is not coordinately reduced, positive sodium balance leads to a progressive increase in extracellular fluid (ECF) volume. This ECF volume increase exacerbates the edema by further diluting plasma albumin. These relationships are summarized in Figure 1.

The neurohumoral mediators of renal sodium retention in underfill edema are predicted to be the same as in any other condition of plasma volume contraction. These include increased activity of the sympathetic nervous system, particularly efferent renal nerve activity (ERNA). Increases in ERNA directly lead to increases in the tubular reabsorption of filtered sodium, and also stimulate renin secretion (3). Activation of the renin-angiotensin system is another hallmark of underfill states; angiotensin II itself increases reabsorption in the proximal tubule, and has a further salt-retaining action by virtue of its effect to stimulate aldosterone secretion from the adrenal gland. Finally, atrial natriuretic peptide (ANP) secretion, governed chiefly by atrial stretch or distension, in turn determined by intravascular blood volume, is decreased in hypo-

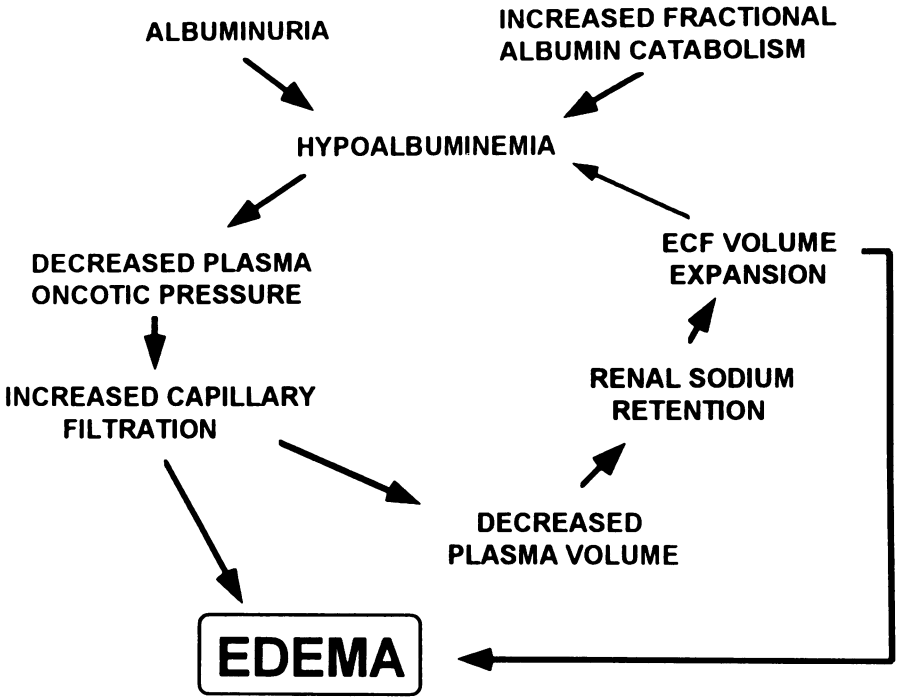

FIG. 1. Schematic presentation of the pathogenesis of underfill edema.

volemic states so that any effect it would contribute to sodium excretion is reduced, thereby facilitating salt retention.

The underfill model of nephrotic edema would therefore predict that plasma volume should be diminished, and that the neurohumoral responses just described should occur in patients or experimental animals with nephrotic syndrome. Evidence on these points is equivocal. Measurement of plasma volume in hypoalbuminemic patients is technically complicated because of exaggerated effects of upright posture and the uncertainty of a reference volume in edematous patients. Studies examining these issues have concluded that, in aggregate, plasma volume measurements in nephrotic subjects can be accurate, and that a range of values is observed, with occasional patients having reduced, many having normal, and some having plasma volumes that are actually increased (4, 5). These results do not therefore support uniformly the underfill model of nephrotic edema formation. Evidence suggesting sympathetic nervous system activation has been presented in children with nephrosis (6, 7, 8) and in rats with proteinuria resulting from the administration of adriamycin which were challenged with a saline load (9). However, we have been unable to demonstrate an improvement in blunted volume expan-

sion natriuresis in nephrotic rats with bilateral renal denervation, suggesting that increased ERNA was not the sole determinant of the blunted response (10). Stimulation of the renin-angiotensin system has also been difficult to demonstrate regularly in either clinical or experimental nephrotic syndrome (11, 12, 13). Aldosterone has been implicated in nephrotic sodium retention in some studies (14) but not others (12). Finally, plasma ANP concentration as a marker of intravascular volume in nephrosis has also revealed mixed results. Although low in some patients (15), it is normal in others (16), again indicating that a uniform view of underfill nephrotic edema must confront a number of inconsistencies.

MECHANISMS AND CHARACTERISTICS OF OVERFLOW EDEMA

Because of these inconsistencies, it is not surprising that an alternative hypothesis regarding the pathogenesis of nephrotic edema has been advanced. This hypothesis argues that sodium retention in nephrotic syndrome occurs as a primary manifestation of the renal disease to result in edema by an overflow mechanism. Since this is thought to be the case in the sodium retention of acute glomerulonephritis, it is often called nephritic edema. In this mechanism, some consequence of the proteinuric state leads to enhanced tubular sodium reabsorption, most likely in the distal nephron. In the face of a normal sodium intake, positive sodium balance occurs, leading to progressive ECF volume expansion. In peripheral capillaries filtration increases as a consequence of an increase in ΔP; when the defense mechanisms are exhausted, edema develops on an overflow basis. Thus, this mechanism places less emphasis on reduced plasma oncotic pressure and plasma volume. Rather, it predicts that plasma and ECF volume expansion must precede the development of edema and coexist with it (1, 4). As discussed above, actual measurements of plasma volume in nephrosis are variable, with some being elevated and many in the normal range, thus providing some support for the overflow hypothesis. Additional support derives from measurements of renal hemodynamics, which indicate that filtration fraction is reduced in patients with nephritic edema as opposed to the increase is observed in hypovolemic sodium retention (4). Moreover, plasma renin activity and aldosterone are likewise suppressed rather than stimulated in this condition (5).

THE BASIS FOR ANP RESISTANCE IN EXPERIMENTAL NEPHROSIS

Thus, available evidence leads to the conclusion that sodium retention in nephrosis cannot be accounted for by underfill or overflow mechanisms

on a uniform basis. This has led to the viewpoint that certain types of clinical nephrotic syndrome may result in edema by one mechanism while other types are characterized by the other mechanism (1, 4, 17). It seemed possible to us that each of these postulated mechanisms of nephrotic edema formation could act via a common underlying abnormality in sodium metabolism which could help to reconcile a number of the conflicting observations mentioned earlier. In particular, we focused on the possibility that an abnormality in the renal actions of ANP could represent a fundamental defect leading to nephrotic sodium retention. Nephrotic rats are known to be resistant to the natriuretic actions of ANP through a strictly intrarenal mechanism (18), and also exhibit a sodium transport abnormality in terminal nephron segments by micropuncture studies (19). The terminal nephron includes the inner medullary collecting duct (IMCD), a nephron segment highly responsive to ANP. Although the exact role of ANP in sodium homeostatis is controversial (20), there is widespread agreement that it is the major mediator of the natriuresis resulting from acute intravascular volume expansion. Since nephrotic rats and humans also exhibit blunted volume expansion natriuresis (9, 10, 12, 15), we evaluated the relationship between sodium excretion and plasma ANP concentration after acute volume expansion in rats made proteinuric from the intravenous injection of adriamycin (7 mg/kg). Three to five weeks after injection, this agent produces high-grade proteinuria without severe impairment of GFR or marked histologic damage, and is a useful model of human minimal change nephropathy or early focal glomerulosclerosis. We subjected both anesthetized normal control and nephrotic rats to an acute infusion of normal saline, 2% body weight given intravenously over a five-minute interval. Over the next 30 min, a clear difference emerged between normal and nephrotic rats: normal rats exhibited a brisk natriuresis, rising from a baseline rate of sodium excretion of 1.38 ± 0.29 to a peak of 13.69 ± 1.54 μeq/min 20 min after the saline infusion, whereas the nephrotic rats, although starting from an only modestly reduced baseline, had a strikingly blunted natriuretic response 0.51 ± 0.09 to 5.29 ± 0.51 μeq/min. This last figure was significantly less than the value in normals after the volume expansion ($p < .005$) (10).

The nephrotic rats thus had blunted volume expansion natriuresis. This was not due to an impaired concentration of ANP in plasma after the volume expansion, since the plasma ANP concentration in nephrotic rats (311 ± 35 pg/ml) was actually higher than in normals (201 ± 27 pg/ml) after volume expansion ($p < .05$), and both were higher than values in hydropenic control animals. We consequently sought to determine if resistance to the renal actions of ANP occurred in nephrosis by measuring urinary excretion (UV) of cyclic guanosine-3′,5′-monophosphate

(cGMP) before and after volume expansion. There is general agreement that cGMP is the major intracellular second messenger of ANP (21), and $U_{cGMP}V$ appears to be a reasonable marker of the renal actions of ANP (22). There was no difference in the baseline rate of $U_{cGMP}V$ between normal and nephrotic rats, but nephrotic animals had a markedly blunted increase after volume expansion compared to normals. This paralleled the blunted volume expansion natriuresis described above, and indicated that nephrotic kidneys were indeed resistant to the renal actions of endogenous ANP stimulated by the saline infusion (10).

We then examined this blunted ANP responsiveness in the context of the known steps involved in the biological actions of ANP (Figure 2). As with any endocrine system, secreted hormone must reach its receptors in target tissues, bind to them, and activate an intracellular signaling pathway(s) responsible for the biological effect; in the case of ANP, the intracellular messenger is cGMP and one of its actions is to act on a luminal sodium channel to inhibit sodium entry (21). The cGMP is subject to degradation by a cGMP-specific phosphodiesterase, which thus curtails the hormone signal.

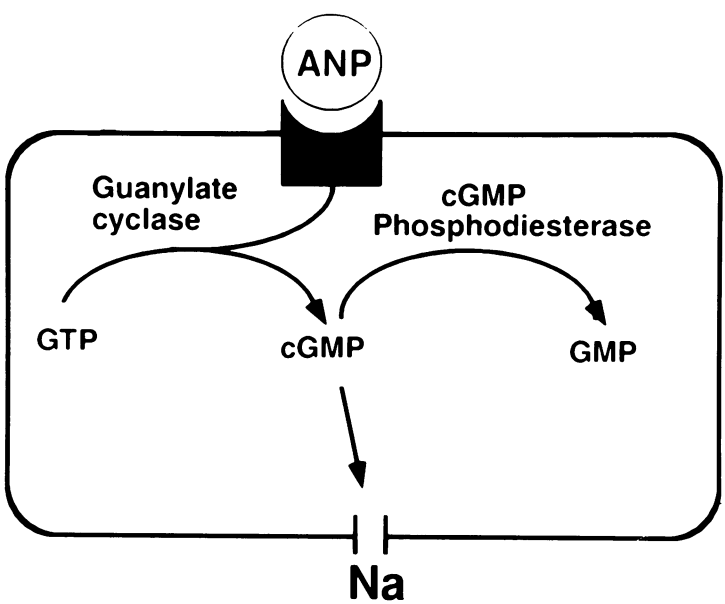

FIG. 2. Outline of the pathways involved in ANP signaling in target cells such as those in the inner medullary collecting duct. The ANP receptor contains a guanylate cyclase in its intracellular domain; interaction of ANP with its binding site on the cell surface activates the enzyme, which then converts GTP to cGMP. cGMP then acts to inhibit a sodium channel in the luminal membrane. The signal is terminated when cGMP is inactivated to GMP by a cGMP phosphodiesterase.

Our results could be explained by a failure of ANP to reach its target site in the IMCD despite the elevated plasma concentration, or by altered binding of ANP to its renal receptors. To test the former possibility, we measured the responsiveness to exogenous ANP of glomeruli and IMCD cells isolated from normal or nephrotic rat kidneys. The results are shown in Figure 3. Both isolated glomeruli and freshly dispersed IMCD cells from normal rat kidneys exhibited a concentration-dependent increase in cGMP accumulated in the medium in response to added ANP. Nephrotic tissues also demonstrated ANP-dependent increased cGMP accumulation, but the response in each tissue was markedly blunted compared to normal (Figure 3). Thus, abnormal responsiveness *in vivo* was paralleled by qualitatively similar diminished cGMP accumulation *in vitro*, indicating that impaired access of circulating ANP to its receptor sites in target tissues in the kidney could not explain the blunted volume expansion natriuresis observed *in vivo*. We carried out studies of the binding of ^{125}I-ANP to freshly dispersed IMCD cells to determine if alterations in the interaction of the peptide with its receptors could account for our results. ANP receptors in these cells are primarily of the biologically active type with intrinsic guanylate cyclase activity (21). We

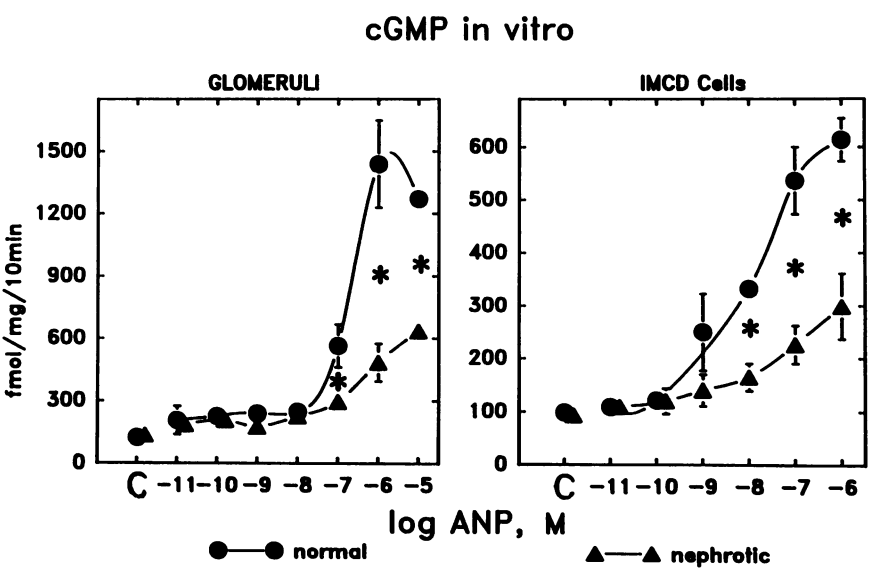

FIG. 3. Accumulation of cGMP in vitro by glomeruli and IMCD cells isolated from normal and nephrotic rat kidneys in response to incubation with ANP in the concentrations indicated. Nephrotic glomeruli and IMCD cells had a markedly blunted rate of cGMP accumulation compared to the normal tissues. Reproduced from Valentin et al. (10) with permission of the American Society of Clinical Investigation.

found no difference between normal and nephrotic cells in either the receptor affinity, as indicated by the K_d, or receptor number, reflected by the B_{max}, demonstrating that altered binding or receptor characteristics are inadequate to account for ANP resistance in this form of experimental nephrosis. A similar conclusion was reached by Perico and associates (18).

Because biologically active renal receptors for ANP possess intrinsic guanylate cyclase activity, binding of the peptide to these receptors results in increased cGMP synthesis. The lack of difference in ANP binding between normal and nephrotic cells consequently argues against a receptor abnormality to account for the impaired ANP-dependent cGMP accumulation we observed. We therefore considered the possibility that ANP resistance in nephrotic rats was related to enhanced phosphodiesterase activity (Figure 2). When glomeruli or IMCD cells isolated from normal or nephrotic rats were incubated with the nonspecific phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX, 10^{-3} M), baseline cGMP accumulation was elevated, and ANP-dependent cGMP accumulation was also markedly increased over control tissues without added IBMX (Figure 4). However, the blunted cGMP accumulation present in nephrotic tissues incubated without IBMX was normalized in the presence of IBMX. A similar result occurred when these tissues were incubated with zaprinast (M&B 22,948, 10^{-3} M). Zaprinast is a specific inhibitor of the cGMP phosphodiesterase; basal cGMP accumulation in the presence of this agent was only modestly increased over controls, but a dose-dependent increase occurred with added ANP (Figure 4). As was true in the presence of IBMX, the blunted ANP-dependent cGMP accumulation of nephrotic tissues was completely normalized by zaprinast (10).

These results indicated that blunted ANP responsiveness in nephrotic renal tissues was corrected by inhibitors of phosphodiesterases, suggesting that enhanced phosphodiesterase activity could contribute importantly to ANP resistance in nephrotic syndrome. This possibility was strengthened by the observation that sodium nitroprusside-stimulated cGMP accumulation was also blunted in nephrotic glomeruli and IMCD cells. This agent activates a soluble guanylate cyclase separate from the particulate enzyme in the cytoplasmic domain of the ANP receptor. Blunted cGMP accumulation to nitroprusside in nephrotic tissues was also corrected by the two phosphodiesterase inhibitors (10).

To test the physiologic relevance of these *in vitro* observations, we repeated the volume expansion protocol in normal and nephrotic rats in which zaprinast was infused into the left renal artery at a dose (10 μg/min) which had no major effect on either basal sodium excretion or $U_{cGMP}V$ (Figure 5). After volume expansion in normal rats, sodium

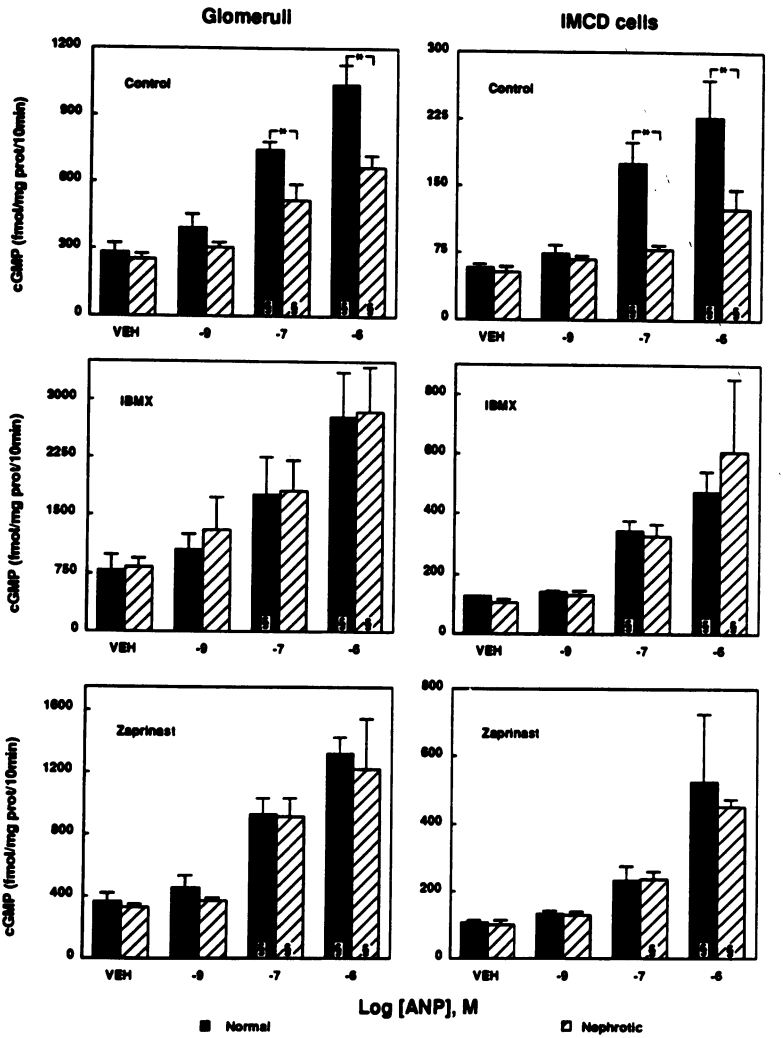

FIG. 4. Effect of incubation with phosphodiesterase inhibitors on ANP-dependent cGMP accumulation by glomeruli and IMCD cells isolated from normal and nephrotic rats. The blunted response of nephrotic tissues compared to normals (top panel) was completely normalized when the nonspecific inhibitor IBMX (10^{-3} M) was added (middle panel). The same result was observed when tissues were incubated with the specific cGMP phosphodiesterase inhibitor zaprinast (bottom panel). From Valentin et al. (10) with permission of the American Society of Clinical Investigation.

excretion rose in the expected manner from the non-infused kidney, and was slightly but not significantly greater than the kidney receiving the zaprinast infusion. In nephrotic animals, the non-infused kidney again exhibited blunted volume expansion natriuresis. However, the infused

kidney had a completely normal response, increasing its rate of sodium excretion to a level that was not different from either the non-infused or zaprinast-infused normal kidney (Figure 5). These results were paralleled by the results of $U_{cGMP}V$ in the same experiments: the zaprinast infusion normalized $U_{cGMP}V$ from the infused kidney just as it had normalized sodium excretion (Figure 5). Thus, inhibition of cGMP phosphodiesterase corrected blunted volume expansion natriuresis, mediated by ANP, *in vivo* just as it had corrected nephrotic ANP resistance *in vitro*.

UNDERFILL AND OVERFLOW REVISITED

These results lead to the hypothesis that abnormal sodium metabolism in nephrotic syndrome may be linked to a defect in the renal ANP-cGMP signaling system referable to heightened activity of a specific cGMP

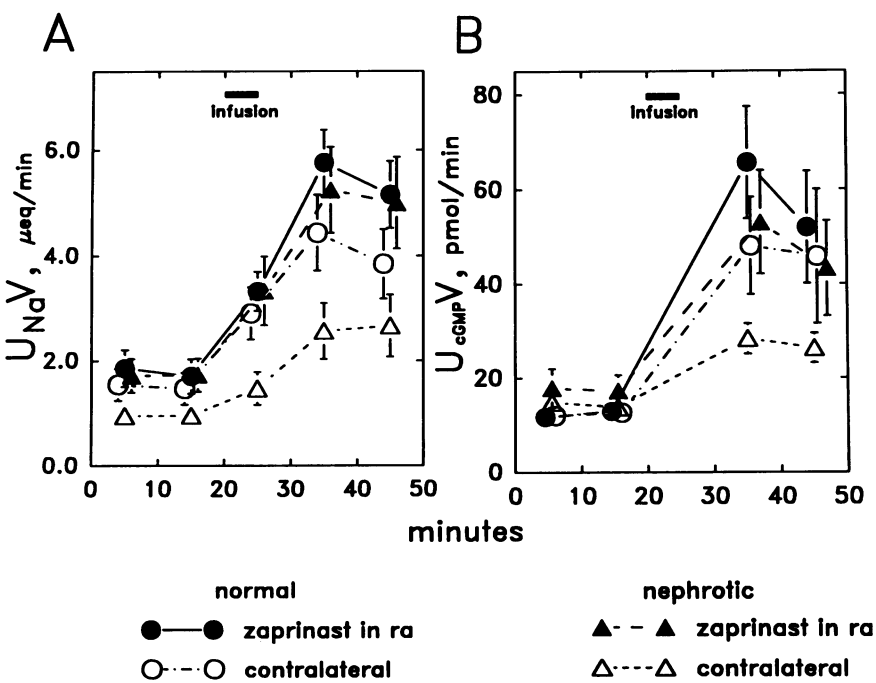

FIG. 5. Effect of intrarenal infusion of zaprinast (10 μg/min) on volume expansion natriuresis and cGMP excretion by normal and nephrotic rats. Noninfused nephrotic kidneys (open triangles) had blunted rates of sodium (left panel) and cGMP (right panel) excretion in response to intravenous infusion of normal saline (2% body weight, black bar). Nephrotic kidneys infused with zaprinast (solid triangles) had responses that were indistinguishable from normal kidneys (circles). From Valentin et al. (10) with permission of the American Society of Clinical Investigation.

phosphodiesterase. It thus may be possible to consider that both underfill and overflow mechanisms of nephrotic edema may share a common underlying pathway. Although at least five families of phosphodiesterases have been identified by molecular cloning, substrate affinity, and inhibitor specificity (23), they share a number of features including sites for modification of activity by cyclic nucleotides themselves and by calcium-calmodulin (23). It may therefore be the case that cGMP phosphodiesterase activity is directly stimulated in circumstances of overflow edema to result in renal resistance to ANP, or indirectly stimulated in underfill conditions by the activated neurohumoral pathways which characterize sodium retention in some circumstances. The cells of the IMCD possess the capability to bind, or to exhibit cellular responses to, numerous vasoactive peptides, steroid hormones, and neurotransmitters; in addition to ANP, these include vasopressin, endothelin, angiotensin II, aldosterone, alpha adrenergic agonists, and dopamine. The cellular actions of many of these agents involve signal transduction pathways which raise intracellular calcium or cyclic nucleotide concentrations one result of which could be stimulation of cGMP phosphodiesterase activity. Consequently, the scheme shown in Figure 6 may help to reconcile the various observations consistent with an underfill mechanism of nephrotic sodium retention (6–9, 17) with the compelling evidence that the retention can be related to a strictly intrarenal defect in sodium handling (18, 19). If the mechanism resulting in nephrotic proteinuria also led to an increase in the cGMP phosphodiesterase in IMCD cells, then primary sodium retention would occur and lead to overflow edema. On the other hand, if the proteinuric state was accompanied by a reduction in plasma volume, the mediators of secondary sodium retention could also activate cGMP phosphodiesterase in IMCD cells. Such agents could include angiotensin II, vasopressin, endothelin, and the α-adrenergic agent norepinephrine, as well as other possible mediators (Figure 6). In either case a common cellular mechanism, resistance to the actions of ANP due to heightened activity of cGMP phosphodiesterase, would participate in nephrotic sodium retention regardless of whatever other renal consequences of the nephrotic state might exist. Finally, it should also be recognized that some consequence of the proteinuria itself could result in such an effect on renal target sites for ANP through a currently unrecognized mechanism.

The observation that a similar abnormality in ANP action can be demonstrated in rats with Heymann nephritis, an immunologic form of renal injury closely resembling human membranous nephropathy (24), suggests that this mechanism may be a generalized one in nephrotic edema. This model therefore offers the hope of providing new insight into nephrotic sodium metabolism. Moreover, it offers possible relevance

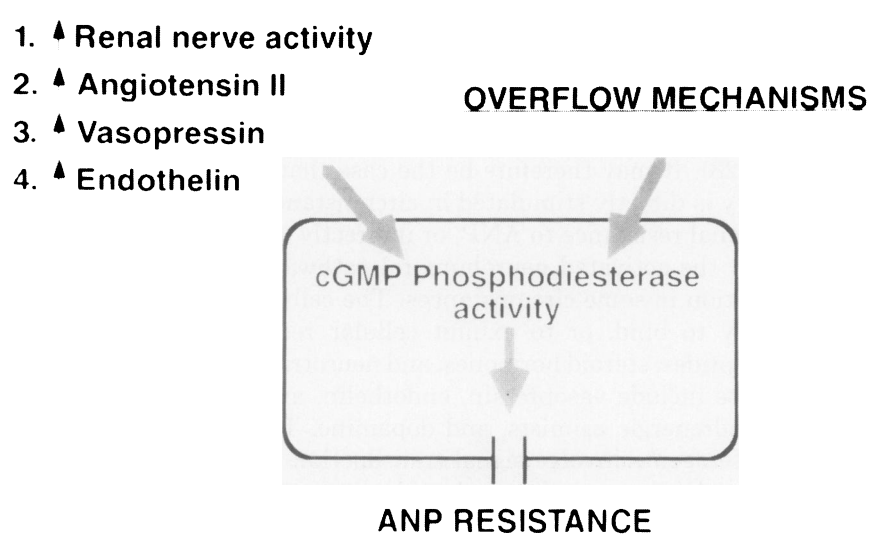

FIG. 6. Hypothetical schema whereby renal resistance to ANP could result from heightened activity of cGMP phosphodiesterase whether the nephrotic state was associated with overflow edema or accompanied by evidence of underfill mechanisms.

to other states of pathological sodium retention such as cirrhosis of the liver and congestive heart failure, which are also characterized by renal resistance to the actions of ANP.

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DISCUSSION

Buckalew, Winston Salem: I enjoyed that very much. As you know, there is some interest in the fact that the kidney makes its own atrial natriuretic factor that may be more important in regulating sodium excretion than the circulating plasma level. It is the so-called urodilatin. Have you had a chance to look in your model at what happens to this particular urodilatin entity? Would this mechanism that you are talking about apply to that as well as to the circulating ANP?

Humphreys: Thank you, Vardy. That's certainly a good question. There is evidence, as you summarized, of an atrial natriuretic peptide gene product's being synthesized within the kidney and perhaps acting within the kidney. We haven't looked at it in this context, except to the extent that urodilatin, as it is called, appears to interact with ANP receptors, just like ANP itself. We can't distinguish the two, either in terms of binding characteristics or in cyclic GMP generation. The nephrotic animals appear to have the same defect as with ANP. In terms of the true mechanism within the animal, we haven't looked at that at all.

Glassock, Lexington: Mike, I enjoyed your presentation very much. I have a question

and also a comment. First of all, I believe the Italian investigators who looked at the unilateral adriamycin model first demonstrated that the resistance to ANP was uniquely localized to the tubules and did not occur in the glomeruli. In other words, following ANP infusion, the glomerular filtration rate rose in the kidney which was nephrotic, whereas your data, and many other data, show that the post-receptor defect in ANP response is found both in the glomeruli and also in the tubules. It's kind of a paradox between the whole animal studies and the in vitro studies. I wonder if you have a potential explanation for the discrepancy between the relative lack of resistance to ANP in the glomeruli when whole animal studies are done.

The second, a comment, is more speculative. We have events occurring in the glomeruli that affect downstream processes, namely innermedullary collecting duct sodium reabsorption. What's the link between glomerular permeability and abnormalities in GMP production or post-receptor events? George F. Schreiner, another member here, showed that cytokines have profound effects on cellular responses in tubules downstream, and one might perhaps speculate that cytokines are being generated in glomeruli and influencing these post-receptor abnormalities in tubules downstream.

Humphreys: Well, these are two excellent points, Dick. With respect to the GFR question, my own view is that the regulation of GFR is so multivariant and has so many determinants that the removal of one, i.e. by blunting this ANP-related effect, may be offset by other forces so that we don't see an overall difference in measured glomerular filtration rate, despite the fact that in the test tube, you can still demonstrate the abnormality. We too are very much interested in what is happening downstream. What's happening to the innermedullary collecting duct to cause it to act this way? If you have any ideas, we are very receptive.

Schrier, Colorado: Thanks for the nice talk, Mike. When one examines other edematous disorders, the hallmark is also a resistance to atrial natriuretic peptides, i.e. cirrhosis and heart failure. In patient studies, exogenous infusion of atrial natriuretic factors has actually shown an increase in cyclic GMP in the urine in both circumstances. In fact, even with endogenous ANP there is a correlation between urinary cyclic GMP and plasma natriuretic peptide levels in heart failure. So I'm wondering if the failure of atrial natriuretic peptide to increase urinary cyclic GMP is specific for your model, adriamycin. Has anyone actually infused atrial natriuretic peptide into patients with nephrotic syndrome and measured urinary cyclic GMP? Does adriamycin have a direct effect on the receptor integrity of the collecting duct?

Humphreys: Well, that's a very good point. Your group, of course, has made the most telling marks in the field in the last decade, looking at this question in other edema-forming states. I can't reconcile the blunted urinary excretion of cyclic GMP that we see in our rats with other circumstances which you've looked at and with other experiments in the rat that deal with other forms of abnormal sodium metabolism. We don't think it's a specific effect of adriamycin. We've carried out these same experiments with the Heymann nephritis model, using Bill Couser's Fx1A antiserum and have exactly the same thing. Of course, that's an immunologically mediated insult as opposed to a toxic insult to the kidney. I think it's a more general phenomenon in the setting of nephrosis.

Schrier: There is no urinary cyclic GMP measurement in nephrotic patients receiving exogenous atrial natriuretic peptide.

Humphreys: No, not that I am aware of.