Atrial Natriuretic Factor Binding to Its Receptor Is Dependent on Chloride Concentration
A Possible Feedback-Control Mechanism in Renal Salt Regulation
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Abstract—Although considerable evidence indicates a role for atrial natriuretic factor (ANF) in renal salt regulation, other studies have found a lack of natriuretic response to high-plasma ANF under certain physiological and pathophysiological conditions. The mechanism for this apparent insensitivity to ANF is unknown. In the present study, it was found that ANF binding to its receptor requires the presence of chloride and occurs in a chloride concentration–dependent manner. ANF binding was measured using the purified recombinant hormone-binding domain of the ANF receptor in the presence of 0.1 mol/L NaCl or other selected salt. High specific binding was detected in the presence of NaCl, KCl, or NH₄Cl. However, binding was undetectable when the salt was replaced with NaHCO₃, CH₃COONa, or CH₃COONH₄, indicating that binding requires the presence of chloride. Chloride dependence was also found with the native receptor in bovine adrenocortical membrane preparations. ANF binding to the recombinant protein was chloride concentration–dependent over a range from 0.05 to 10 mmol/L, and a half-maximum binding was attained at ≈0.6 mmol/L equivalent chloride concentration. Competitive-binding assays at several fixed concentrations of NaCl showed that lowering chloride concentration caused a decrease in maximum binding but did not alter $K_d$ values, suggesting that a loss of chloride turns off ANF binding rather than reducing affinity for ANF. Saturation-binding studies showed that excess ANF cannot overcome loss of binding caused by low chloride. Chloride-dependent ANF-receptor binding may function as a feedback-control mechanism regulating the ANF-receptor action and, hence, renal sodium excretion. (Circ Res. 2000;86:1135-1139.)

Key Words: atrial natriuretic factor ■ receptors ■ chloride ■ sodium ■ kidney
Binding of ANF to Its Receptor Requires the Presence of Chloride

In the presence of 0.1 mol/L NaCl, a significant level of specific binding of 125I-ANF to the purified hormone-binding domain was observed (Figure 1). Similar levels of binding were found when NaCl was replaced with KCl or NH₄Cl. However, when the salt was replaced with sodium bicarbonate, sodium acetate, or ammonium acetate, binding was undetectable. These results indicate that ANF binding requires the presence of chloride, and that other anions such as bicarbonate, acetate, and phosphate contained in the assay medium cannot substitute for the chloride requirement. On the other hand, the type of cation (Na⁺, K⁺, or NH₄⁺) in the medium had no apparent effect on ANF binding. Chloride requirement for ANF binding was also observed with the native ANF receptor in bovine adrenocortical membrane preparations. The specific binding of ANF obtained in the presence of 0.1 mol/L sodium acetate in the assay medium was 16-fold less than that in the presence of 0.1 mol/L NaCl. When the membranes were used without dialysis, the specific binding in the presence of 0.1 mol/L sodium acetate ranged from 70% to 90% of that found in the presence of 0.1 mol/L NaCl.

Figure 1. Effect of various salts on binding of ANF to the purified hormone-binding domain of the ANF receptor. Binding assays were carried out in a medium containing 0.1 mol/L NaCl or other salt. Binding of 125I-ANF was measured in the absence (-) and presence (+) of 0.1 μmol/L unlabeled ANF. Data are mean ± SEM; n = 6.

Figure 2. Chloride concentration dependence of ANF binding to the ANF-receptor hormone-binding domain. 125I-ANF binding was measured with increasing concentrations of NaCl. The half-maximum binding was attained at 0.6 mmol/L NaCl.
Lack of Chloride Completely Abolishes ANF-Receptor Binding

Competitive-binding assays were performed at several fixed concentrations of NaCl by incubations with varying concentrations of unlabeled ANF competing against a constant amount of 125I-ANF tracer (Figure 3). The results showed that lowering chloride concentration caused a decrease in the maximum binding but did not alter binding affinity toward ANF ($K_d$ remained at $\approx$1 nmol/L).

Excess ANF Cannot Overcome Inhibition of ANF Binding by Low Salt

Incubation of the hormone-binding domain in the presence of 0.1 mol/L NaCl with the increasing concentrations of 125I-labeled ANF showed binding saturation at 0.1 $\mu$mol/L ligand concentration (Figure 4). However, in the absence of chloride, binding was undetectable, even when the concentration of the radioligand was increased to 0.1 $\mu$mol/L, a concentration 100-fold higher than the $K_d$ value. These results indicate that loss of ANF binding due to low chloride concentration cannot be overcome by excess ANF.

Discussion

The data presented in this study show that ANF binding to its receptor required the presence of chloride. In the absence of chloride, there was no detectable binding of ANF to the hormone-binding domain of the ANF receptor. Other anions such as bicarbonate, acetate, and phosphate could not substitute for the chloride requirement. On the other hand, the type of cation present showed no effect on ANF binding. Binding was chloride concentration–dependent over the concentration range from $\approx$0.05 to 10 mmol/L with the half-maximal ANF binding being attained at $\approx$0.6 mmol/L equivalent chloride concentration. Chloride requirement for ANF binding was also observed with the partially purified and dialyzed bovine adrenocortical membranes, although a low level of ANF binding was still observed when NaCl was replaced with sodium acetate. The residual ANF-binding activity may have been due to the presence of chloride ions bound to or trapped in membrane vesicles in the adrenocortical membrane preparations. The chloride dependence of ANF binding to the receptor is consistent with our recent finding of a buried, protein-bound chloride ion in the structure of the ANF receptor hormone-binding domain as determined by X-ray crystallography (K.S. Misono, X. Zhang, F. van den Akker, V.C. Yee, unpublished data, 2000).

Competitive ANF-binding assays performed at several fixed concentrations of NaCl showed that lowering chloride concentration caused a decrease in maximum binding but did not alter $K_d$ values. This indicates that a loss of chloride completely abolishes ANF binding rather than reducing binding affinity. Furthermore, saturation-binding data showed that inhibition of ANF binding by low salt could not be overcome by excess ANF. Physiologically, these results suggest that the chloride-dependent ANF binding provides an on-off mechanism for the receptor, and that loss of ANF binding on chloride deficit cannot be overcome by excess levels of ANF.

The chloride dependence of ANF binding demonstrated in this study suggests a possibility that changes in chloride concentration at the target sites for this hormone may modulate hormone-receptor binding and regulate physiological responses. In the kidney, this chloride-mediated regulation of ANF-receptor binding may function as a feedback-control mechanism in ANF-induced natriuresis to maintain normal salt and fluid balance. In the loop of Henle and the distal tubule, sodium reabsorption is mediated by Na$^+$ and Cl$^-$ cotransport systems and, hence, is accompanied by chloride reabsorption. Thus, the chloride concentrations at these tubular sites are tightly coupled with the state of electrolyte and
water transport. In the medullary collecting ducts immediately downstream (the main tubular site of ANF action), the tubular chloride concentration may reach millimolar ranges under certain physiological and pathophysiological conditions. For example, aldosterone treatment lowers the tubular fluid chloride concentration to ≤4 mmol/L, which falls within the range where ANF-receptor binding is chloride concentration-dependent. Additionally, the ANF receptor is located in both apical and basal membranes of the medullary collecting ducts regulating sodium reabsorption and vasopressin-stimulated water reabsorption. ANF natriuresis and diuresis may then be regulated through control of ANF-receptor binding by the chloride concentrations in the tubule and peritubular microcirculation. It is probable that such a control mechanism plays an important role in renal regulation of electrolyte and fluid homeostasis.

In edematous disease states such as congestive heart failure, nephrotic syndrome, and liver cirrhosis, the plasma levels of ANF are markedly elevated, yet sodium is retained. Congestive heart failure is characterized by activation of the renin-angiotensin-aldosterone system, increased vasopressin secretion, and heightened sympathetic nervous activity. ANF, elevated because of volume expansion, is thought to be one of the principal factors opposing these activities. Although studies using the ANF-receptor antagonist HS-142-1 have demonstrated the suppressive effects of ANF on the sympathetic and renin-angiotensin-aldosterone systems as well as on sodium reabsorption, the high plasma levels of ANF fail to produce correspondingly enhanced natriuresis in congestive heart failure. In such an edematous state, the proposed chloride-mediated feedback mechanism for the ANF receptor may function to prevent excessive salt loss. The chronically elevated plasma ANF in this state would be expected to activate the ANF receptor and induce persistent ANF natriuresis, which would consequently reduce the tubular chloride concentration. ANF natriuresis might then be turned off by inhibition of ANF binding by insufficient chloride when the tubular chloride concentration falls below a threshold level. This chloride-mediated feedback-control mechanism may be responsible, aside from the counterregulatory action of the renin-angiotensin system, for the attenuated response to high-plasma ANF observed in the edematous states. Such a salt-conserving feedback mechanism may likewise operate in ANF-overexpressing transgenic animals, in which salt is also retained in spite of high plasma levels of ANF.

The lack of natriuretic response to high-plasma ANF observed in these states and animals is consistent with our finding in vitro that excess ANF cannot overcome the loss of ANF binding caused by low chloride. Additionally, it has been found that KCl infusion or acute volume expansion by isotonic saline infusion in ANF-overexpressing transgenic animals causes exaggerated natriuretic responses. This exaggerated natriuresis may also be explained by the chloride-mediated feedback mechanism operating in the kidney. With KCl infusion or acute volume expansion, the resultant increase in the filtered chloride concentration may unblock this chloride-mediated inhibition of ANF binding to the receptor, allowing maximal natriuresis to occur in response to high plasma levels of ANF. It has also been reported that high-dose ANF infusion in normovolemic animals induces only modest natriuresis. The suppressed natriuresis in response to high-dose ANF infusions may similarly have resulted from the chloride-mediated feedback mechanism, in which a decreased tubular chloride concentration after acute ANF natriuresis may have prevented further natriuresis by inhibiting binding of ANF to its receptor.

In animal models of congestive heart failure and nephrotic syndrome, ANF infusion lowers the arterial mean pressure and increases the glomerular filtration rate to levels similar to those in the respective control animals, whereas ANF natriuresis is markedly blunted. In addition, in ANF-overexpressing transgenic animals, high-plasma ANF is accompanied by systemic hypotension but normal salt excretion is maintained. These findings suggest that the chloride-mediated feedback control operates at renal tubular sites but not at the renal or systemic vascular sites.

Attenuated natriuretic response to ANF has also been observed in animals on a low-salt diet. The NaCl deficit in low-salt animals is associated with marked elevation in sodium reabsorption in the medullary collecting ducts. ANF infusion in these animals does not reduce this sodium reabsorption. On the other hand, the systemic hypotensive effect of ANF is still present. On the basis of these findings, it has been suggested that a salt-conserving counterregulatory mechanism operates in the kidney and overcomes ANF natriuresis under salt-deprived conditions. It was also found that the lack of responsiveness to ANF in these salt-deficient animals is reversed within 1 hour of salt repletion, suggesting that this counterregulatory mechanism is not mediated by downregulation of the ANF receptor. Additional studies showed that this salt-conserving mechanism operates primarily in the medullary collecting duct and functions independently of the sympathoadrenergic and renin-angiotensin-aldosterone systems. The suppressed natriuretic response to ANF could not be explained on the basis of reduced renal perfusion pressure or glomerular filtration rate. These findings are consistent with direct inhibition of the ANF receptor by chloride deficit as demonstrated in this study and are in agreement with the present hypothesis that chloride-mediated feedback control of the ANF receptor occurs at the renal tubular sites, regulating renal salt excretion.

In summary, binding of ANF to the ANF-receptor hormone-binding domain was found to require the presence of chloride and occur in a chloride concentration-dependent manner. It is proposed that chloride-mediated feedback control of the ANF receptor occurs in the kidney and plays a role in the regulation of ANF-mediated natriuresis and, hence, renal salt excretion. In light of this possible feedback-control mechanism in ANF natriuresis mediated by chloride, the physiological and pathophysiological roles of ANF in salt and volume homeostasis warrant additional investigation.

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References


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