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Role of atrial natriuretic factor in volume control

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Role of atrial natriuretic factor in volume control. Atrial natriuretic factor (ANF) is a 28 amino acid polypeptide hormone secreted mainly by the heart atria in response to atrial stretch. ANF acts on the kidney to increase sodium excretion and GFR, to antagonize renal vasoconstriction, and to inhibit renin secretion. In the cardiovascular system, ANF antagonizes vasoconstriction, and shifts fluid from the intravascular to the interstitial compartment. In the adrenal gland, ANF is a powerful inhibitor of aldosterone synthesis. ANF participates importantly in the natriuretic response to acute and chronic volume overload. ANF's property of shifting fluid from the vascular to the interstitial compartment acts as a buffering device, guarding against excessive plasma volume expansion in face of an increased total extracellular fluid volume. ANF is also a physiological modulator of GFR, and mediates nephron hyperfiltration and natriuresis when salt excretion is threatened by a reduction in the number of nephrons. Guanylyl cyclase (GC_A) receptors mediate the effects of ANF by generating cGMP. Clearance receptors remove ANF from the circulation by receptor-mediated endocytosis, and serve as a hormone buffer system to impede large inappropriate fluctuations in plasma levels of ANF. The specific structure-function-dynamics relationships of these receptors serve to modulate the role of ANF in pressure-volume homeostasis.

In 1981 de Bold et al published the decisive experiment demonstrating that administration of a crude acid extract of rat atria to anesthetized rats led to a powerful natriuretic response, sodium excretions increasing more than fortyfold above baseline levels, whereas a similarly prepared ventricular extract was without effect [1]. The natriuretic substance(s) was named atrial natriuretic factor (ANF). Our studies in the isolated perfused rat kidney demonstrated that the natriuretic effect of atrial extract is due in part to its direct actions on the kidney. Atrial extract increased GFR, constricted efferent arterioles, and had unique renal vascular action, acting as a powerful antagonist of vasoconstriction with a small agonist (vasoconstrictive) action of its own [2]. Studies with atrial extract were short lived because by the end of 1983 and early 1984 several laboratories, including our own, purified ANF to completion, and determined its chemical structure [3]. Since then there has been a veritable explosion of research in the field, with more than 6,000 articles published to date. In this article I will briefly describe the main properties and the functions of ANF and its receptors in effecting volume regulation. For an inclusive recent review on ANF and its receptors, with extensive literature citations, the reader is directed to [3].

The chemical nature of ANF

As shown in Figure 1, ANF is a polypeptide hormone of 28 amino acids (ANF28) with a core sequence between disulfide linked cysteines, and a C terminal extension that confers biological activity to the peptide [3]. Among different mammalian species, ANF differs by a single amino acid in position 12, such as isoleucine in the rat, methionine in humans [3]. ANF is a member of an ever growing family of so-called natriuretic peptides with a conserved central core and variable N and C terminal sequences (Fig. 1). Brain natriuretic peptide (BNP), in spite of its name, is present at the highest concentration in atrial and ventricular muscle. In normal conditions, the concentrations of BNP in heart tissue and plasma are far lower that those of ANF. However, in ventricular hypertrophies the expression of this peptide increases and may reach levels similar to those of ANF. BNP shares the same receptors and have the same effects as ANF when administered to experimental animals. C-type natriuretic peptide (CNP) is present mostly in brain and in endothelial cells, and in spite of its name, it does not have significant natriuretic activity in mammals. ANF 32, better known as urodilatin, has been detected in urine and distal tubular cells. When administered to mammals, urodilantin effects are undistinguishable from those of ANF. The physiological roles of BNP, CNP and urodilatin are essentially unknown, and these peptides will not be further considered in this short review.

The main effects of ANF in the mammalian organism

Figure 2 schematically illustrates the main effects of ANF in mammalian organisms. The stimulus for the secretion of ANF is atrial stretch or pressure. Thus, increases in plasma volume, and systemic and pulmonary pressures are the physiologic stimuli for ANF secretion. The main actions of ANF are: (i) direct and indirect effects on the kidney to alter renal hemodynamics, and to increase fluid and electrolyte excretion; (ii) functional antagonism of the renin-angiotensin-aldosterone system by inhibiting synthesis and/or release of renin and aldosterone, and by antagonizing all known effects of angiotensin; (iii) functional antagonism to all modalities of humoral or autonomic induced vasoconstriction; and (iv) shift of fluid from the intravascular to the interstitial compartment by increasing capillary hydraulic permeability. It is not difficult to surmise that these combined effects of ANF are essentially geared to regulate pressure-volume homeostasis.

Renal actions of ANF

Figure 3 schematically illustrates the several nephron sites that are targets for ANF effects (a detailed review and specific references are in [3]). ANF increases single nephron (SN) GFR in proportion of total GFR. The increase in GFR is due mainly to an increase in glomerular capillary hydrostatic pressure that results

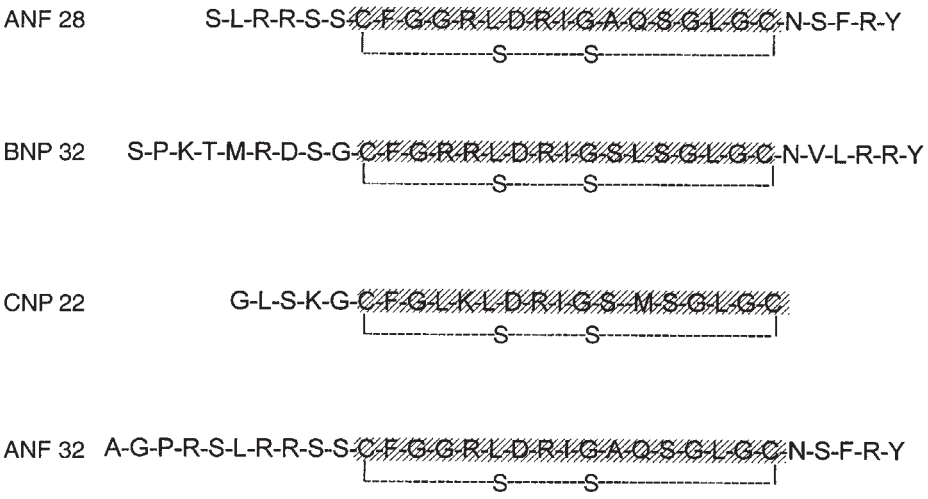

Fig. 1. *Amino acid sequence of ANF (ANF 28) and other members of the natriuretic peptide family.* The peptides have a conserved central core between disulfide linked cysteines (shaded) and variable C and N terminals. See text for details.

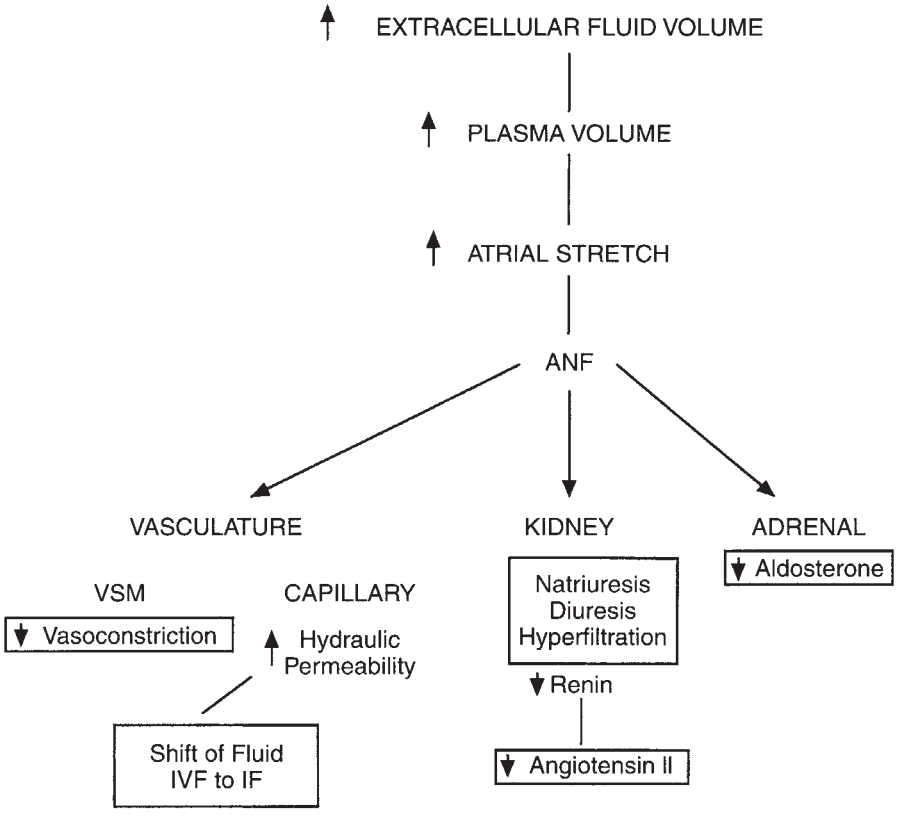

Fig. 2. *The main actions of ANF in the mammalian organism.* See text for description. Abbreviations are: VSM, vascular smooth muscle; IVF, intravascular fluid volume; IF, interstitial fluid volume.

from efferent arteriolar constriction and afferent arteriolar dilation. In normal conditions, ANF does not alter or even slightly decreases SN and total renal blood flow (RBF). ANF is the only known endogenous substance that may increase GFR in face of a decrease in blood pressure, and an unchanged or even decreased RBF. In vasoconstricted kidneys, however, ANF markedly increases RBF due to its generic vasorelaxant property (see below).

ANF markedly increases the load of sodium to the base of the inner medullary collecting duct (IMCD), an effect that is essential for a robust natriuretic response, and subsequently disrupt load-reabsorption balance in this nephron segment. The reasons for the increase in sodium load to the IMDC are multiple, and include: (i) increase in GFR; (ii) decrease in inner medullary hypertonicity that decreases passive fluid efflux from the thin limbs of Henle's loop; (iii) direct tubular effects of ANF that decrease sodium reabsorption in nephron segments proximal to the IMDC. The disruption of load-reabsorption balance in the IMDC also has multiple causes, including a decrease in sodium

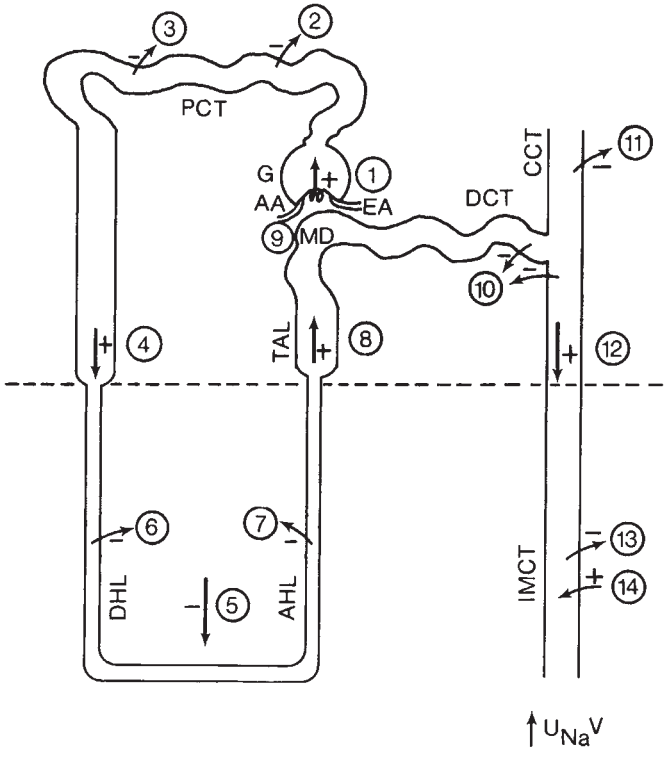

Fig. 3. *Postulated nephron sites of action of ANF.* 1. Increase in GFR by constriction of efferent arterioles (EA) and dilation of afferent arterioles (AA). 2. Inhibition of Na reabsorption in deeper nephron proximal convoluted tubules (PCT). 3. Inhibition of angiotensin-stimulated Na reabsorption. 4. Increase in sodium load to descending limb of Henle's loop (DHL). 5. Decrease in hypertonicity and increase in pressure in inner medullary interstitium. 6. Decrease in passive water efflux from DHL. 7. Decrease in passive Na reabsorption from thin ascending limb (AHL). 8. Increase in Na load to thick ascending limb (TAL) and macula densa (MD). 9. Inhibition of renin secretion. 10. Inhibition of sodium reabsorption in distal tubule (DCT) and cortical collecting duct (CCT) by ANF-induced inhibition of aldosterone secretion. 11. Inhibition of thiazide-sensitive NaCl reabsorption. 12. Increase in Na load to the inner medullary collecting duct (IMDC). 13. Inhibition of amiloride-sensitive Na reabsorption. 14. Increase in Na influx by stimulation of Na-K-2Cl cotransport and passive forces. Reproduced with permission from Oxford University Press [3].

efflux by inhibition of an amiloride-sensitive cation channel, and an increase in sodium influx by stimulation of a basolateral furosemide-sensitive Na^+-K^+-$2Cl^-$ cotransporter, and by an increase in pressures in the inner medullary interstitium. Because ANF is a powerful inhibitor of the renin-angiotensin-aldosterone system, it will indirectly act on tubular sites that are targets of this system, including proximal tubular sites (angiotensin) and distal nephron sites (aldosterone).

Although the relative contribution of each of the nephron actions of ANF to its final natriuretic action remains to be elucidated, it is clear that hemodynamic factors, including but not limited to the increase in GFR, are paramount for a robust natriuretic response. We and others have demonstrated that when renal hemodynamic effects are precluded by early and late renal clamp experiments in dogs or rats, ANF fails to elicit an important increase in sodium excretion [3]. Abnormal renal hemodynamics explain to a great degree, if not totally, the resistance of the kidney to the natriuretic effect of endogenous or administered ANF in some pathological cases of volume retention such as congestive heart failure or the nephrotic syndrome.

ANF and the renin-angiotensin-aldosterone system

ANF infusion markedly inhibits renin secretion by the kidney and aldosterone secretion by the adrenal, resulting in a decrease in plasma levels of these antinatriuretic hormones [4, 5]. The decrease in plasma aldosterone is due to the decrease in plasma renin activity, and to a direct effect of ANF on adrenal zonal glomerulosa to inhibit aldosterone synthesis. The most likely explanation for the decrease in renin secretion is the increase in sodium load to the macula densa, as ANF fails to decrease renin when the increase load to the macula densa is impeded, such as in renal clamp experiments [3]. ANF also antagonizes all of the known effects of angiotensin II, including its peripheral vasoconstrictive effect, its growth promoting activity in vascular smooth muscle cells, its stimulation of proximal sodium fluid reabsorption, and its central dipsinogenic effect [3]. These antagonistic effects are likely due to the ANF-induced decrease in cytosolic calcium that, at least in vascular smooth muscle cells, is due to cGMP-dependent stimulation of sarcolemmal Ca^{2+} ATPase activity.

The physiological importance of the inhibition of the renin-angiotensin-aldosterone system for the overall role of ANF in volume control has been suggested by results of a dose-effect study in humans [3]. In this study, ANF infusion at rates that barely increased plasma levels of the hormone, and led to only slight, if any, increase in fluid and electrolyte excretion, markedly decreased plasma renin activity and plasma aldosterone levels. Maximal inhibition was obtained at lower doses than those needed for a maximal natriuretic and diuretic effect.

ANF and cardiovascular hemodynamics

The effects of ANF on cardiovascular hemodynamics are complex and depend on the status of cardio-circulatory parameters. Administration of ANF decreases blood pressure slightly but consistently in normotensive experimental animals and humans, and markedly in several models of hypertension in laboratory animals [3]. In normotension, ANF decreases blood pressure by decreasing cardiac output (CO), while calculated total peripheral resistance remains unaffected [6]. ANF decreases CO by lowering plasma volume and central venous pressure, the decrease in plasma volume being due to a shift of fluid from the intravascular to the interstitial compartment (see below) [5, 7]. Similarly, in experimental models of volume-dependent hypertension (such as DOC-salt hypertension in rats), the ANF-induced decrease in blood pressure is due mainly to a decrease in CO. However, in renin-dependent models of hypertension (such as 2K-1C, Goldblatt hypertension in rats), ANF decreases blood pressure mainly by counteracting the vasoconstriction, and CO may be even slightly increased due to the relieve in afterload [3]. From the studies referred to above, and from our earlier studies in the isolated perfused rat kidney [2], it is clear that ANF is not a vasodilator in a strict sense, but an antagonist of vasoconstriction. This property, together with the effect of ANF on the redistribution of extracellular fluid volume (see below), explains to a great extent the complex and sometimes apparently contradictory effect of ANF on cardiovascular dynamics.

ANF and the distribution of extracellular fluid volume

In experiments in anesthetized dogs in 1984, we demonstrated that after the administration of ANF, hematocrit rose in spite of religious replacement of external fluid losses. We postulated then that ANF may shift fluid from the intravascular to the interstitial compartment [5]. The definitive proof of this unique effect of ANF came from experiments in nephrectomized rats, in which administration of ANF leads to a significant decrease in blood pressure, an increase in hematocrit and a corresponding decrease in plasma volume. Sodium nitroprusside, at doses that decrease blood pressure to the same degree as ANF, did not cause a change in hematocrit or plasma volume, demonstrating that these effects of ANF were not due to a redistribution of fluid induced by the decrease in blood pressure [7]. These findings were confirmed by several groups of investigators, and subsequent experiments in isolated mesenteric capillaries demonstrated conclusively that ANF markedly increases capillary hydraulic permeability leading to an efflux of fluid without changes in net ultrafiltration forces [8]. In the lung, ANF may be having a protective effect against the edema because an increase in hydraulic permeability in the low pressure lung capillaries will favor absorption rather than filtration of fluid.

The physiological importance of this unique effect of redistributing extracellular fluid volume is illustrated by the results of a very recent study using an ANF knockout mouse model. In this study heterozygous animals, which have approximately one-half the plasma concentration of ANF compared to normal mice, already had a significantly decreased hematocrit, in spite of normal salt excretion. The hematocrit was further decreased in homozygous animals with no detectable ANF in plasma [9].

Role of ANF in adaptive increases in GFR and sodium excretion

Until recently, in spite of the compelling evidence partially described above, the physiological role of ANF as a modulator of GFR, and the participation of the hormone in volume homeostasis has been questioned. Recently, Matsuda and Morishita developed a specific non-peptidic GC receptor antagonist with which to reinvestigate these fundamental issues. The antagonist named HS-142-1, competes with ANF for binding to the guanylyl cyclase (GC) receptors, and does not affect ANF binding to clearance receptors. HS-142-1 does not have any known action of its own, and antagonizes all known cardiovascular, renal and adrenal effects of the hormone [10].

In conscious and anesthetized rats fed a normal or a high salt diet, but not a low salt diet, administration of HS-142-1 significantly decreases GFR, demonstrating that ANF is necessary to maintain a normal level of glomerular filtration [11, 12]. The lack of effect of HS-142-1 on GFR in rats fed a low salt diet, an experimental condition characterized by very low plasma levels of ANF, further attests to the specificity of the GC receptor antagonist in blocking the GFR modulatory role of this hormone [12]. Moreover, HS-142-1 blocked the hyperfiltration in the remnant kidney after ⅘ nephrectomy, and the hyperfiltration in streptotozin-induced diabetes in rats, demonstrating that ANF plays an important role in the adaptive increases in GFR in compensatory glomerular hypertrophy [12, 13].

Use of HS-142-1 and of the ANF knockout mouse model also furthered our understanding of the role of ANF in the adaptation to experimentally-induced volume expansion and high salt intake. Thus, HS-142-1 markedly blunted the natriuresis of acute saline expansion, and the natriuresis of mineracorticoid escape phenomenon [10, 14]. Moreover, in the homozygous ANF knockout mouse, with no detectable plasma levels of ANF, adaptation to a high salt diet was impaired, resulting in "salt-sensitive" hypertension [9].

The recent studies described above provide unequivocal evidence that ANF is significantly involved in the physiological modulation of GFR, and that ANF has a significant role in the natriuretic response to acute volume expansion. Perhaps more important, these studies show that ANF is not simply an "emergency" hormone involved in the response to acute volume overload, as has been assumed by some investigators, but that it has a significant role in the adaptation to chronic volume expansion. Furthermore, ANF is significantly involved in the adaptations of renal functions, including maintenance of total GFR, when adequate salt excretion is threatened by a loss in the number of functioning nephrons.

Receptor functions and dynamics modulate ANF actions

There are two biochemically and functionally distinct classes of ANF receptors whose structure-function-dynamics characteristics, schematically represented in Figure 4, effect the role of ANF in pressure-volume homeostasis [3, 15, 16]. The cytoplasmic domain of GC receptors contains a guanylyl cyclase sequence and a "kinase-like" sequence that modulate the activity of guanylyl cyclase [16]. Thus the GC receptor is unique as it contains in a single molecule the acceptor (ligand binding sites), effector (guanylyl cyclase), and modulator (kinase-like domain) functions. The GC receptor is a bonafide membrane resident protein that does not undergo rapid endocytosis. When GC receptors are unoccupied, the kinase-like domain represses the activity of guanylyl cyclase. ANF binding derepress the guanylyl cyclase moiety, leading to the generation of cGMP, the main if not sole messenger of all known actions of ANF [16]. This event is accompanied by loss of receptor affinity, resulting in a very rapid receptor-ligand dissociation [17]. Thus, GC receptors function in a "staccato" mode with very rapid ligand on and off rates. The rapid association of ANF with GC receptors, together with the presence of the acceptor-effector-modulator functions in a single receptor molecule, allows for immediate and powerful responses upon increases in plasma levels of ANF. The extremely rapid receptor-ligand dissociation leads to the prompt termination of responses when plasma levels of ANF fall.

Plasma levels of ANF are determined by the balance between its rate of secretion by the atria, and its metabolic clearance rate at peripheral tissues. A specific class of ANF receptors, appropriately named clearance (C) receptors, is responsible for the fast metabolic clearance and very short plasma half-life (1 to 3 min) of the hormone [18, 19]. Structurally, C receptors have a very short cytoplasmic domain (37 amino acids), a characteristic feature of all clearance and/or transport receptors described to date [15]. C receptors do not mediate any of the known cardiovascular, renal and adrenal effects of ANF described in this review. Accordingly, *in vivo* blockade of C receptors by specific ligands decreases the metabolic clearance of endogenous ANF resulting in elevated plasma levels of the hormone [15, 18]. The mechanism of the clearance function of C receptors is by receptor-mediated endocytosis and delivery of ANF to lysosomes where it is hydrolyzed to

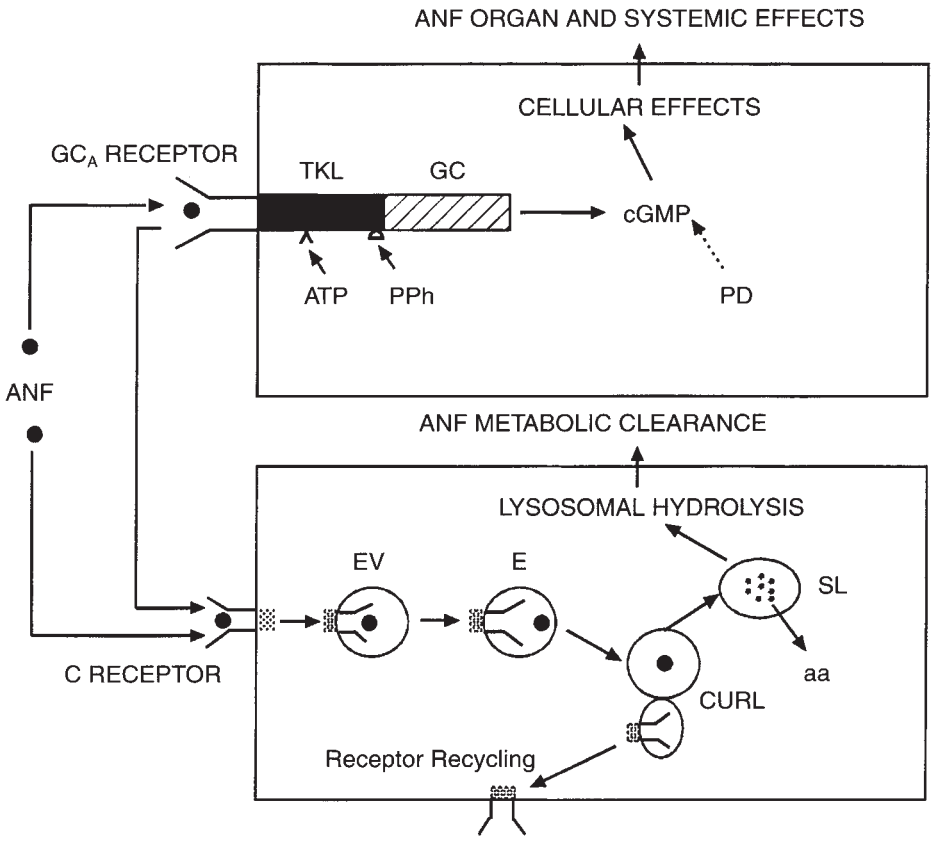

Fig. 4. *Schematic representation of the structure, functions, and dynamics of the two classes of ANF receptors.* See text for description. Abbreviations are: GC_A, guanylyl cyclase type A receptor; TKL, kinase-like domain; GC, guanylyl cyclase domain; PPh, protein phosphatase(s); PD, phosphodiesterase(s); C, clearance receptor; EV, endocytic vesicle; E, endosome; CURL, sorting endosome; SL, secondary lysosome; aa, amino acids.

completion, while the internalized receptors are recycled to the cell surface (Fig. 4) [20]. ANF dissociates very slowly from surface C receptors, resulting in sufficient resident time for the endocytosis of receptor-ligand complexes [15]. Thus, C receptors function in a "continuo" mode, continuously delivering ANF to lysosomes, and returning to the cell surface to mediate additional cycles of removal of ANF from the circulation. In this manner, C receptors act as a hormone buffer system to impede large and inappropriate plasma fluctuations of ANF, to rapidly bring plasma levels of ANF to basal levels once the stimulus for its secretion ceases, and to dispose of ANF molecules dissociated from GC_A receptors. The combination of the "staccato" and "continuo" modes of GC and C receptor functions, respectively, allows for a precise modulation of the role of ANF in pressure-volume homeostasis.

The two arms of the participation of ANF in volume control

Under normal conditions, the role of ANF in the regulation of extracellular fluid and plasma volume is brought about by the two arms of its action. On one hand, ANF increases renal sodium excretion by its direct actions of the kidney, and by inhibiting secretion and end-organ effects of antinatriuretic hormones, particularly angiotensin and aldosterone. On the other hand, ANF decreases plasma volume by shifting fluid from the vascular compartment. This action helps to relieve—albeit incompletely—the increase in plasma volume brought about by an increase in total salt content and extracellular fluid volume. In the presence of enhanced antinatriuretic impulses, and abnormal renal hemodynamics, such as in congestive heart failure or in the nephrotic syndrome, the excretory arm of ANF's role is markedly blunted but its buffering effect on plasma volume may remain intact. In the last instance, the major physiological role of ANF is to defend against excessive plasma volume expansion, even at the cost of a relative expansion of the interstitial fluid volume.

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