Functional Properties and Dynamics of Natriuretic Peptide Receptors (44043)

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Since the discovery of atrial natriuretic factor (ANF or ANP) in 1981 by De Bold *et al.* (1), more than 6500 articles on the subject have appeared in the literature. For an extensive review on ANP and its receptors, the reader is directed to Reference 2. In this short review, we will consider in a noninclusive manner the dynamics and functional properties of natriuretic peptide receptors. ANP is a 28–amino acid polypeptide hormone secreted mainly by the heart atria in response to atrial stretch that results from an increase in central venous pressure, or systemic and pulmonary hypertension. ANP is a member of a family of natriuretic peptides, which include B-type or brain natriuretic peptide (BNP), C-type natriuretic peptide (CNP), and urodilatin. The physiological role of BNP, CNP, and urodilatin remains essentially unknown.

The main actions of ANP in the mammalian organism are geared toward the regulation of volume-pressure homeostasis (2, 3). In the kidney, ANP exerts hemodynamic and tubular actions that lead to diuresis and natriuresis. In the cardiovascular system, ANP acts as a strong antagonist of vasoconstriction and also decreases plasma volume, a combination of events that reduces blood pressure. The decrease in plasma volume is due to a shift of fluid from the intravascular to the interstitial compartment brought about by an increase in hydraulic permeability of systemic capillaries. ANP is also a powerful functional antagonist of the renin-angiotensin-aldosterone system through inhibiting renin secretion by the kidney, aldosterone synthesis by the adrenal, and by antagonizing all known peripheral actions of angiotensin II. The inhibition of the activity and effects of renin-angiotensin-aldosterone contributes substantially to the cardiovascular effects of ANP. Several *in vivo* studies in the past 8 years, including studies on inhibition of ANP metabolism by blockade of C receptors, administration of specific guanylyl cyclase receptor antagonists, and, more recently, studies on gene deletion of ANP and guanylyl cyclase type A receptors in mice, conclusively demonstrated that ANP, through guanylyl cyclase type A receptors, play a major role in pressure-volume homeostasis (2–5).

There are at least two biochemically and functionally distinct classes of natriuretic peptide (NP) receptors, which are schematically depicted in Figure 1. Clearance (C) receptors are by far the most abundant class of NP receptors, and in some cells and tissues (e.g., cultured vascular smooth muscle cells, cultured fibroblast, lung, and kidney tissues) amount to more than 95% of the total population of NP receptors (2, 6, 7). The major known function of these receptors is to remove ANP and possibly other NP from the circulation. In this manner C receptors contribute to the plasma homeostasis of the hormone. Guanylyl cyclase (GC) receptors are signaling receptors that mediate all known cardiovascular and renal effects of NP in the mammalian organism *via* the generation of cGMP (8). GC-A is the physiological receptor for ANP and probably also for BNP and urodilantin, whereas GC-B is the probable physiological receptor for CNP (9).

Functional Properties and Dynamics of Clearance Receptors of Natriuretic Peptides

C receptors were the first of the NP receptors to be biochemically characterized (10, 11). They are composed of a unit containing 496 amino acids and are present in the cell membrane mostly in the form of homodimers of ~120 kDa, with a small proportion of ~60-kDa monomers. C receptors have a putative single membrane spanning domain, a large extracellular domain that binds with high affinity all members of the NP family, as well as truncated synthetic analogs of ANP (2, 6, 7, 10, 12). The extracellular domain of C receptors has considerable homology (33%) with that of GC-A receptors but no significant amino acid sequence homology with other proteins (10). The outstanding structural feature of C receptors is its very

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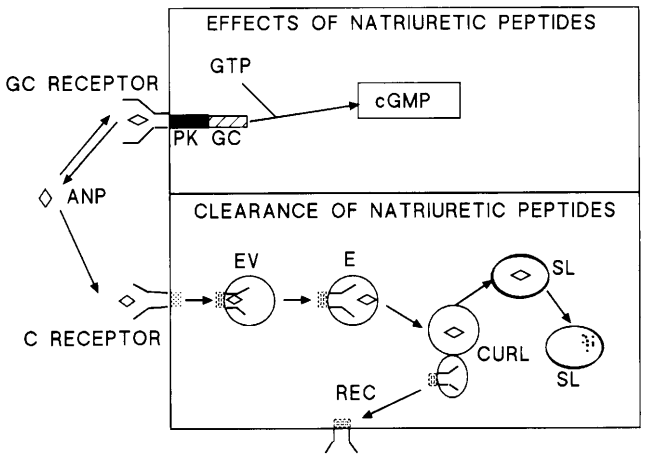

Figure 1. The two main classes of natriuretic peptide receptors. Guanylyl cyclase (GC) receptors mediate the cellular, organ, and systemic effects of natriuretic peptides. GC-A receptors are the physiological receptors for ANP, and possibly for BNP, whereas GC-B receptors are the likely physiologic receptors for CNP. GC receptors are composed of three domains: an extracellular binding domain, an intracellular domain with guanylyl cyclase activity (GC), and a domain interposed between the single transmembrane span and GC (PK) that has homology with protein kinases and modulates the activity of GC. GC receptors mediate the cellular, organ, and systemic effects of natriuretic peptides by the generation of cGMP upon ligand binding. The GC-A receptor does not undergo endocytosis, and the response is terminated by the rapid dissociation of receptor-ligand complexes. Clearance (C) receptors have as a major function the removal of natriuretic peptides from the circulation. C receptors have a large extracellular binding domain and a very short cytoplasmic tail of only 37 amino acids. Receptor-mediated endocytosis is the mechanism by which C receptors exert their clearance function. Receptor-ligand complexes are endocytosed *via* chlatrin-coated pits and segregated in endocytic vesicles (EV) and endosomes (E). In the acidic environment of E, receptor-ligand complexes are dissociated and then are sorted in vesicles named sorting endosomes or compartment for uncoupling receptor and ligand (CURL). The ligand (ANP) is directed to lysosomes (SL), where it is hydrolyzed to its constitutive amino acids (represented as dots within SL), which are then returned to the medium. The unoccupied C receptor is recycled to the cell membrane (REC), where it can mediate additional cycles of internalization. See text for more detailed description and for literature references.

short cytoplasmic domain of 37 amino acids. This characteristic is common to all known clearance and/or transport receptors, whereas classic biological signaling receptors usually have very large cytoplasmic domains (2, 6, 7).

C receptors bind with high affinity all members of the NP family, as well as truncated synthetic analogs of NP with as little as five amino acids of their ring structure (2, 6, 7, 13). The minimal amino acid sequence for high-affinity binding to C receptors is Arg-Ile-Asp-Arg-Ile-NH_2 (13). The clearance function of C receptors was discovered in our laboratory in 1987 in experiments in functioning isolated perfused rat kidneys and in intact rats (14). In the isolated kidney, a truncated synthetic analog of ANP, C-ANF_{4-23}, specifically bound with high affinity to >90% of the binding sites of ANP in total kidney tissue and kidney cortex. In spite of its high affinity for ANP receptors, C-ANF_{4-23} did not have effects on its own and did not alter the dose-response of ANF_{1-28} on any of the measured renal function parameters, including glomerular filtration rate, fluid and electrolyte excretion, and vasorelaxation of pre-constricted kidneys. This led us to conclude that C-ANF_{4-23} interacted with a class of receptors that did not mediate any of the known renal and vascular effects of ANP. When administered to intact rats, C-ANF_{4-23} led to an increase in plasma levels of endogenous immunoreactive ANP, which in turn resulted in natriuretic and blood pressure lowering effects that followed pari-passu the rise and fall in plasma levels of endogenous ANF. This led us to postulate that the receptors occupied by C-ANF_{4-23} have as a major function the removal of ANP from the circulation, and, accordingly, we named them clearance receptors (14, 15).

Figure 2 shows the effects of an infusion of C-ANF_{4-23} in anesthetized rats on plasma levels of endogenous immunoreactive ANP, renal functions, and blood pressure. As can be seen, the effects of the infusion of C-ANF_{4-23} were undistinguishable from those of the infusion of ANF_{1-28} that raised plasma levels of ANP to the same extent as that obtained with C-ANF_{4-23}. In addition to supporting the postulate that the receptors occupied by C-ANF_{4-23} have a clearance function, the results shown in Figure 2 have two major implications. First, they show that small increases in plasma levels of endogenous ANP are able to elicit appropriate renal and cardiovascular responses for the maintenance of volume-pressure homeostasis. Second, they open the potential therapeutic possibility of using C receptor ligands to increase plasma levels of endogenous ANP.

Based on results of *in vitro* studies in isolated membranes, other investigators have postulated that C receptors, or a subtype of C receptors, may also have a signaling activity, by inhibiting adenylyl cyclase *via* activation of a pertussis-sensitive Gi protein, and/or by increasing phospholipase C and phosphoinositite turnover (16, 17). The physiological role of this signaling, if present, is unknown. Whatever the case, there is compelling evidence to indicate that C receptors do not mediate any of the known renal, adrenal, and cardiovascular effects of natriuretic peptides. In addition to our studies described above, more recent work with the GC receptor antagonist HS-142-1 (reviewed in Ref. 3) and, more important, with a mouse model with genetic deletion of GC-A receptors (4) demonstrates conclusively that GC-A receptors fully account for mediation of the physiologic responses to ANP that modulate volume-pressure homeostasis.

Definite proof that C receptors have a major clearance function at the systemic level came from pharmacokinetics experiments in the rat demonstrating that C-ANF_{4-23}, as well as other specific ligands of C

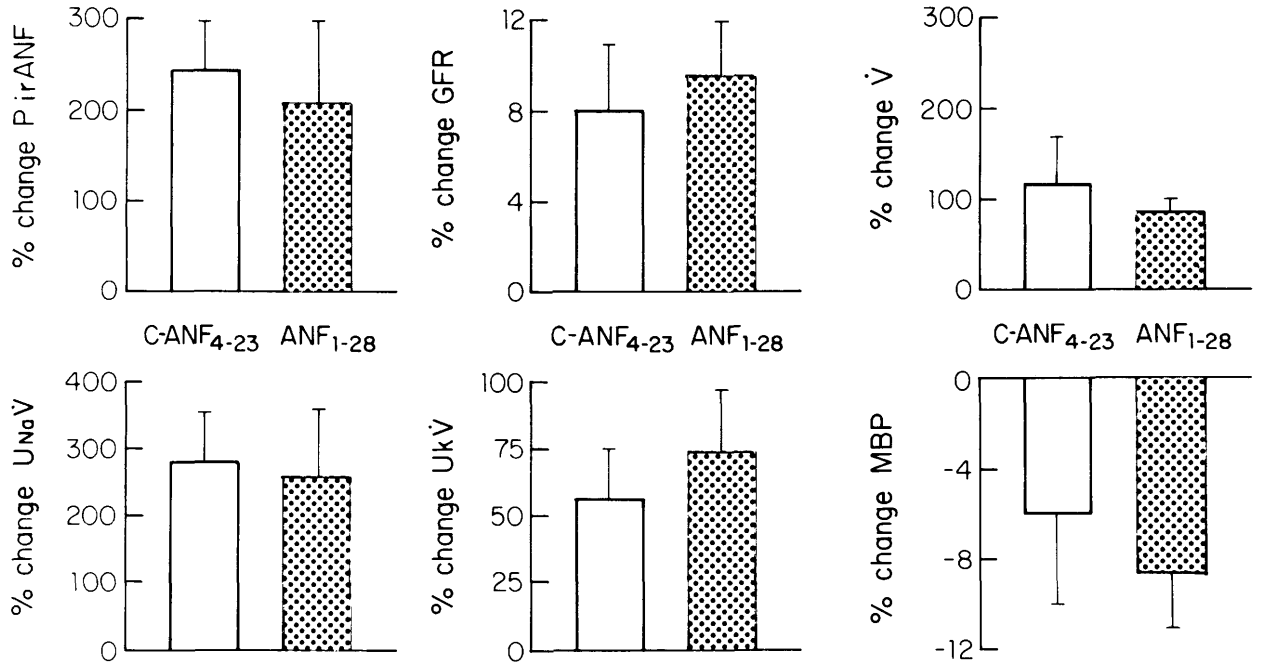

Figure 2. Comparative renal and blood pressure-lowering effects of similar increases in plasma levels of immunoreactive ANF by infusion of a C receptor ligand (C-ANF_{4-23}) or ANF_{1-28} in anesthetized rats. Infusion of 1 μg/min/kg body wt of the specific C receptor ligand C-ANF_{4-23} increased plasma levels of endogenous ANF by ~250%, an increase that is due to a decrease in the metabolic clearance of endogenous ANF (see Fig. 3). Rat ANF_{1-28} was infused (0.02–0.03 μg/min kg body wt) to increase plasma levels of immunoreactive ANF (P irANP) to levels similar to those obtained by C-ANF_{4-23}. In these conditions, C-ANF_{4-23} and ANF_{1-28} slightly but significantly decreased mean atrial blood pressure (MPB) and markedly increased glomerular filtration rate (GFR), urine flow rate ($\dot{V}$), sodium excretion ($U_{Na}\dot{V}$), and potassium excretion ($U_k\dot{V}$). There were no significant quantitative differences between the effects of C-ANF_{4-23} and ANF_{1-28}. Based on data in Ref. 14.

receptors that do not bind to GC receptors, markedly decreases the metabolic clearance of ANP (18, 19). ANP and other natriuretic peptides are also metabolic substrates for neutral endopeptidase (NEP) (20). Under normal conditions, this enzyme has a relatively minor role in the metabolic clearance of ANP. However, in conditions in which a large fraction of C receptors are occupied by high plasma levels of endogenous ANP or by the administration of synthetic ANP or C receptor ligands, NEP contributes importantly to the metabolism of ANP (19, 21). Thus, as shown in Figure 3, administration of C-ANP_{11-15}, a specific C receptor ligand, markedly decreases the metabolic clearance (MCR) of ANP to approximately one-third its control value and slightly increases plasma half-life ($t_{1/2}$). The NEP inhibitor phosphoramidon alone has no significant effect on MCR or $t_{1/2}$ of ANP. However, phosphoramidon has a major synergistic effect when C receptors are blocked by C-ANP_{11-15}, further decreasing MCR to approximately one-fifth its control value, and increasing $t_{1/2}$ by approximately 3-fold. From these results, it may be postulated that the most effective manner of therapeutically increasing plasma levels of endogenous ANP is by the combined administration of a C receptor blocker and a NEP inhibitor.

Cellular Mechanisms of the Clearance Function of C Receptors. Studies in our laboratory in cultured bovine vascular smooth muscle cells demonstrated that the clearance function of C receptors is the result of receptor-mediated endocytosis of ANP, with a subsequent lysosomal hydrolysis of ANP, and rapid and efficient recycling of internalized C receptors to the cell surface (22). More recently, we found that the endocytic rate of cloned wild-type human kidney C receptors stably transfected into cultured Chinese hamster ovary (CHO) cells was similar to that described for native C receptors in vascular smooth muscle cells (23). Thus, the cellular mechanisms of the clearance function of C receptors depend on the properties of the receptor rather than of a specific cell type in which the receptor is expressed.

Figure 4 shows the results of a typical chase experiment in recombinant CHO cells transfected with wild-type human C receptors. The results are shown together with a schematic representation of the cellular pathways of receptor-ligand internalization, lysosomal hydrolysis of ANP, and recycling of C receptors to the cell surface. C receptors internalize at a rate of 5%–7% of occupied receptors per minute. This process is constitutive in nature and does not depend on ligand binding, a property that is also shared by other clearance and/or transport receptors (6, 22). The process of internalization is completely blocked by hypertonic sucrose, a maneuver known to disrupt endocytosis *via* chlatrin-coated pits (23). Internalized receptor-ligand complexes are dissociated in the acidic

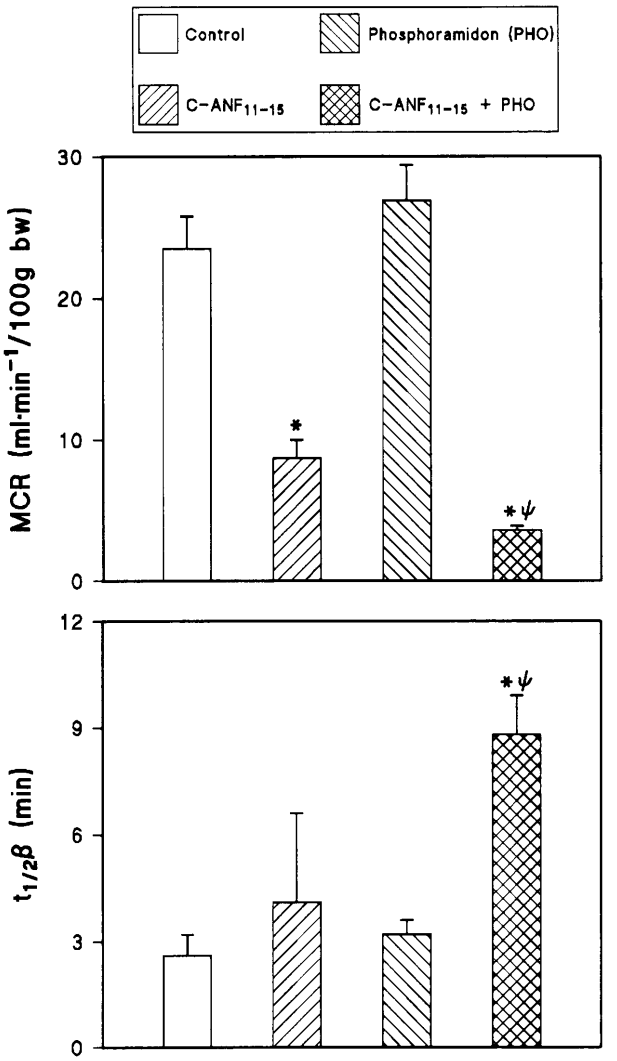

Figure 3. Synergistic role of clearance receptor and neutral endopeptidase in the metabolic clearance and plasma half-life of ANP. The metabolic clearance (MCR) and plasma elimination half-life ($t_{1/2}\beta$) of administered ^{125}I-ANP$_{1-28}$ were determined in anesthetized rats in control conditions, or in conditions of blockade of C receptors by C-ANP)$_{11-15}$ alone, inhibition of neutral endopeptidase (NEP) alone by phosphoramidon, and a combination of administration of C-ANP$_{11-15}$ and phosphoramidon. Blockade of C receptors alone by C-ANP$_{11-15}$ leads to a major decrease in MCR and a slight increase in $t_{1/2}\beta$. Inhibition of NEP alone by phosphoramidon has no effect on MCR and $t_{1/2}\beta$. Addition of NEP inhibition to C receptor blockade produced a major synergistic effect, further decreasing MCR to approximately one-fifth its control value, and increasing $t_{1/2}\beta$ by ~3-fold. The data show that under normal conditions C receptors have a major role in removing ANP from the circulation. While NEP has little effect in this process, it markedly potentiates the effects of C receptors when they are nearly saturated. *$P < 0.01$ versus control; $\Psi P < 0.01$ versus C-ANP$_{11-15}$ or phosphoramidon alone. Based on data in Ref. 19.

environment of endosomes, and dissociated ANP is directed toward lysosomes, where it is metabolized to its constituent amino acids, which are then returned to the medium. This process can be completely blocked by lysosomotropic weak bases such as NH_4Cl (22, 23). Internalized dissociated receptors are rapidly and efficiently recycled to the cell surface, where they may mediate additional cycles of ANP removal from the medium. Consequently, there is no detectable homologous downregulation of surface C receptors even upon prolonged exposure of the cells to saturating concentrations of ANP (22, 23). This attribute greatly contributes for the efficient role of ANP receptors in removing natriuretic peptides from the circulation.

Molecular Mechanisms of the Clearance Function of C Receptors. Recently we undertook a detailed study on the molecular determinants of the clearance function of C receptors using recombinant CHO cells stably transfected with wild-type or mutated human kidney C receptors (23). The top panel of Figure 5 depicts schematically the amino acid sequence of the juxtamembrane regions of the C receptor, and the mutations that were performed in these regions. The bottom panel of Figure 5 shows the effects of the mutations on the net endocytic rate of the

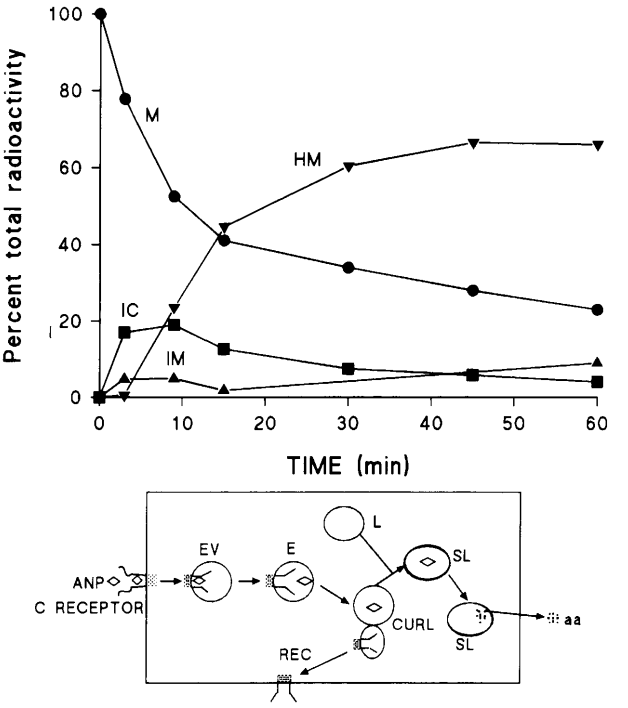

Figure 4. Clearance receptor-mediated endocytosis and lysosomal hydrolysis of ANP. (top) ^{125}I-ANP$_{1-28}$ was loaded onto surface C receptors at 4°C in monolayers of recombinant CHO cells stably transfected with human kidney C receptors. Then, the monolayers were transferred to 37°C, and at several time intervals the amount of radioligand remaining in the membrane (M), taken up in the intracellular compartment (IC), metabolized and released to the medium (HM), and released intact to the medium (IM) was determined. The results show that the radioligand bound to C receptors is rapidly internalized and then undergoes hydrolysis to its constitutive amino acids which are then released to the medium. There is minimal dissociation of surface receptor-ligand complexes during the period of incubation. The hydrolysis of radioligand is completely blocked by 10 m*M* NH_4Cl, demonstrating that it takes place in lysosomes (not shown). (bottom) schematic illustration of the receptor-mediated endocytic process and lysosomal hydrolysis of ANP. EV, endocytic vesicle; E, endosome, CURL, sorting endosome; L, lysosome; SL, secondary lysosome; aa, amino acids. Based on data in Ref. 23.

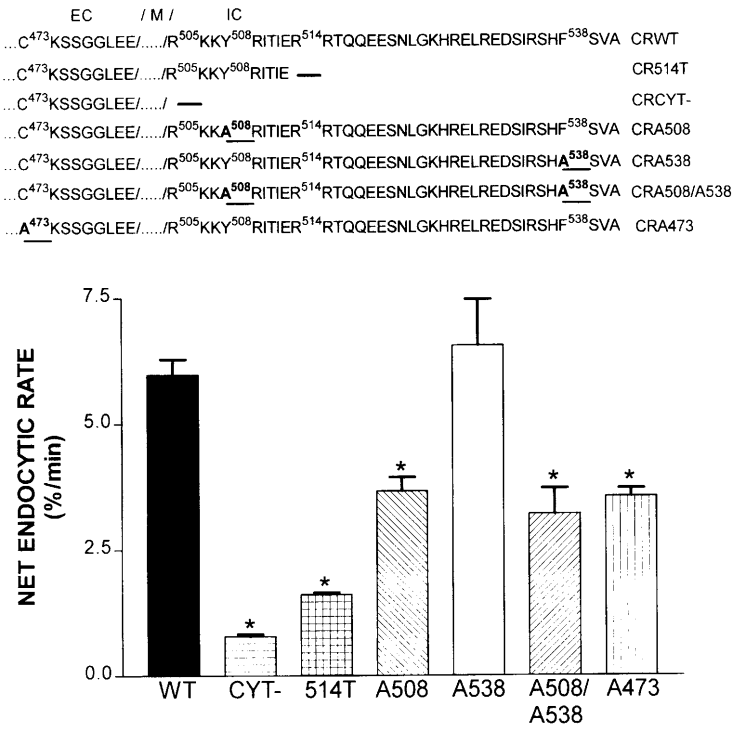

Figure 5. Molecular determinants of the clearance function of C receptors. Wild-type (WT) and mutated human kidney C receptors were stably transfected into CHO cells. Net endocytic rates of C receptor-ANP complexes were determined as described in Ref. 23. (top) Juxtamembrane domains of cloned wild-type and mutated C receptors. EC, extracellular domain, M, transmembrane domain, IC, cytoplasmic domain. Amino acids are designated by single letters, mutations heavy lines (deletions) or underlines (point mutations). (bottom) Net endocytic rates of the wild-type and mutated receptors. Total deletion of the cytoplasmic domain (CYT-) practically abolished endocytosis, whereas large deletion of the cytoplasmic portion (514T) reduced endocytic rate to approximately one-fourth that in WT. Within the cytoplasmic domain substitution of Y508 by A, decreased endocytic rate by ~50%, whereas substitution of the other aromatic amino acid F538 by A had no effect. The double mutant A508/A538 had an endocytic rate that was not significantly different from the single mutant A508. Substitution of the unpaired C473 in the extracytoplasmic domain by A, a mutation that impedes the formation of homodimers, reduced endocytic rate by ~50%, demonstrating that homodimers internalize at twice the rate of monomers. For more detailed description and conclusions see text and Ref. 23. Based on data in Ref. 23.

transfected receptors in recombinant CHO cells. Similar to all other receptors studied to date, the fundamental internalization signal for C receptors lies in the cytoplasmic domain. Only complete deletion of this domain (CRCYT-) virtually halts chlatrin coated pit internalization, reducing the net endocytic rate by ~10-fold. Deletion of the terminal 28 amino acids (CR514T) of the cytoplasmic domain also markedly reduced endocytosis to approximately one-fourth that observed for CRWT. This reduction is not due to the presence of phenylalanine in this region, because the net endocytic rate of the mutant CRA538 was not significantly different from that of CRWT. Within the cytoplasmic domain, tyrosine 508 plays an important role in endocytosis because substitution of this aromatic amino acid by alanine (CRA508) reduced net endocytic rate by ~50% of that of CRWT. The double mutant CRA508/A538 had a similar net endocytic rate than the single mutant CRA508.

Cytoplasmic domain tyrosine within the context of internalization signals (e.g., NPXY or YXXZ, in which X is any amino acid and Z is a large hydrophobic amino acid) has been shown to play an important role in endocytosis of membrane receptors (24). In the C receptor, tyrosine is not flanked by recognizable sequences that form part of internalization signals in other receptors. The finding that tyrosine still has an important role in the endocytosis of C receptors further strengthens the concept that internalization motifs are degenerate and that the role of tyrosine is that it lies on a β turn, conferring a proper secondary structure for the interaction of the cytoplasmic domain with clathrin adaptor proteins (23, 24). Lysosomal hydrolysis of internalized ligand and recycling of internalized receptors were not altered by the mutations, demonstrating that the signals for endocytosis are independent of the further intracellular processing of endocytosed receptor-ligand complexes (23).

An unexpected finding of this study was that constitutive dimerization of C receptors plays an important role in endocytosis (23). When dimerization is impeded by substituting the unpaired cysteine 473 in the extracellular domain by alanine, the net endocytic rate of the recombinant C receptors is reduced by ~50% (Fig. 5). Thus, homodimers of C receptors, the major form in which they are expressed at the cell surface, increase the rate of internalization of ANP by 4-fold compared with monomers because they bind the double of ANP molecules and internalize at twice the rate of monomers.

Summary of Properties of C Receptors That Contribute to Their Efficient Clearance Function. The properties of C receptors that contribute to their efficient clearance function may be summarized as follows:

1. C receptors have a very high affinity for natriuretic peptides. This property allows for receptor occupancy by ANP even at normal plasma concentrations of the hormone, and permits a sufficient resident time of ANP on surface receptors for the endocytosis of receptor-ligand complexes.

2. C receptors are distributed at high densities in tissues and cells that receive a large proportion of the cardiac output (e.g., vascular cells, kidney cortex,

lung), allowing for rapid removal of ANP from the circulation.

3. C receptors are endocytosed at rates that far exceed the dissociation of surface receptor-ligand complexes, allowing for an efficient delivery of ANP to lysosomes where it is hydrolyzed.

4. Internalized C receptors are recycled to the cell surface without significant homologous downregulation in the density of surface receptors upon prolonged exposure to ANP. This allows a single C receptor to mediate several cycles of removal of ANP from the circulation.

5. The state of constitutive dimerization of C receptors markedly enhances the ability of C receptors to remove natriuretic peptides from the circulation by binding the double of ANP molecules and by internalizing at twice the rate of monomer forms.

6. In combination the properties described above permit C receptors to function as a hormone buffer system, precluding large inappropriate shifts in plasma levels of ANP, rapidly removing ANP from the circulation once the stimulus for secretion is terminated, and eliminating from the extracellular medium ANP molecules that dissociate from signaling guanylyl cyclase receptors.

Dynamics of Guanylyl Cyclase Receptor-Ligand Interactions

Guanylyl cyclase receptors of natriuretic peptides are unique among biological receptors as they contain in a single molecule the acceptor (ligand binding), effector (enzyme-guanylyl cyclase) and modulator (a kinase-like domain interposed between the transmembrane domain and the guanylyl cyclase domain) functions that in other receptors (e.g., G protein coupled receptors) are performed by separate molecules (9, 25, 26). The current model of explaining GC receptor function is that in absence of ligand binding, the kinase-like domain represses the activity of guanylyl cyclase. Upon ligand binding (ANP and BNP for GC-A receptors; CNP for GC-B receptors), there is an allosteric change that permits the interaction of a cytosolic factor, most possibly ATP, with the kinase-like domain, leading to an allosteric derepression of guanylyl cyclase and the consequent generation of cGMP, the second messenger of natriuretic peptide actions (9). The structure and signaling mechanisms of GC-A receptors are reviewed by M. Chinkers in this issue (27). Here, we will consider only the dynamics of GC-A receptors and their interactions with ANP.

Lack of Endocytosis of GC-A Receptors. Studies from our laboratory in cultured glomerular mesangial and renomedullary interstitial cells have demonstrated that, contrary to C receptors and most other polypeptide hormone receptors, the GC-A receptor is a bonafide membrane resident protein that does not undergo rapid endocytosis and does not mediate lysosomal hydrolysis of ANP (28). Similar results were obtained by other investigators in cultured neuroblastoma cells (29) and in our laboratory in recombinant CHO cells stably transfected with cloned human GC-A receptors (Maack T, Porter G, Silva M, Nikonova L, unpublished results). The lack of rapid endocytosis of GC-A receptors is consistent with the particular molecular structure of this receptor. Thus, GC-A receptors do not contain signal sequences that are usually associated with rapid endocytosis, and there are no aromatic amino acids in the cytoplasmic domain near the transmembrane domain. Moreover, because GC-A receptors contain in a single molecule acceptor-effector-modulator sequences (see above), their mode of operation does not necessarily depend on receptor mobility on the plane of the membrane, a phenomenon that in other receptors may lead to their entrapment in coated pits with subsequent rapid endocytosis.

Lack of substantive endocytosis protects GC-A receptors from homologous downregulation (7). Nevertheless, prolonged exposure to high levels of ANP leads to desensitization of ANP induced activation of guanylyl cyclase, most possibly by GC-A receptor dephosphorylation (30). In the absence of endocytosis, however, other processes must account for the termination of the interaction of ANP with GC-A receptors. As we will describe below, studies in our laboratory demonstrated that one of these processes is the rapid dissociation of receptor-ligand complexes upon ANP binding to GC-A receptors under physiological conditions (28).

Termination of the Interaction of ANP with GC-A Receptors. Figure 6 compares the temperature-dependence of the off-rate (K_{off}) of ANP from GC_A and C receptors in monolayers of cultured glomerular mesangial or vascular smooth muscle cells. The dissociation of radiolabeled ANP from both receptors at 4°C is very slow, less than 1.5%/min. At 37°C, the dissociation of ANP from C receptors increases modestly, an increase that is expected on thermodynamic grounds by the rise in temperature alone. In contrast, there is a disproportionate increase in the offset of ANP from GC_A receptors between 30° and 37°C, resulting in a K_{off} at 37°C that is greater than 85%/min. The Q_{10} of the dissociation of ANP from GC-A receptors between 30° and 37°C is >6.8, whereas, between 4° and 30°C it is only 2–3 (28). This phenomenon is not observed in isolated membranes in which the K_{off} of radioligand from GC-A is similar to that from C receptors and is very slow at room temperature and at 37°C.

The results described above demonstrate the existence of a physiological process that markedly increases the dissociation of ANF from GC_A receptors. This process depends on a cytosolic factor, as it is not

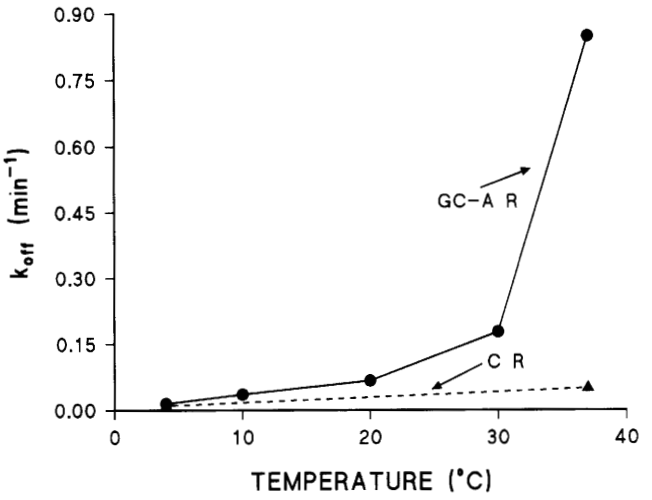

Figure 6. Physiological modulation of the dissociation of ANP from surface GC-A receptors. The off-rates (k_{off}) of ^{125}I-ANP$_{1-28}$ from native GC-A receptors in monolayers of cultured glomerular mesangial cells and from native C receptors in monolayers of cultured vascular smooth muscle cells were determined as described in Ref. 28. The dissociation rates at 4°C were very slow and similar for GC-A and C receptor-radioligand complexes. There is a disproportionate increase in the K_{off} from GC-A receptors between 30° and 37°C, a phenomenon that does not occur with C receptor-radioligand complexes. At 37°C, dissociation rate of ANP from GC-A receptors was ~85%/min, whereas it was less than 5%/min from C receptors. The disproportionate increase in the dissociation of GC-A receptor-ligand complexes at physiological temperatures occurs only in intact cells and not in isolated membranes preparations, indicating that it depends on the interaction of an active metabolite with the cytoplasmic domain of these receptors. Data are from Ref. 28 for GC-A receptors and from Ref. 2 for C receptors. Modified from Ref. 15.

observable in isolated membranes, and depends on active metabolism, because it is detectable only in intact cells at physiological temperatures. As will be described below, it is possible, even likely, that ATP mediates the sudden increase in receptor-ligand dissociation.

De Lean and co-workers were the first to show that ATP increases the K_{off} of ANP from GC-A receptors more than it increases K_{on}, resulting in an increase in the apparent equilibrium dissociation constant K_d (31, 32). These authors also demonstrated that amiloride decreased the dissociation of GC-A receptor-ligand complexes in membranes from bovine adrenal zona glomerulosa cells and postulated that this is due to an interaction between amiloride and ATP in modulating the dissociation of receptor-ligand complexes (31). High concentrations of amiloride (0.5 mM), markedly decreases the K_{off} of ANP from GC$_A$ receptors in monolayers of cultured glomerular mesangial cells, by more than 10-fold (28). Recently, we found that R5-substituted analogs of amiloride were significantly more potent than amiloride in decreasing the K_{off} of ANP from cloned human GC-A receptors stably transfected into CHO cells, with hexamethylamiloride (ED_{50} = 1.7 ± 0.9 μM) being ~10-fold more potent than amiloride (ED_{50} = 19 ± 6 μM) (Friedman O, Nikonova LN, Maack T, unpublished results).

The phenomenon of rapid dissociation of ANP from GC$_A$ receptors has previously escaped the attention of investigators, including ourselves, because binding experiments where either performed in intact cells at subphysiological temperatures, or in nonphysiological conditions in isolated membranes. The experiments described in the preceding paragraphs carry the important lesson that binding experiments performed in nonphysiological conditions may not reflect the true kinetics of binding of polypeptide hormones to their receptors in the intact environment of the cell.

Physiologic Consequences of the Rapid Dissociation of ANP from GC-A Receptors. The rapid dissociation of ANP from GC-C receptors is an important component of the stimulus-response homeostasis for this hormone (Fig. 1). When the free concentration of ANP falls, a fast dissociation favors a prompt termination of the response. The dissociated hormone can then be rapidly removed by the abundant C receptors. Moreover, rapid dissociation also increases the availability of unoccupied GC-A receptors, whose density contrary to that of C receptors, is limited. In this manner, it favors a heightened response when plasma ANP increases. As described above, ATP is likely to play a major modulatory role in this process because it increases ligand offset (and to a smaller degree onset) and at the same time magnifies the ANP-induced increase in guanylyl cyclase activity. The combination of these events is likely to result in a rapid and powerful response when plasma concentrations of ANP rise and a prompt termination of responses when plasma concentrations of the hormone fall (28).

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