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Structure, signaling mechanism and regulation of natriuretic peptide receptor-guanylate cyclase

Kunio S. Misono[1], **John S. Philo**[2], **Tsutomu Arakawa**[2], **Craig M. Ogata**[3], **Yue Qiu**[1], **Haruo Ogawa**[1,†], and **Howard S. Young**[4]

[1] University of Nevada School of Medicine, Reno, NV, U.S.A

[2] Alliance Protein Laboratories, Thousand Oaks, CA, U.S.A

[3] Advance Photon Source, Argonne National Laboratory, Argonne, IL, U.S.A

[4] Department of Biochemistry, University of Alberta, Edmonton, AL, Canada

Summary

Atrial natriuretic peptide (ANP) and homologous B-type natriuretic peptide (BNP) are cardiac hormones that dilate blood vessels and stimulate natriuresis and diuresis, thereby lowering blood pressure and blood volume. ANP and BNP counterbalance the actions of the renin-angiotensin-aldosterone and neurohormonal systems, and play a central role in cardiovascular regulation. These activities are mediated by the A-type natriuretic peptide receptor (NPRA), a single transmembrane segment, guanylate cyclase (GC) linked receptor that occurs as a homodimer. Here we present an overview of the structure, possible chloride-mediated regulation, and signaling mechanism of the NPRA and other receptor-GCs. Earlier, we determined the crystal structures of the NPRA extracellular domain with and without bound ANP. Their structural comparison has revealed a novel ANP-induced rotation mechanism occurring in the juxtamembrane region that apparently triggers transmembrane signal transduction. More recently, the crystal structures of the dimerized catalytic domain of green algae GC Cyg12 and that of cyanobacter GC Cya2 have been reported. These structures closely resemble that of the adenylate cyclase catalytic domain consisting of C1 and C2 subdomain heterodimer. AC is activated by binding of $G_s\alpha$ to C2 and ensuing 7° rotation of C1 around an axis parallel to the central cleft, thereby inducing the heterodimer into a catalytically active conformation. We speculate that, in the NPRA, the ANP-induced rotation of the juxtamembrane domains, transmitted across the transmembrane helices, may induce a similar rotation in each of the dimerized GC catalytic domains, leading to the stimulation of the GC catalytic activity.

Keywords

natriuretic peptides; atrial natriuretic peptide receptor; guanylate cyclase; single transmembrane segment receptor; transmembrane signal transduction; X-ray crystallography; single particle electron microscopy; hormone binding; structural motif; allosteric regulation

Corresponding author: Kunio S. Misono, Department of Biochemistry, University of Nevada School of Medicine, Reno, Nevada 89557. Tel: 775-784-4690, Fax: 775-784-1419, kmisono@unr.edu.
[†]Present address: Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo, Japan

Enzyme:
guanylate cyclase, EC 4.6.1.2

Natriuretic peptides

Atrial natriuretic peptide (ANP, Figure 1) is secreted by the atrium of the heart in response to blood volume expansion. ANP stimulates salt excretion [1] and dilates blood vessels [2,3], thereby lowering blood pressure and reducing blood volume. ANP counterbalances the renin-angiotensin-aldosterone system and plays a central role in cardiovascular homeostasis. ANP also suppresses cardiac hypertrophy and fibrosis and is involved in remodeling of the heart and the vascular system [4–8]. The B-type natriuretic peptide (BNP) is mainly produced in the ventricle and has activities similar to ANP [9]. The C-type natriuretic peptide (CNP) occurs in the brain [10], vascular endothelium [11], cartilage [12], and other peripheral tissues, and plays a variety of local regulatory roles. Physiologic and pathophysiologic roles of natriuretic peptides and receptor systems are reviewed in this series by Kishimoto et al. [13] and Pandey [14]. The detailed structure-activity relationship for ANP has been studied by peptide synthesis approach and is summarized in [15].

Natriuretic peptide receptors – Molecular topology

The hormonal activities of ANP and BNP are mediated by the A-type natriuretic peptide receptor (NPRA). The NPRA is a single transmembrane segment receptor linked to its intrinsic guanylate cyclase (GC) activity in the intracellular domain (Figure 2). Binding of ANP or BNP stimulates the GC activity and elevates intracellular levels of cGMP, which in turn elicits physiologic responses through cGMP-regulated ion-channels, protein kinases, phosphodiesterases, and possibly other effector proteins. CNP activities are mediated by the B-type natriuretic peptide receptor, which has a molecular topology similar to that of the NPRA.

The NPRA exists as a homodimer of a single-span transmembrane polypeptide, which contains an extracellular ligand-binding domain (ECD), a transmembrane domain, and an intracellular domain (ICD) consisting of a protein kinase-like domain (PKLD) and a GC catalytic domain (GCD; Figure 2) [16]. The ECD contains a highly conserved chloride binding site near the ECD dimerization interface [17,18]. The ECD also contains in its juxtamembrane region a highly conserved structural motif, referred to as receptor-GC signaling motif [19]. Single-residue mutations in this motif either render the receptor unresponsive to ligand binding or cause constitutive activation of the GC activity [20], suggesting that this conserved structure plays a critical role in transmembrane signal transduction. ATP is thought to bind to the PKLD and augment GC stimulation by ANP [21,22]. The PKLD is phosphorylated [23,24] and its dephosphorylation leads to receptor desensitization [25,24].

In the NPRA genes in human [26] and in rat [27], roughly the exons 1–6, 8–15, and 17–22 encode the ECD, PKLD, and GCD, respectively. The intervening sequences, the transmembrane sequence and a linker region between the PKLD and the GCD are encoded by exons 7 and 16, respectively.

ANP receptor extracellular domain — Biochemical and biophysical properties

We expressed the ECD of rat NPRA in mammalian cells (COS cells and CHO cells) and purified it by ANP affinity chromatography [28]. The purified ECD bound ANP with an affinity (K_d ~1 nM) comparable to that of the full-length NPRA purified previously from bovine adrenal membranes [29]. The ECD contains three disulfide bonds Cys60-Cys86, Cys164-Cys213 and Cys423-Cys432 in a 1–2, 3–4, and 5–6 linkage pattern (Figure 3) [30]. Of these, the disulfide bond Cys60-Cys86 occurs in the chloride binding site (see below) and

the disulfide bond Cys423-Cys432 occurs in the juxtamembrane receptor-GC motif. Both disulfide bonds are conserved among the A- and B-type natriuretic peptide receptors. The ECD also contains five N-linked oligosaccharides [31]. Correct glycosylation is essential for expression of the functional NPRA: deletion of any one of the five glycosylation sites by mutagenesis reduces or abolishes NPRA expression [32]. On the other hand, de-glycosylation of the native or expressed ECD with endoglycosidase F_2 or H has no effect on ANP binding [20]. Together, these findings suggest that glycosylation is essential for folding or transport of the nascent receptor polypeptide to the cell membrane but, once the active receptor is formed, the glycosyl moieties are not involved in ANP binding. This notion is consistent with the crystal structure of the ANP-ECD complex [17], which shows that glycosyl moieties or the glycosylation sites are located away from the ANP-binding site.

By sedimentation equilibrium analyses, we found that the ECD undergoes dimerization with a dissociation constant K_d of ~500 nM. In the presence of ANP, ECD dimerization was strongly enhanced (K_d ~10 nM) [18].

Crystal structures of the ECD with and without ANP

We have determined the crystal structures of the apo ECD dimer and the ANP-ECD complex (Figure 4a,b, respectively) [33, 17]. Each ECD monomer has the membrane-distal and membrane-proximal subdomains connected by three stretches of the polypeptide backbone. The apo ECD occurs as a homodimer associated via the membrane-distal subdomain [34, 35]. In the ANP-bound complex, two ECD monomers bind one ANP molecule, forming a 2:1 complex (Figure 4b) [17]. The structure reveals detailed ANP binding interactions (Figure 4c) that include: i) Arg14 of ANP hydrogen bonding with Glu119 of ECD monomer A (Glu119A) and Asp62 of ECD monomer B (Asp62B). Arg95A and Asp62B are also hydrogen bonded, contributing to the stability of the complex; ii) Phe8 of ANP makes a hydrophobic contact with a hydrophobic pocket formed by Tyr154A, Phe165A, Val168A, and Tyr172A; and iii) the C-terminal peptide backbone of ANP (Asn24-Ser25) forms a short parallel β-sheet with the receptor protein backbone (Glu187B-Phe188B). These binding interactions identified in the ANP-ECD crystal structure are consistent with the structure-activity relationship data reported for ANP [15].

Chloride-mediated control of the NPRA

A protein-bound chloride atom occurs near the dimer interface (Figure 4d) [17]. This chloride is reversibly bound [18], being consistent with the finding that ANP binding to the receptor requires chloride and is chloride concentration-dependent [36]. We have proposed that chloride may allosterically regulate the NPRA in the kidney and control ANP-induced natriuresis.

The natriuretic activity of ANP has been well documented experimentally. Yet, the physiological role of ANP as a natriuretic hormone continues to be debated because there are certain physiologic and pathologic conditions where salt is retained despite elevated plasma ANP [37–39]. For example, in normovolemic animals, infusion of high-dose ANP does not cause a corresponding increase in natriuresis [40]. In edematous diseases such as congestive heart failure, nephrotic syndrome, and hepatic cirrhosis, plasma levels of ANP are markedly elevated, yet sodium is retained [41–43]. In ANP-overexpressing transgenic animals, plasma ANP levels are markedly elevated, yet salt is retained [44,45]. The insensitivity to the natriuretic effects of ANP is also observed in salt-depleted rats, which occurs independent of the renin-angiotensin-aldosterone (RAA) and sympathetic systems, and is not due to receptor down-regulation [46]. Although it is beyond the scope of this review to analyze individual cases, there apparently exists a common mechanism that can independently switches off natriuresis irrespective of ANP presence, and sodium retention

supersedes ANP natriuresis in situations where the RAA system is activated either as a normal physiologic response or as a compensatory (often aggravating) response in disease states such as heart failure. We speculate that the chloride-control of the NPRA occurs in the kidney on the luminal surface of the collecting duct and that this mechanism switches off the NPRA (hence prevents ANP natriuresis) in response to a reduced luminal chloride concentration.

In vitro, ANP-binding to the NPRA is chloride-sensitive over a chloride concentration range 0.1 mM to 10 mM [36,18]. When volume depletion activates the RAA system, for example, the luminal chloride concentration at the collecting duct may decrease to <1 mM [47–49]. At such low chloride levels, the receptor is unable to bind ANP, blocking natriuresis. Indubitably, for this mechanism to operate, both the NPRA and the ligand ANP must be localized on the luminal side of the renal tubule (but not on the basolateral side where the chloride concentration is stable at 90 to 110 mM). There is a plethora of evidence in support of this view.

The NPRA as well as other natriuretic peptide receptors (NPRs) are expressed along the nephron tubule. The subcellular localization (or polarization) of the NPRs has been studied by immuno-fluorescence staining using anti-receptor antibodies. Although the results are not in complete agreement, the NPRA is found localized predominantly on the apical (or luminal) membrane of the medullary collecting duct cells [50], where it is proposed to regulate sodium transport [51,52]. On the other hand, the NPRB is localized on the apical membrane of intercalated cells, where it is thought to interact with CNP and regulate acid-base homeostasis [53].

The presence of natriuretic peptides in the urine (and hence the luminal fluid) has long been known [54–59]. Urodilatin, originally discovered and isolated from human urine, is a 32-residue natriuretic peptide derived from the common ANP precursor polypeptide differently processed in the kidney (review in [50]). It is synthesized in the tubular cells, luminally secreted, and regulates tubular sodium transport by binding to the luminal surface NPRA. It has been proposed that urodilatin, rather than ANP (of cardiac origin), is mainly responsible for natriuretic and diuretic regulations [51]. Similar tubular synthesis and urinary excretion of CNP has also been reported [60].

In addition to urodilatin, ANP and other natriuretic peptides of cardiac origin may also be present in the luminal fluid and contribute to the regulation. It is well established that small proteins and peptides (with the molecular weight of less than ~20,000) efficiently filtere through the glomerulus into the tubular lumen (for review see [61,62]). Small proteins are reabsorbed mainly by endocytosis and intracellularly hydrolyzed. Small peptides are hydrolyzed by proteases on the luminal brush border membrane of the proximal tubule [63] and the metabolites are rapidly absorbed. Because of these activities, it is often assumed that no peptide reaches the distal site of the nephron. It is necessary to note, however, that certain peptides are resistant to hydrolysis and enter the urine intact. Studies by microperfusion of radiolabeled peptides into the nephron (either the surface nephron in vivo or the isolated nephron in vitro) showed that vasopressin, oxytocin, and insulin, all containing disulfide bridge(s), are not hydrolyzed at the luminal brush border and are recovered intact in urine or the collecting fluid, while small linear peptides such as angiotensin I and II, bradykinin, glucagon, and luteinizing hormone-releasing hormone are hydrolyzed and recovered as amino acids or small fragments [64–66]. To our knowledge, no similar study has yet been reported for ANP or other natriuretic peptides. Natriuretic peptides may be similarly resistant to brush border hydrolysis (in vivo) and reach the distal tubule intact at least fractionally. Consistent with this view, ANP, BNP, and CNP are known to be excreted in the urine [54–59], and their levels are higher in heart failure [67,68], apparently corresponding

to their elevated plasma levels. Indeed urinary natriuretic peptides, especially BNP [68] and N-terminal BNP [67], have been proposed as non-invasive diagnostic and prognostic biomarkers for heart failure.

ANP inhibits sodium reabsorption (thus stimulating natriuresis) via the second messenger cGMP by inhibiting the cGMP- and amiloride-sensitive cation channels on the luminal membrane of collecting duct cells (by direct inhibition of the channels by cGMP and by suppression via cGMP-dependent protein kinase and Gi) [69] and by inhibiting Na^+/K^+-ATPase on the basolateral membrane [70]. The former is believed to function in rapid and direct control of sodium transport, while the latter functions in a slower and longer-term regulation. It has been pointed out that the affinity of cGMP-regulated channels for cGMP is weak with K_ds 20 μM or greater, while the cGMP levels in most cells are below 100 nM [71]. It is likely then that the channels on the luminal membrane are inhibited by local elevation of cGMP by activation of the NPRA also on the luminal membrane by luminal natriuretic peptides.

Taken together, it is clear that both the NPRA and natriuretic peptides (ANP, BNP, CNP, and urodilatin) are present in the luminal side of the collecting duct where the final and rate-limiting regulation of sodium reabsorption occurs. The ANP-NPRA regulatory mechanism may then be governed by the change in the chloride concentration in the lumen.

The kidney filters the volume some 60 times of plasma or more than 10 times of the total extracellular fluid volume per day and, consequently, almost all of the filtered salts and water must be returned to the circulation [48]. Preventing excessive salt loss in the process is essential for survival. It is conceivable then that there exists a mechanism that enables sodium reabsorption to override natriuretic stimuli when necessary. Deactivation of the NPRA at low luminal chloride concentrations (which change in parallel with sodium concentrations) allows *for* sodium reabsorption even in the presence of high natriuretic peptide levels.

It needs to be acknowledged that, at present, the direct evidence for the proposed chloride-control of the ANP-NPRA system is limited to the observation of the chloride effects on ANP binding and cGMP production in vitro [36,18] and the conserved chloride-binding site identified in the X-ray structures [17,18]. However, it is worthy to note that the data existing in the literature are consistent with and strongly suggest the proposed mechanism operating in the kidney. This control mechanism may account for the renal insensitivity to ANP long recognized but left unexplained to date. Additional focused studies are necessary to verify and understand how this control mechanism may operate in vivo and ultimately to utilize such knowledge for improved cardiovascular disease therapy.

ANP-induced structural change in the ECD

Binding of ANP to the ECD does not cause appreciable intra-molecular structural changes (root mean square deviation of the assigned 426 Cα atoms in ECD between the ANP-bound and unbound structures, 0.64 Å) [17]. ANP binding, however, causes a large change in the ECD dimer quaternary structure. The ECD monomers undergo a twisting motion (Figure 5a) [17,72], which causes the two juxtamembrane domains in the dimer to translate in opposite directions. This movement alters the relative angular relationship between the two juxtamembrane domains, that is equivalent to rotating each domain by 24° counter-clockwise (looking toward the cell membrane (Figure 5b). We have proposed that this ligand-induced rotation mechanism in the juxtamembrane region triggers transmembrane signal transduction [17,35].

The structures of the apo ECD dimer and the ANP-ECD complex were also observed by single particle electron microscopy (Figure 5c) [72]. This method allows determination of the native structures in the absence of crystal contacts and in closer to native buffer solution conditions. The three-dimensional reconstructions of the apo ECD dimer and the ANP-ECD complex revealed an ANP-induced conformational change similar to that identified based on the X-ray structures. These electron microscopy data confirm that the ECD occurs as a pre-formed homodimer in the head-to-head configuration and undergoes the large and distinct quaternary structural change, as seen in X-ray structures, in response to ANP binding.

Rotation mechanism for transmembrane signaling by the NPRA

We speculate that the ANP binding-induced rotation of the juxtamembrane domains in the dimerized receptor is transmitted across the transmembane helices and reorients the two intracellular domains into the active conformation, thereby enabling GC catalysis [17,34,35] (Figure 6; taken from the textbook *Biochemistry* by Garret and Grisham, 2009 [73]). This is the first example of the rotation mechanism for receptor signaling that has been structurally demonstrated.

The NPRA belongs to the family of membrane-bound receptor-GCs. Receptor-GCs and receptor-protein kinases represent two major families in the super-family of single transmembrane segment, enzyme-linked receptors. Signaling by the receptor-protein kinases is thought to be mediated by agonist-induced “association” mechanisms, while signaling by the receptor-GCs may be mediated by agonist-induced “rotation” mechanisms.

Protein kinase-like regulatory domain (PKLD)

The intracellular domain consists of the PKLD and the GCD (Figure 2). The PKLD is thought to be the site for ATP binding. ATP is a positive allosteric effector of the NPRA, which augments GC activation by ANP [21,22]. Contrary to this model, others have reported the absence of such stimulatory effects by ATP [74]. The PKLD is phosphorylated at multiple sites [23,24]. Desensitization of the NPRA in cultured cells upon extended exposure to ANP is accompanied by dephosphorylation [25,24]. The PKLD structure has been modeled based on sequence homology to protein tyrosine kinases [75]. This model has found some support by site-directed mutagenesis studies. However, the actual structure of the PKLD has not been reported. Thus, the structure and the regulatory role of this domain remain largely unsolved.

The PKLD is connected to the C-terminal GCD by a ~50 residues long linker region. Deletion mutagenesis studies have suggested that this region is necessary for receptor dimerization and the GC activity [76]. From its amino acid sequence, this region has been predicted to form an amphipathic helix in the monomer and a coiled-coil structure in the receptor dimer. On the other hand, more recent studies by systematic site-directed mutagenesis of the guanylin receptor (or guanylate cyclase C) have suggested that this region does not contain a coiled coil structure nor is necessary for dimerization [77]. Thus, the structure and role of this linker region remains uncertain.

Guanylate cyclase catalytic domain (GCD)

Recently, two independent groups have reported the crystal structures of GC catalytic domains: a 188-residue GC catalytic core of Cyg12, a GC from eukaryote unicellular green algae [78], and a 202-residue catalytic core of Cya2, a GC from prokaryote cyanobacter [79]. These are the first structures for any GCs that have been reported more than 10 years after the first reports of the adenylate cyclase (AC) structures [80–82]. GCs and all known ACs belong to the class-III nucleotide cyclase family and share a high sequence similarity

[83]. By amino acid sequence comparison, Cyg12 is homologous to mammalian soluble GCs, while Cya2 appears to be related to membrane-bound GCs.

Both Cyg12 GCD and Cya2 GCD were expressed in *E. coli*, and without the putative linker region discussed above. Yet, both formed and crystallized as dimers. The Cyg12 GCD had a specific activity of 2.8 μmol/min/mg in the presence of Mn^{2+} but much lower activity (less than 1%) in the presence of Mg^{2+} [78], as generally observed for mammalian GCs [84] and ACs [85]. The activity of the Gya2 GCD was reported significantly lower at 1.5 nmol/min/mg [79]. Yet, the GC activity showed a similar metal ion-dependence exhibiting a significantly higher specific activity in the presence of Mn^{2+} than in the presence of Mg^{2+}, and even higher when both metal ions were present. In eukaryotes, manganese is a trace element and magnesium ions are assumed to be the physiological active-site ions. The enhancement of the catalytic activity by Mn^{2+} ions is considered unlikely to have any physiological meaning [85]. Nevertheless, the observed homodimerization of the GCD and the metal ion-dependence of the catalytic activity support the integrity of the expressed proteins.

As expected from the high sequence homology with ACs, both Cyg12 GCD and Gya2 GCD monomers have the same protein fold as the mammalian AC catalytic domain. Each GCD monomer contains a 7-stranded β-sheet surrounded by several α-helices. In the dimer, two GCD monomers are related by a two-fold symmetry axis that runs through the central dimer cleft and form a wreath-like structure (Figure 7a, Cyg12 GCD dimer). The central cavity between the two monomers contains two symmetric active sites. The catalytic residues in each active site are supplied jointly by both monomers. The active-site residues in each monomer are located at positions homologous to their counterparts in ACs. Such conserved active site residues include two metal-binding Asp residues, ribose-orienting Asn residue, transition state-stabilizing Arg residue, triphosphate-binding Arg and Lys residues [78]. The guanine base-recognition residues Glu and Cys in Cyg12 [78] and Glu and Gly residues in Cya2 [79] are similarly conserved at the positions close to the locations of adenine base-recognizing Lys and Asp in ACs [86].

The AC catalytic core consists of a C1 and C2 subdomain heterodimer. In the AC catalytic core, C1 and C2 domains, related by a pseudo two-fold symmetry, form a heterodimeric wreath-like structure. By structural comparison, the dimer structure of Cyg12 GCD (Figure 7a) is similar to the open, inactive conformation of the AC catalytic domain, which must close to be catalytically active [78]. On the other hand, the Cya2 GCD dimer is in a closed conformation that must open in order to bind the substrate GTP for catalysis [79]. This closed structure of the Cya2 GCD dimer may explain its low specific GC activity.

Interestingly, the specific activity of the Cyg12 GCD at 2.8 μmol/min/mg is roughly comparable to those observed for the full-length receptor-GCs purified from various tissues and species, which range from 1.8 μmol/min/mg to 23 μmol/min/mg [87–89,29]. Together, these data seem to suggest that the structure of the Cyg12 GCD dimer may reflect the structure of the GCD in the dimerized full-length NPRA in its basal state.

Possible mechanisms for GC activation and NPRA signaling

Signaling by G-protein coupled receptors may involve stimulation of AC by $G_s\alpha$ that is released from the heterotrimeric G-protein upon receptor activation by a ligand. A possible mechanism for this AC activation by $G_s\alpha$ has been proposed based on the crystal structures of the AC catalytic domains [80–82]. In the proposed mechanism, $G_s\alpha$ binds to the C2 domain of the AC C1/C2 heterodimer. This binding causes a 7° rotation of the C1 domain around an axis that runs through the C1 domain and roughly parallel to the central cleft axis.

This movement brings the catalytic residues on the C1 domain closer to the catalytic residues on the C2 domain, thereby forming the catalytically competent active site [80].

In the Cyg12 GCD crystal structure, two GCD monomers are reported to be in an inactive, open conformation (Figure 7a) [78]. It has been suggested that activation of the Cyg12 GCD may be mediated by a domain rotation similar to that of the AC C1 domain rotation induced by $G_s\alpha$ binding to C2. The Cyg12 GCD monomer contains a surface groove similar to the groove on the AC C2 domain to which $G_s\alpha$ binds. In the dimerized Cyg12 GCD, binding of certain regulatory elements, similar to the H-NOX sensor domain in soluble GC, to this groove may cause a domain rearrangement or rotation in the GCD monomers leading to stimulation of GC activity (Figure 7b) [79, 78].

The NPRA and other receptor-GCs exist as homodimers. Their GCDs are similarly expected to form homodimer structures. We speculate that the ANP-induced rotation of the two juxtamembrane domains in the ECD [17,35] may be transduced across the transmembrane helices and through the PKLD, causing a rotation of each of the two GCD domains [17,35] (Figure 5a, 6). This rotation may bring the GCD dimer into a closed and active conformation (modeled in Figure 7c), thereby enabling GC catalysis. In this signaling process, the PKLD may play a regulatory role by binding with the allosteric effector ATP or by its phosphorylation state. The actual and detailed mechanism of GC activation by ANP, namely the signal transduction mechanism, must await determination of the NPRA's GCD structure and ultimately the structure of the full-length NPRA with and without bound ANP.

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Abbreviations

ANP	atrial natriuretic peptide
BNP	B-type natriuretic peptide
CNP	C-type natriuretic peptide
NPRA	ANP receptor (A-type natriuretic peptide receptor)
NPR	natriuretic peptide receptor
RAA	renin-angiotensin-aldosterone
GC	guanylate cyclase
ECD	extracellular domain
ICD	intracellular domain
PKLD	protein kinase-like domain
GCD	guanylate cyclase domain
AC	adenylate cyclase

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Figure 1.
Amino acid sequences of ANP, BNP and CNP from rat. Two Cys residues in each peptide form an intra-molecular disulfide-bond, which is essential for the activity [90]. Conserved residues are shaded.

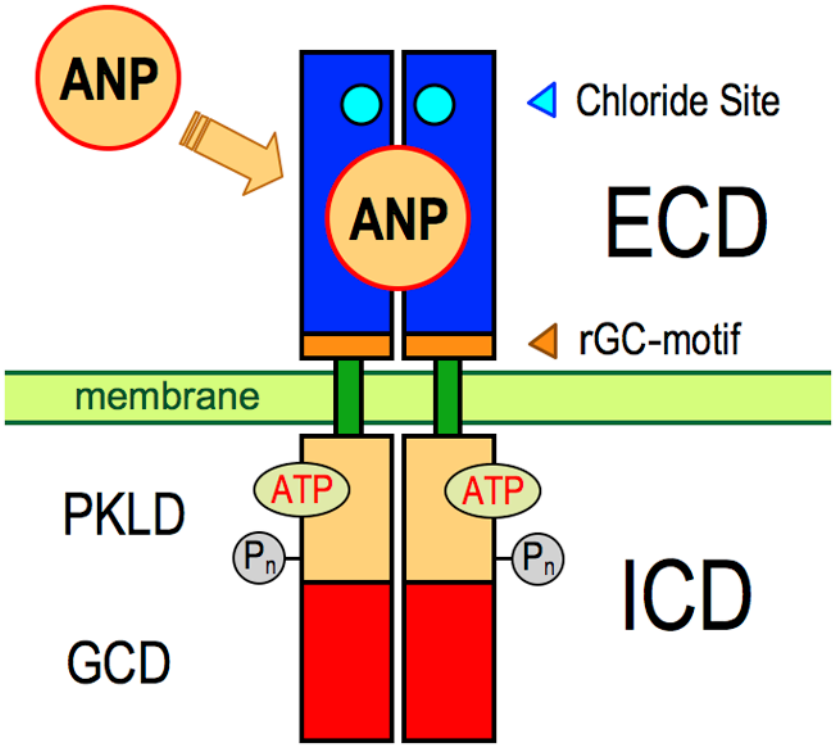

Figure 2.
The molecular topology of the NPRA. The NPRA occurs as a pre-formed homodimer. Each monomer contains an extracellular ANP-binding domain (ECD), a transmembrane domain, and an intracellular domain (ICD) consisting of a protein kinase-like domain (PKLD) and a GC domain (GCD). The ECD contains a highly conserved chloride binding site [33,17,18] and a juxtamembrane GC-signature motif [20]. Bound chloride (cyan ball) is essential for ANP binding [36]. The juxtamembrane GC-signature motif plays a critical role in transmembrane signal transduction. The PKLD binds the positive allosteric effector ATP [21,22] and is phosphorylated at multiple sites [23].

Figure 3.

(a) Diagram illustrating the covalent structure of the ECD. The ECD contains five N-linked oligosaccharides (boxes) [31] and three disulfide-bonds (orange lines) [30]. The glycosylated Asn residues and disulfide-bonded Cys residues are indicated. No free Cys residue is present in the ECD.

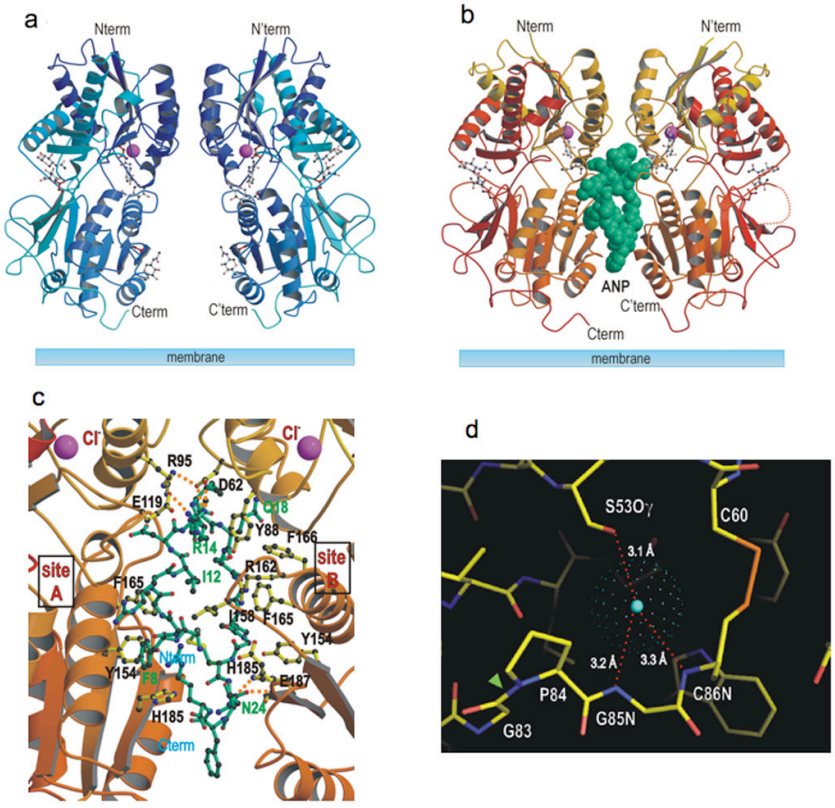

Figure 4.

(a,b) Crystal structures of apo ECD dimer (PDB: 1DP4) and ANP-ECD complex (PDB: 1T34) [33,17]. ANP is shown in green. Protein-bound chloride atoms are shown by magenta balls. **(c)** Close-up view of ANP binding interactions. Major interactions are shown circled. **(d)** Close-up view of the chloride binding site in the apo ECD [33,18]. Chloride is hydrogen bonded to the hydroxyl-group of Ser53, and the backbone NH moieties of Gly85 and Cys86. The binding site also contains the only *cis*-peptide bond in the ECD (green arrow head) and the Cys60-Cys-86 disulfide bond. The van der Waals radius of the chloride atom is represented by a green dotted ball.

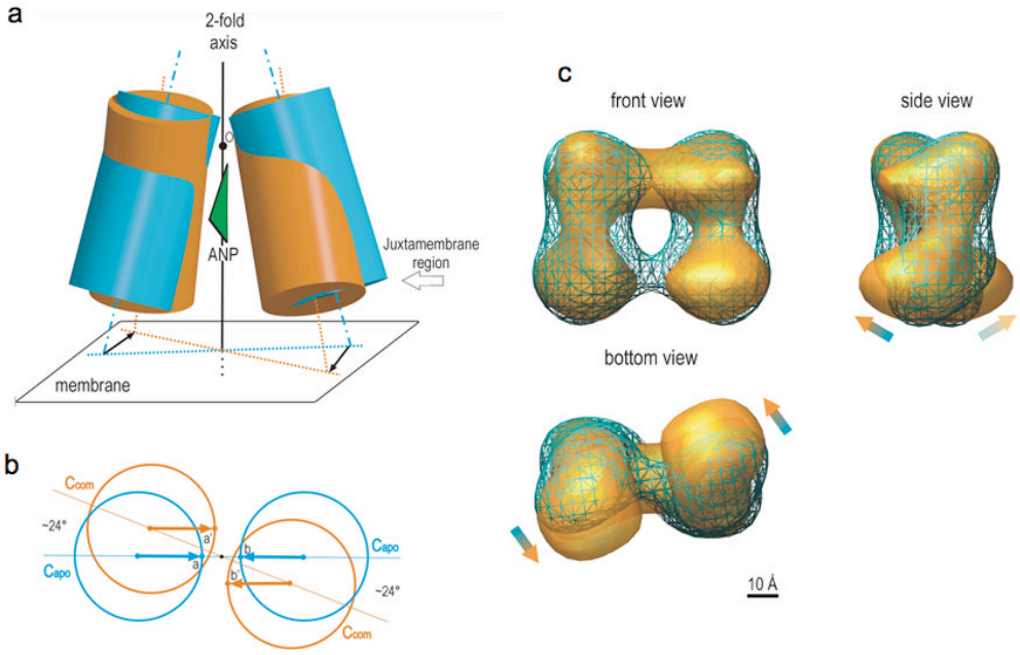

Figure 5.

(a) Schematic illustration of ANP-induced change in the ECD dimer structure. ANP binding causes a twisting motion of the two ECD monomers from the apo position (blue) to the complex position (orange) [17,35]. **(b)** Viewed toward the membrane, the juxtamembrane domains in the apo form (blue circles) translate to the complex position (orange circles) with essentially no change in the inter-domain distance. The arrows depict parallel translocation. This motion causes a change in the angular relationship between the two domains equivalent to rotating each domain by 24° counter-clockwise. Because the dimerized receptor is free to spin or move about in the cell membrane, this rotation motion occurring in the juxtamembrane domains would be the only structural change that is "recognized" by the receptor upon ANP binding. **(c)** ANP-induced conformational change observed by single-particle electron microscopy [72]. Reconstruction of the apo ECD dimer (blue mesh) is superimposed onto that of the ANP-ECD complex (gold surface). The reconstructions are rendered at 70% of the correct molecular volume for clarity.

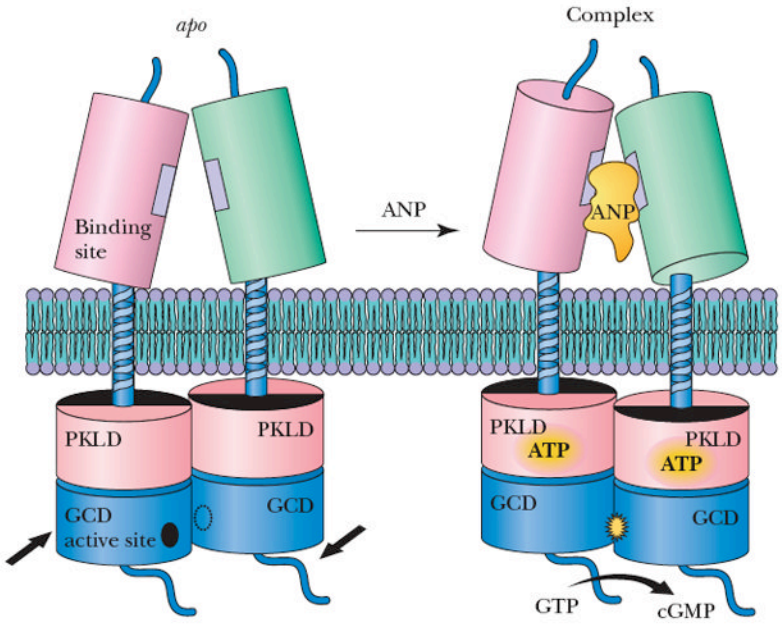

Figure 6.
Rotation mechanism proposed for transmembrane signaling by the NPRA. Taken from a textbook, *Biochemistry* by Garrett and Grisham, 4th edition, 2009 [73] (drawing adapted from [35]). The details are in the text.

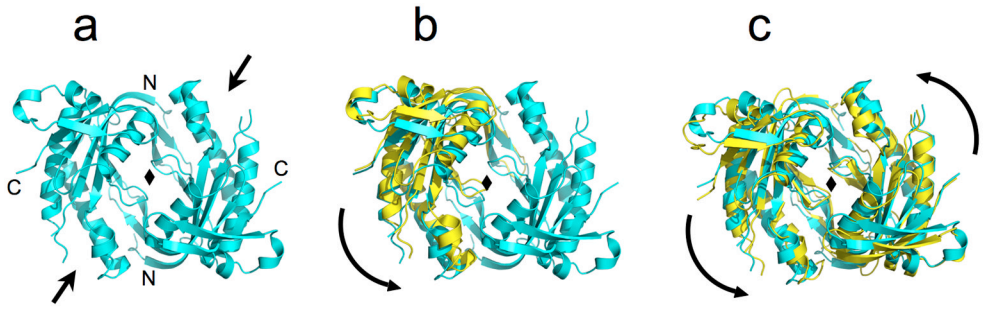

Figure 7.

(a) Structure of Cyg12 GCD dimer (PDB: 3ET6), which is an open inactive conformation [78]. The arrows show the surface grooves in the GCD that correspond to the $G_s\alpha$ binding site in AC C1 domain [80]. The N- and C-terminal ends of each monomer are labeled. The 2-fold symmetry axis in the dimer runs perpendicular to the plane of the page. The dimer structure is seen from the C-terminal end. (b) Model for GC activation [78]. The GCD monomer (yellow) was aligned to the C1 domain of the activated $G_s\alpha$-AC complex [82] (PDB: 1CJU) and overlaid onto the open-inactive GCD dimer (cyan, PDB: 3ET6). (c) Model of the closed active GCD dimer conformation (yellow) overlaid onto the open inactive GCD dimer (cyan). The rotation of each of the two domains (around each own axis) leads to the closed and active conformation (arrows).