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Advances in the Mechanism of Action of Natriuretic Peptides at a Cellular Level

Gurdeep S. Mannu[1*] and Angeline Bhalerao[2]

[1]John Radcliffe Hospital, Oxford University Hospitals NHS Trust, UK.
[2]Ipswich General Hospital, Norfolk, UK.

Authors' contributions

This work was carried out in collaboration between both authors. Author GSM designed and wrote the search protocol. Authors GSM and AB conducted the systematic search and interpretation and both authors produced the final manuscript.

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Review Article

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ABSTRACT

Background: Natriuretic peptides have a vast array of different actions at numerous sites throughout the human body. There have been rapid advances in understanding their mechanism of action in recent years and this review aimed to collate all available information on this field in order to present the current evidence-base.
Method: A two-step process utilising a Medline/PubMed systematic search was conducted. The initial search was undertaken using elementary phrases. The search produced over 4000 published papers on the topic of the mechanisms of action of natriuretic peptides. The resultant abstracts were analysed and appropriate papers were selected. The secondary search was performed by (1) using the reference lists of the chosen articles and (2) by using PubMed weblink for related articles. The studies were selected if they were in English language published in the past 30 years (1983-2013) and included the appropriate topics. All of the reports regarding the intracellular and pharmacological mechanisms of action of natriuretic peptides were selected.
Conclusions: This review has collected all information on the recent advances in our understanding of the intracellular pathways that allow these peptides to bring about natriuresis, vasodilatation and their many other effects. Although offering significant pharmacological potential,

**Corresponding author: Email: Gurdeepmannu@gmail.com;*

this field remains poorly understood and there is a need for more research using newer research techniques.

Keywords: Natriuretic peptides; pharmacology; mechanism of action; cardiovascular physiology; natriuresis.

1. INTRODUCTION

Atrial natriuretic peptide, ANP (present in the cells of the heart atria) and brain natriuretic peptide, BNP (so named as it was first discovered in the brain, despite being more abundant in the atria and ventricles of the heart) are stored in cardiac myocytes as 'pre-hormones', in an inactive form. Their release is triggered by high blood pressure which causes the cardiac muscle to be stretched, liberating these peptides into the systemic circulation as fully active hormones [1]. The circulation takes them to a host of target sites, where they exert their numerous actions [2].

The first documented target site for these peptides was the kidneys, where they have a natriuretic effect and increase the excretion of sodium ions from the blood into the urine [3]. In fact it was the observation of natriuresis resulting from the injection of atrial tissue extracts into rats by *de Bold* in 1981, which led him to originally discover these concealed peptides. The excretion of sodium leads to a corresponding movement of water which osmotically follows these ions from the blood into the urine. This in turn results in a reduced blood volume, and thus a subsequent decrease in blood pressure [4]. As a result of this, a negative feedback loop is completed, and consequently these hormones are known as 'natriuretic peptides' [2].

Since the 1980s, three different types of natriuretic peptide have been discovered, namely ANP, BNP, and CNP (CNP is abbreviated from C-type natriuretic peptide) [5]. The actions of these peptides are numerous and their targets span the entire body [6]. Although there has been much research into various facets of natriuretic peptide action, there remains a paucity of information in the literature integrating the intracellular mechanisms of these peptides. In light of this, this review will aim to collate all available information on the intracellular mechanisms of these peptides and to present the recent advances made in understanding how these peptides exert such a vast array of effects.

2. METHODOLOGY

A two-step process utilising a Medline/PubMed systematic search was conducted. The initial search was undertaken using elementary phrases including "natriuretic peptides", "ANP", "BNP", "CNP", "mechanisms of action", and "natriuresis". Only the most recent literature in the field was required so the time-window for the literature review was restricted to the past 30 years (1983-2013).

The resultant abstracts were analysed and appropriate papers were selected. The secondary search was performed by (1) using the reference lists of the chosen articles and (2) by using PubMed weblink for related articles. The studies were selected if they were in English language and included the appropriate topics and if they were in the English language. The search produced over 4000 published papers on the topic of the mechanisms of action of atrial natriuretic peptides. All of the reports regarding the intracellular and pharmacological mechanisms of action of natriuretic peptides were selected.

3. RESULTS AND DISCUSSION

3.1 Current Status of Knowledge

Firstly, in order to understand the cellular mechanisms by which these peptides act, it is necessary to initially understand their chemical structure and to appreciate the differences between them. All natriuretic peptides are formed as pre-hormones. In order to activate them a section of the peptide chain must be cleaved off to leave the natriuretic peptide chain. The 3 natriuretic peptides (ANP, BNP and CNP) range in molecular sizes from 22 amino acids (CNP) to 32 amino acids long (BNP), with ANP being in the middle with 28 amino acids in total [7] as Fig. 1 illustrates.

Despite the difference in length between natriuretic peptides, there is a central region of 17 amino acids which is surprisingly well conserved in all 3 peptides. This area is known

as the 'primary structure' and has only 6 locations of variation along its entire length. This high degree of conservation indicates that this region of the peptide must have a vital role in the action of all natriuretic peptides. The primary structure may have something to do with the general binding of peptide to its complementary receptor molecule.

However, natriuretic peptide receptors (NPRs) may bind to more than one natriuretic peptide (although their affinities to each differ). It is this central region that allows this generalisation. The difference in affinities between receptors for different peptides may be explained by the structure of the peptide chain outside this primary structure. The two ends of this primary structure are identified by the location of a cysteine amino acid (Fig. 1). The natriuretic peptide structure arises from the folding of the peptide chain so that the 2 cysteines in each peptide touch. This leads to the formation of cysteine bridges, thus giving these peptides their characteristic 'Ω' shape (as shown in Fig. 2).

3.2 Natriuretic Peptide Receptors

Each natriuretic peptide receptor is connected to different intracellular cascades. It is by virtue of this that the effects of each natriuretic peptide depend on which receptor they bind to. There are 3 types of natriuretic peptide receptor (NPR), namely, NPR-A, NPR-B, and NPR-C. As mentioned earlier, each of these 3 receptors has different affinities for each of the 3 natriuretic peptides ANP, BNP and CNP. Specifically, NPR-A has the greatest affinity for ANP [8], then BNP, and the least affinity to CNP. The NPR-B receptor appears to favour CNP and binds with this peptide with greatest affinity, before ANP, and with least affinity to BNP. The NPR-C also has least affinity to BNP, instead preferring ANP over CNP (this is summarised in Table 1). One thing seems clear however, ANP seems to be favoured by all receptors before BNP. This may be either because of the peptide structure (being 4 amino acids shorter than BNP), or due to the natriuretic receptor tertiary structure being innately complementary to ANP specifically.

The NPR-A and NPR-B receptors themselves have a number of similarities, in fact, their structural homology in the extra cellular domain is as high as 44% [9]. Both these receptors, when binded to their respective ligands, dimerise with adjacent like receptors and cross phosphorylate each other using ATP, thus activating the guanylate cyclase domain. Concerning the intracellular domain, both NPR-A and NPR-B are linked to guanylate cyclase secondary messenger which activates a number of downstream enzymes [10]. This proves essential for the sheer host of numerous cascades which stem from these receptors, which will be discussed later. NPR-C on the other hand is not guanylate cyclase linked, but is instead a clearance receptor, much shorter than the other two receptors, and is responsible for the removal of the natriuretic peptides which bind to it [11].

3.3 Receptor Physiological Functions

The actions of each receptor and the respective intracellular conducting mechanisms are diverse. However, before exploring these intracellular cascades, it is first necessary to gain an understanding of what the main physiological outcomes of their stimulation are.

The most obvious function of NPR-A as mentioned earlier, is natriuresis [12] which is directly related to a corresponding diuresis [6]. However, this diuresis not only occurs by the increased loss of water by osmosis, but also by the induction of intracellular aquaporins which migrate and fuse into the cellular membrane (aquaporin 2 in renal epithelial cells [8,9,13]). Both of these mechanisms lead directly to NPR-A's role in the control of haemoconcentration.

NPR-A also inhibits the renin-angiotensin-aldosterone-system ('RAAS') [14], whose main function (specifically of the aldosterone component) is to increase sodium reabsorption in the proximal tubule of the kidney nephron; thus, by blocking it NPR-A potentiates sodium loss. Sodium and potassium are linked by an anti-port transport system. If sodium is absorbed, then potassium is lost, hence if sodium is not absorbed i.e. it is lost, then potassium is retained. It is due to this fact that NPR-A increases potassium retention by the body.

The inhibition of the RAAS system (specifically of the angiotensin II component which is chiefly responsible for vasoconstriction) lowers the total peripheral resistance and hence lowers blood pressure. The blood pressure lowering effect of NPR-A stimulation is further exacerbated by its direct vasodilatation/relaxation action [15,16], which in addition, indirectly increases vessel permeability. Hence, in high concentrations natriuretic peptides increase movement of fluid from plasma, into the interstitial space.

There is evidence of many other actions resulting from the stimulation of these receptors; namely decreased cell proliferation [17], apoptosis [7,18], decreased cardiac fibrosis [19], as well as effects on cardiovascular remodelling. NPR-A stimulation may even play a role in immune dysfunction and airway inflammation in conditions such as allergic asthma [20]. NPR-B has vasodilator effects [21], and is also associated with cell proliferation [19] and bone growth [22]. However, NPR-C is specifically a clearance receptor involved in the removal and clearance of the natriuretic peptides which bind to it [23]. NPR-C has been shown to be a shortened receptor since it totally lacks any guanylate cyclase activity (unlike the other two receptors). The main functions of each receptor are summarised in Table 1.

The mechanism by which ANP can achieve such a variety of different effects has been the source of focused research. It can be explained by the fact that there are many different types of ANP, collectively referred to as the atrial peptides. As discussed earlier, the natriuretic peptides are formed as pre-hormones, and the different forms of ANP are named from where they are cleaved along the peptide chain. Each section acts as an agonist for a different physiological effect. Namely, these sections include the long acting natriuretic peptide (types 1-30), vessel dilator type (ANP 31-67), kaliuretic peptide (ANP 79-98- which is the active form, produced via secondary cleavage from ANP 68-98), and the first discovered; atrial natriuretic factor, ANF (ANP 99-126) [22]. Fig. 3 illustrates this cleavage.

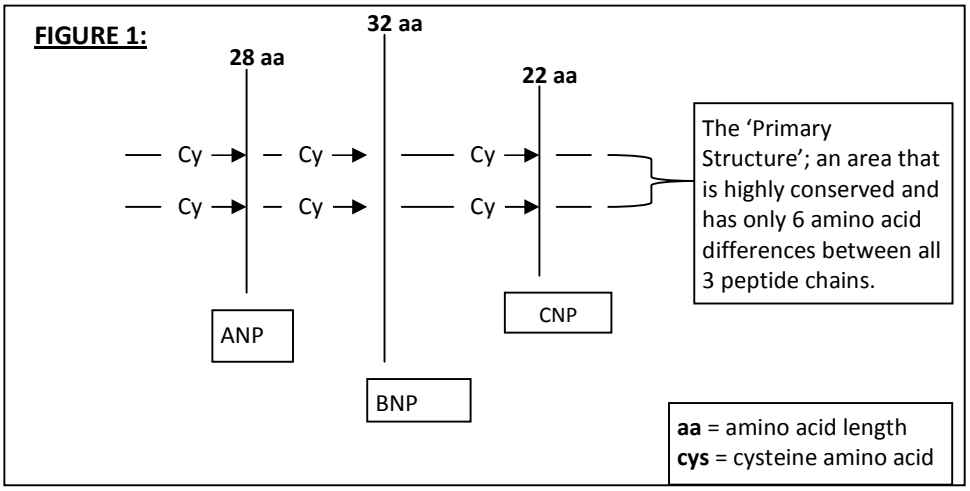

Fig. 1. The molecular sizes of the three natriuretic peptides ranges with CNP (22 amino acids), BNP (32 amino acids) and ANP (28 amino acids). However a conserved 6 amino acid length segment marked with cysteine amino acids is present in all three peptides and demarcates the two ends of this primary structure

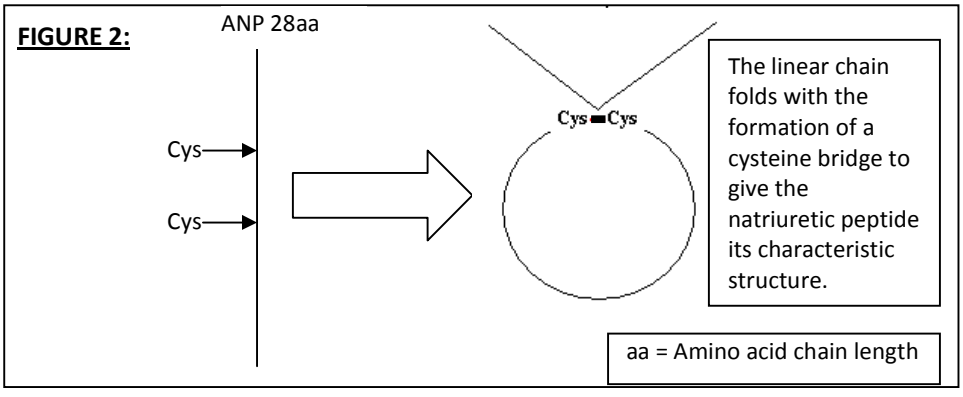

Fig. 2. The two cysteine amino acids in the natriuretic peptide leads to the formation of cysteine bridges, thus giving these peptides their characteristic 'Ω' shape.

Table 1. Each natriuretic peptide receptor is connected to different intracellular cascades and subsequently has different physiological outcomes. This table summarises the known functions of the natriuretic peptides. GFR = Glomerular Filtration Rate

Main functions	NPR-A Natriuresis (6)	NPR-B Vasorelaxation (1)	NPR-C Clearance of natriuretic peptides (14)
	Vasorelaxation (8) (increase GFR?) Inhibition of the Renin-Angiotensin-Aldosterone-System ('RAAS') (7) Apoptosis and antigrowth effects (10) Decreased cell proliferation (9) Decreased cardiac fibrosis (11) Increased uptake of renal dopamine (15)	cell proliferation (11) Bone growth (13)	
Natriuretic peptide preferred. (with greatest affinity first)	ANP>BNP>CNP	CNP>ANP>BNP	ANP>CNP>BNP
Intracellular signalling type:	Protein kinase, with Gunaylate Cyclase	Protein kinase, with Gunaylate Cyclase	No gunaylate cyclase activity,thus a - shortened receptor

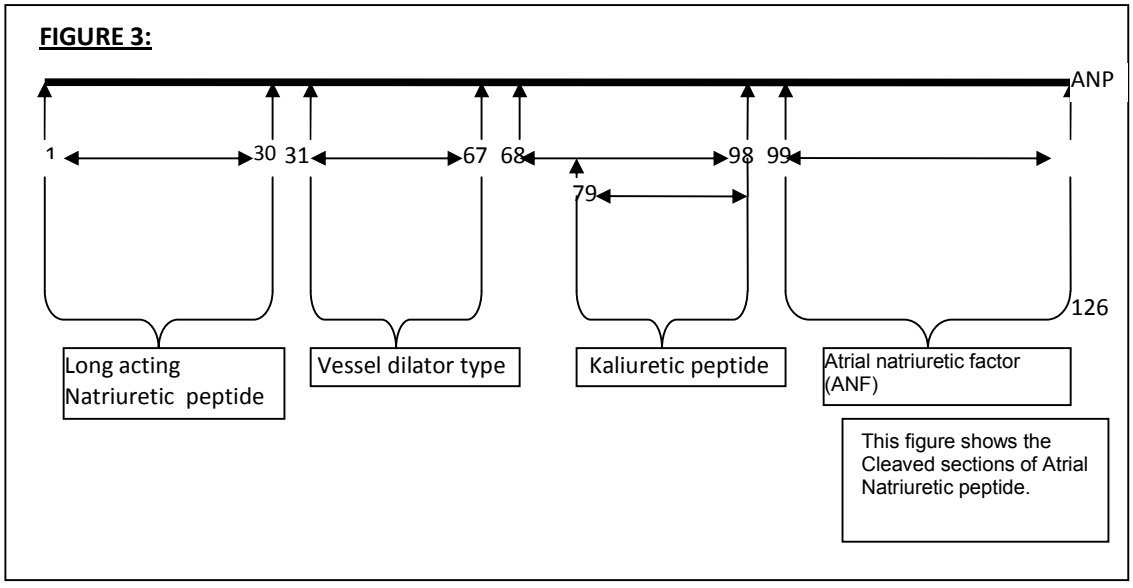

Fig. 3. The natriuretic peptides are formed as pre-hormones, and the different forms of ANP are named from where they are cleaved along the peptide chain. Each section acts as an agonist for a different physiological effect

3.4 Intracellular Mechanisms

The physiological effects of each of the natriuretic receptors are produced by complex cellular mechanisms. The fact that the same effect may be brought about by more than one receptor does not necessitate a different mechanism in each case. In fact, despite the difference in receptors, there is the same common conduction cascade involved ubiquitously via guanylate cyclase [24]. There has been recent interest in the intracellular interaction between ouabain and natriuretic peptides. It has been shown that ANP and ouabain may interact on Na(+)-K(+)-ATPase signalling and cardiac function [25]. There is significant recent evidence suggesting that endogenous ouabain is a natriuretic hormone [26] and stimulates atrial ANP secretion [27]. However this section will focus on ANP, BNP and CNP intracellular endogenous signalling.

3.5 Guanylate Cyclase Types

All of the resultant cascades and mechanisms from these peptides share certain similarities. These similarities are called 'constant cascades', and are certain substrates and reactions in the cascade sequence which are commonly co-existent in all 3 receptors (NPR-A, B and C). Each of these receptors (with the exception of NPR-C[6]) is bound intracellularly to the catalytic enzyme guanylate cyclase.

There are 2 distinct forms of guanylate cyclase. The first form is a soluble form (sGC) which is used in the intracellular signalling that results from nitric oxide ligand interaction with its receptor on vascular smooth muscle. This results in calcium uptake by sarcoplasmic reticulum, decreasing intracellular calcium concentration and thus resulting in vasodilatation. Another mechanism by which vasodilatation is brought about is via the second form of guanylate cyclase, or the 'membrane bound guanylate cyclase' (or 'mGC'). It is this membrane bound guanylate cyclase which is attached to the intracellular side of the short transmembrane part of the natriuretic peptide receptor of both NPR-A and NPR-B. Hence it is this form which leads to all of the signal transduction pathways resulting in the effects that these peptides produce. The actual enzyme family of the membrane bound guanylate cyclase is made up of 7 types; GC-A to GC-G. Although all of these enzymes are attached to receptors on the cell surface, only 3 of them have had ligands identified (namely GC-A, GC-B and GC-C), the other 4 have, as yet, no discovered complementary ligand agonist.

When considering the resultant effects signalled by ANP/BNP ligand binding GC-A (see Table 1 for ANP/BNP binding actions) [28], it is not surprising that the main tissue distribution of this mGC is in vascular smooth muscle cells, heart, kidney, adrenals, endothelium, central and peripheral nervous system, and the spleen [29]. CNP however, is the ligand for GC-B and results primarily in restoration of vascular tissue as well as the replacement of hyaline cartilage by bone tissue (i.e. endochondral ossification) [29]. Thus, it is distributed mainly in fibroblasts and other tissues.

GC-C is the signal transducer for guanylin [30] and uroguanylin, (as well as heat-stable enterotoxins) [31] and results in increased intestinal and renal water and electrolyte transport [7,31]. As a result, it is logically situated in intestinal epithelium and liver (if regenerating). As mentioned previously, the other 4 mGC's do not have identified ligands as yet, hence are referred to as 'orphan receptors'. Their functions are also unclear, but their locations are clearly identified. GC-D is present in olfactory neuroepithelium, thus it would not be too farfetched to hypothesise that it may be used in some type of cascade resulting in odour recognition. GC-E and GC-F are present in the Retina, hence may be involved in vision. GC-E is also present in the pineal gland [7] situated deep within the brain, and thus may be involved in the secretion of melatonin from this gland. As such, this mGC may play a role in the body's sleep-regulating cellular machinery. The distribution of GC-G is much more erratic; being located in skeletal muscle, lung and intestine tissues, hence it leaves no obvious clues about its physiological function.

With the mapping of the human genome, experimental approaches into establishing the functions of these 4 mGC's, and their roles in bringing about these effects are made possible. One possible experiment would involve 'knocking-out' the genetic variation coding for GC-D, GC-E and F in mice or other mammals and observing the result in terms of visual acuity and their effect on odour recognition in contrast to control 'normal' parameters. This may give a better idea as to the importance of these mGC's, as well as give possible indications for the mechanism by which they act if the intracellular

concentration of various substrates where to be systematically measured. This approach will seem unlikely to be very useful in the case of GC-G, due to its vast distribution throughout the body. However if these tissues where to be isolated and extracted from living mammals with GC-G gene deactivated or deleted, then such an experiment may prove to be quite a useful study.

When concerned with natriuretic peptides, only GC-A and GC-B are relevant. The structure of GC-A is summarised in Fig. 4. The signal transducing apparatus of the NPR-mGC is ATP-coordinated through a certain modularly domain known as the ATP-regulated module ('ARM') [32], located just above the kinase-like domain of the receptor (which also requires ATP). This module has been shown to be vital in both ATP binding, as well as in intracellular signalling of guanylate cyclase following ANP ligand binding to the NPR. ARM is present intracellularly in the sequence about 40 amino acids [32] after the transmembrane domain. It is the glycine-rich cluster this module contains which proves vital in ATP binding and thus intracellular signalling of the mGC [33]. Thus it is this glycine rich cluster which is referred to as the ATP-regulated module (ARM). It was later found that the same ARM was present in both GC-A and GC-B, i.e. the same section was involved for not only ANP and BNP signalling, but that of CNP too.

Thus ATP is vital in natriuretic peptide intracellular signalling no matter which peptide is involved, which receptor is involved, or even which mGC is used! Further research would be needed to establish whether ATP is involved in all mGC signalling. But what exactly is ATP's role? It was found that ATP is involved in mediating the transmission of the intracellular signal via the ARM by turning the receptor 'on' or 'off' [32]. ATP does this by causing an allosteric change in both the catalytic site intracellularly and extracellularly [30]. Intracellularly, this results in the guanylate cyclase's reaction which converts guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP) being hastened. This in turn produces more second messenger cGMP to initiate other intracellular cascades. Extracellularly this results in the NPR being deactivated and the natriuretic peptide ligand dissociating from the receptor and floating away. This subsequently stops further stimulation and resets the signal machinery for another ligand (see Fig. 4).

3.6 Receptor Deactivation

Although what exactly brings about this allosteric change in the NPR-A linked Guanylate cyclase A? How is this linked to the deactivation of the extracellular NPR-A? The answer lies in the increased cGMP levels produced as a result of a ligand (e.g. ANP) binding to the extracellular NPR-A portion of the receptor and stimulating guanylate cyclase A to increases conversion of GTP to cGMP. It is this cGMP which is ironically key to the receptors own deactivation. cGMP initiates serine-threonine protein kinase G (PKG) activation, and it is this enzyme PKG which then phosphorylates phosphatase PP5 by converting adenosine triphosphate to adenosine diphosphate (note: ATP's involvement again!). Phosphatase PP5, via a negative feedback loop, dephosphorylates GC-A (see Fig. 4). The desensitization of GC-A, logically decreases cGMP level. It is these series of events which results in the characteristic initial rapid increase in cGMP concentration on ANP stimulation, but on maintained ANP stimulation results in decreases in cGMP levels.

This mechanism has the advantage of being directly responsive to the outside environment. If there is high blood pressure, the heart myocytes will release more natriuretic peptide, increasing its concentration in the blood plasma. The higher the concentration, the greater the interaction with NPR, leading to increased ATP-ARM interaction, and hence more cGMP produced. Elevated cGMP levels mean increased effects, whether that is increased natriuresis, or increased vasodilatation. The point here is that the size of the effect produced is directly proportional to the concentration of natriuretic peptide in plasma, and thus directly proportional to the heart myocyte excitation or stretch. (Note, this is true up until cGMP level rises past a threshold, after which the negative feedback loop described above causes the ANP effects to subside). Overall this proves to be an incredibly simple, yet very robust mechanism [32]. It is necessary to next understand how the effects of the receptor (NPR) stimulation, mGC signalling, and cGMP second messenger formation cause each of the natriuretic peptide effects listed in Table 1.

3.7 Vasorelaxation

As discussed, the vasodilatory effect of natriuretic peptides is mediated by NPR-A and NPR-B via the ATP mediated mGC-A cascade producing cGMP, directly related to the

extracellular ANP/BNP plasma concentration. The involvement of cGMP is the same as that in the transduction of nitric oxide mediated vasodilatation. So the immediate question posed in the research community is: How does cGMP bring about the relaxation of vascular smooth muscle? In other words, we know that the resultant effect of this will be an increase in the vessel diameter, and hence increased flow and decreased blood pressure generally, but how is this vasodilatation biochemically brought about?

The answer lies in a combination of mechanisms. The first mechanism (see Fig. 5), mediated by cGMP, involves the formation of active protein kinase G by cGMP [2]. It is this protein kinase G which directly phosphorylates ion channels in the cell membrane and thus alters the ion gradients by affecting the movement of ions across the membrane.

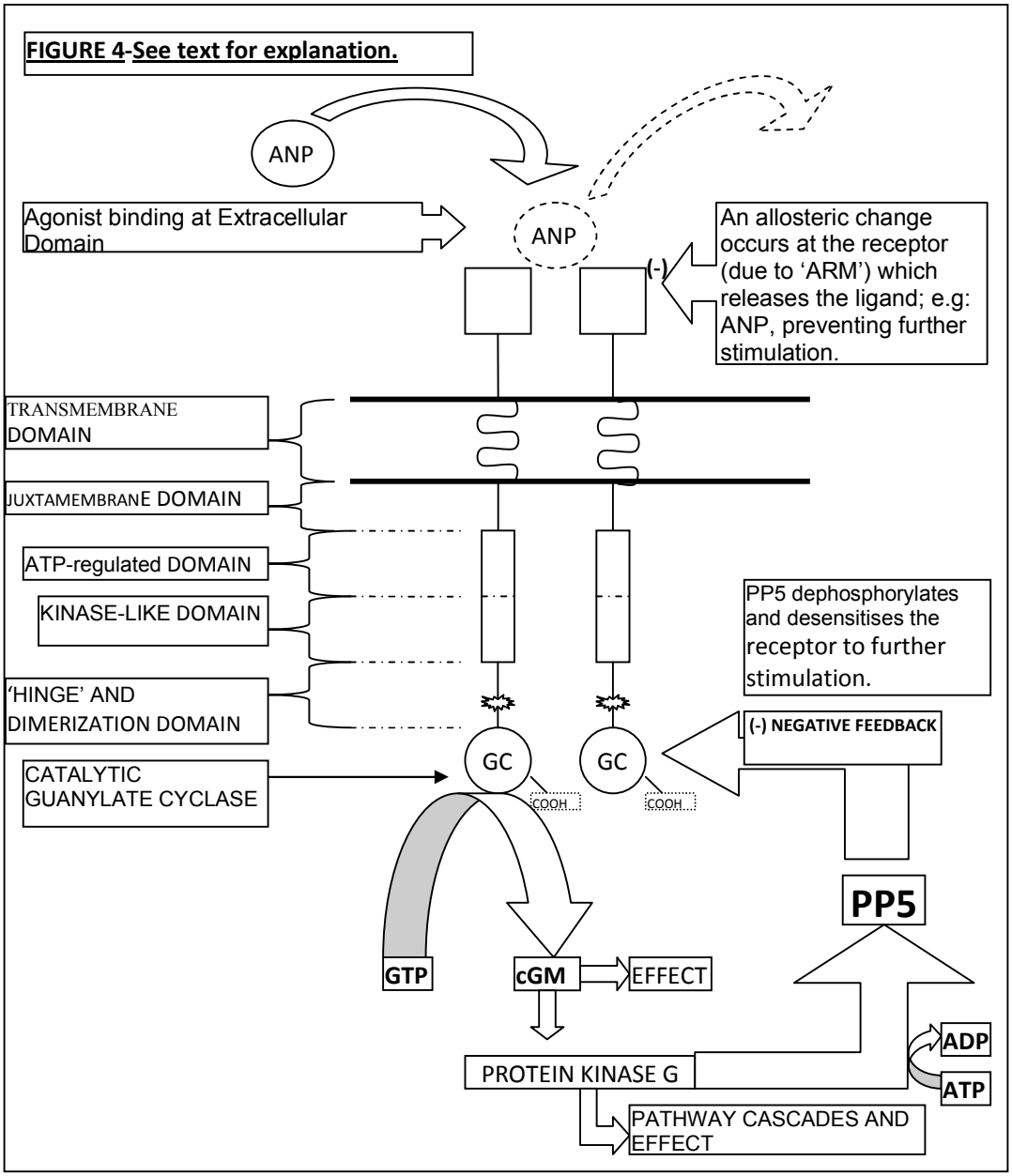

Fig. 4. Demonstrates the structure of the membrane bound guanylate cyclase receptor in addition to the intracellular transduction pathway

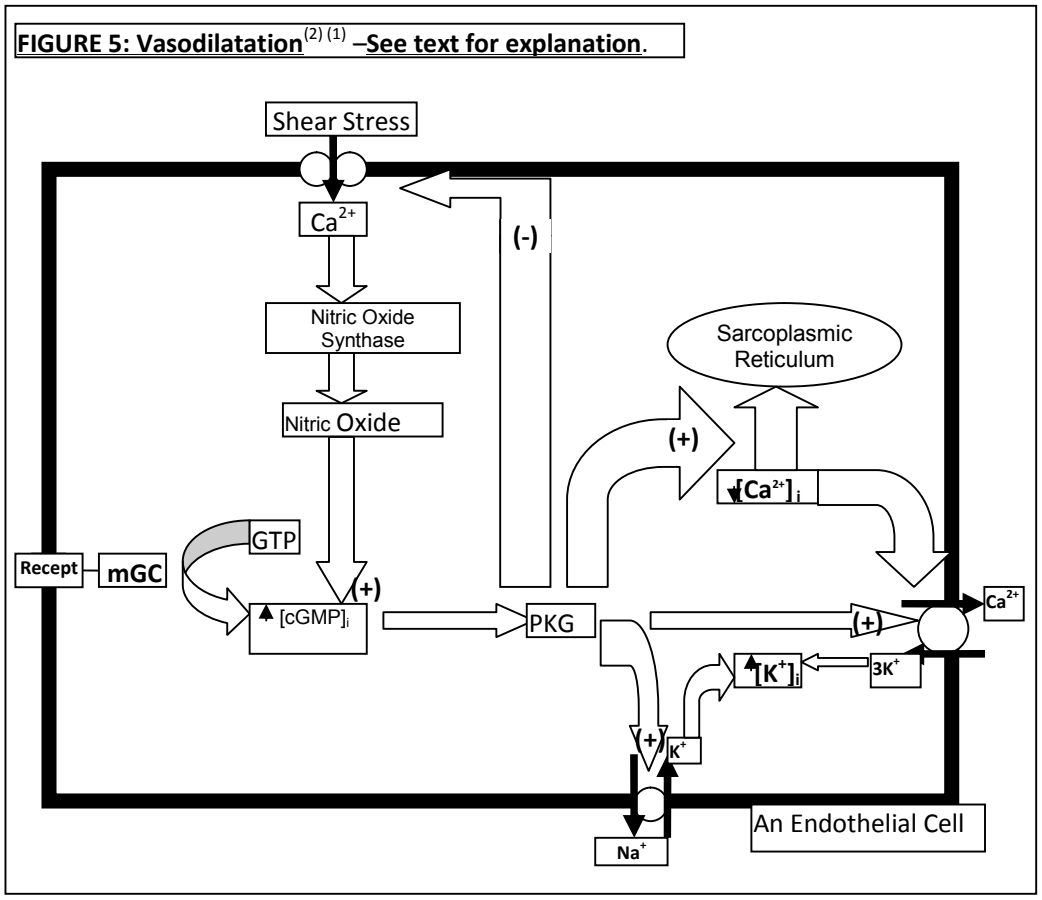

Fig. 5. Demonstrates in the intracellular cascade resulting in vasodilatation

The main ion channels affected seem to be the calcium and potassium transporters. By activating the calcium-potassium co-transport channels, the cell actively pumps calcium out of the cell against its diffusion gradient (there is 12,000 times more calcium outside the cell than inside!). Hence by decreasing intracellular calcium concentration, vasodilatation occurs. The obvious end result is that intracellular potassium concentration increases. Protein kinase G may also have some effect on the sarcoplasmic reticulum to increase cytoplasmic calcium uptake, thus potentiating the decrease in intracellular calcium, a substrate vital for the smooth muscle actin and myosin filaments contractile mechanism, thus the result is a decrease in vasocontraction. However further research is needed in this field concerning the finer mechanisms of this action.

Experiments, however, have shown that cGMP-dependent protein kinase activates calcium-activated potassium channels in cerebral artery smooth muscle cells [34]. In this experiment, it was shown that when the intracellular surface was exposed to cGMP-PK, with ATP and cGMP directly, the channel activity increased by approximately eight times. Other experiments testing related areas of this field have found similar results. One such experiment found that an endogenous PKG activates calcium channels by decreasing the Ca^{2+} and voltage activation thresholds independently of sensitivities [35]. All of these experiments however, suggest that protein kinase G is vital for the transduction of vasorelaxation.

Other experiments however, show that another mechanism involved is the inhibition of independent potassium channels; thus inhibiting re-polarisation hence resulting in inhibition of contraction. This was found in pulmonary vascular endothelial cells [15] and involves the inhibition of inwardly rectifying potassium channels [15]. What's more, this experiment shows it is possible for vasorelaxation to occur

without the involvement of PKG, in what is termed 'PKG independent vasorelaxation'. This is very interesting as PKG has long been thought of as a prime transduction enzyme when considering more studied forms of vasorelaxation, i.e. Nitric Oxide induced [21].

In an experiment a protein kinase G-sensitive channel propagated flow-mediated calcium entry into vascular endothelial cells [21]. Although the stimulus for vasorelaxation was different (in this case it was shear stress), the standard NO mechanism involved used the PKG as would be expected (Fig. 5). So these new findings suggest there is more to cGMP calcium affecting signal transduction than was otherwise expected, however what all of these studies show is that this certainly is an area for clarification and further study. Fig. 5 shows this concept as well as serving as an integrated summary of all of the processes discussed above.

As well as being involved in vasodilatation, ANP also conducts vasoconstriction in specialised parts of the body; as experiments on rat kidney show [36]. In this experiment, it was proved that in a rat kidney, ANP resulted not only in pre-glomerular vasodilatation, but also post-glomerular vasoconstriction. This seems like a very perplexing idea of how the same ligand can cause two opposite effects in two different parts of the same tissue. The mechanism behind this is as yet still a very mysterious area of research. Experiments which may help our understanding of this problem could involve 'knocking-out' (deactivating the genes coding for) the downstream substrates directly linked to cGMP and investigated the physiological result on the two areas of the glomeruli and seeing whether vasoconstriction or vasodilatation is affected. This may give us a key insight into the biochemical differences in the mechanisms of each action, and aid our understanding of the differential intracellular pathways involved. What we can consider, however, is why these opposite effects are significant? Well, the post glomerular constriction increases the filtration pressure and thus helps in natriuresis. The results from this study link the vaso-action of natriuretic peptides with their natriuretic properties. This brings us nicely to the section of this review.

3.8 Natriuresis

Natriuresis is the secretion of sodium ions into the urine and thus there loss from the body [37,38]. Natriuretic peptide natriuresis is mediated mainly by ANP via NPR-A activation. It has been established that it is the NPR-A receptor [12] which mediates the natriuretic and diuretic renal responses to acute blood volume expansion [12], and it seems that ANP not only stimulates the secretion of sodium in the collecting duct of the nephron [39] through a prostaglandin E2-dependent mechanism [37], but also prevents its re-absorption.

The mechanism reducing sodium re-absorption involves a pathway mediated by BNP and CNP as well as urodilatin and atrial natriuretic factor, all which, as expected act by stimulating the formation of cGMP from GTP when binded to their respective Guanylate cyclase (either GC-A or GC-B) linked receptors (NPR-A or NPR-B). cGMP in turn can either directly act on transmembrane sodium transporter channel (via the same mechanism as mentioned previously when discussing calcium channels in the initiation of vasorelaxation) [37], or cGMP may go on to initiate the activation of protein kinase G, which in turn may, via various phosporylated proteins [40], act on the cyclic nucleotide –gated cation channel to inhibit intake of sodium by the cell [40]. From further study, it appears that of these two mechanisms, direct cGMP action is the more important factor in inhibiting this channel.

Protein kinase G however, also has another important role, namely in the stimulation of the cell membrane sodium/potassium active transport channel. This is a channel type which uses energy in the form of dephospohorylating ATP to ADP to pump sodium ions out of the cell, and potassium into the cell, against the chemical gradients of both respective ions. The result is obviously an increase in natriuresis by not only this increased sodium secretion, but of the reduced reabsorption too [37]. This particular channel seems to also be mediated by ANP via prostaglandin E2 (PGE2). ANP (cleavage types 1-30, 31-67, and 79-98) acts on its respective receptor, causing the cyclo-oxygenase mediated conversion of arachidonic acid to PGE2 [37]. PGE2 then acts on the sodium/potassium anti-port channel to increase its activity and secretion of these respective ions.

As well as its effects on the actual secretion of sodium, intracellular cGMP has been shown in experiments to be involved in the modulation of permeability of macromolecule across aortic endothelial cells [41]. In this experiment it was shown that depending on $[Ca2+]_i$, cGMP can play opposite roles in endothelial permeability in a

single cell, increasing, or decreasing the cell's permeability, findings which may prove to have direct relevance in the kidneys when considering filtration rates related to pre-glomerular and post-glomerular vessel relaxation and constriction.

Natriuresis brings about a decrease in blood volume; however there may be a more direct way that these peptides decrease blood volume. Recent evidence suggests atrial natriuretic factor may stimulate the insertion of aquaporin 2 in renal epithelial cells by cGMP-dependent membrane, thus increasing the diuretic response following natriuresis [42].

3.9 Inhibition of RAAS

NPR-A mediates the inhibition of the renin-angiotensin-aldosterone-system (RAAS). This system has hypertensive properties and thus is essential in severe haemorrhages since it potentiates events such as preservation of sodium (anti-natriuresis), vasoconstriction, thirst and modulation of glomerular filtration rate (GFR) [43]. However, in times of pathological hypertension, these events would obviously prove detrimental to the homeostasis of constant blood pressure as they will exacerbate an already high blood pressure by further increasing it. The release of natriuretic peptides inhibits all of these effects by directly inhibiting the RAAS system, hence potentiating the reverse of these events and lowering blood pressure [44]. It can therefore be said that ANP is the endogenous antagonist of the renin angiotensin aldosterone system. The two systems together, allow a fine balance between blood pressure and plasma volume to be established within the host [45,46].

The question however, is how do natriuretic peptides inhibit the RAAS? The answer proves to be just as intricate as the RAAS-system itself. The mechanism lies in the actual gene transcription of the renin mRNA and its expression [47]. The formation of aldosterone is by aldosterone synthase, an enzyme coded for by the gene CYP11B2. A recent experiment [14] has shown that natriuretic peptides reduce CYP11B2 mRNA expression in cultured neonatal rat cardiocytes. Hence they may inhibit the cardiac RAAS and restrain cardiac hypertrophy and fibrosis [14]. What's more, recent studies have shown that ANP and BNP also inhibit aldosterone synthase in cultured human adrenal cells [48].

3.10 Cell Proliferation

Apart from the obvious vasorelaxation, natiuretic and diuretic actions of natriuretic peptides, recent evidence suggests more subtle roles for these peptides in the general physiological activities of the body. An example is experimental evidence suggesting ANP may have an important role in the fetal development of the human heart due to its activity in up regulating the RAAS in cardiac fibroblasts [49]. So much so, that studies have shown that mice lacking BNP resulted in increased proliferation [50] of interstitial fibroblasts and cardiac fibrosis [19]. This established BNP to be a potent antifibrotic factor in mice, hence significant in ventricular remodelling in pathological diseases such as acute myocardial infarction [19]. When considering human development, a whole host of new research begins to show that these peptides may be more important than they may seem at first inspection. When considering fetal development, CNP has been shown to be associated with increased endochondral ossification, or bone growth in fetal mouse tibias [22].

Recent experimental results show that natriuretic peptides play a role in increasing neuroblastoma cell proliferation. These natriuretic peptides stimulate this cell proliferation acting via NPR-A and B receptors [51]. However the same experiment has shown that ANP and BNP in higher levels actually have the opposite effect, inhibiting proliferation, acting via a different receptor which isn't, interestingly, guanylate cyclase linked. These results were later confirmed in pulmonary endothelial cells where it was found that when intracellular cGMP concentrations ([cGMP]i) were increased and kept constantly high, they induce apoptosis, or programmed cell destruction as well as inhibited additional cell growth [18].

As well as in pulmonary endothelial cells, ANP had earlier also been shown to be a participating cause of apoptosis in cultured cardiac myocytes. This is interesting as these are the very cells which originally produce this peptide! As the other studies show, this case also seems to act via guanylate cyclase -mediated increases in intracellular cGMP concentration. However, the same study concerning ANP-mediated cardiac myocyte apoptosis showed that noradrenaline seems to potentiate cell survival and that in this case ANP may compete with noradrenaline, -regulating cardiac cell death or growth. This

seems fascinating since noradrenaline acts via adenylate cyclase and cyclic adenosine monophosphate (cAMP), whereas ANP acts by the different, but physiological similar cGMP. This gives the impression that there is a balance between these 2 factors when the cell is not dividing and growing [52].

Contrarily, and quite confusingly, other research, such as that looking into the effects of ANP and BNP on pheochromocytoma cells (a vascular tumour of the adrenal gland) seems to give the opposite results. The study on this cell type indicates that sustained increases in cGMP as a result of these natriuretic peptides actually extend the life of these cells and prevent apoptotic DNA fragmentation, and thus cell death [53].

These results are supported by other studies looking at the ANP and NO triggered increases in intracellular cGMP affecting the growth stimulating noradrenaline action in cardiac myocytes and fibroblasts. The results again showed ANP-cGMP mediated inhibition of growth stimulated by noradrenaline [17].

These contradicting studies give a very mixed and confusing picture about the intracellular mechanism causing the effects of ANP and BNP on cells with relation to cell survival. Most of the established literature tend to agree that there is an increase in cGMP, however what happens after this is not only disputed mechanistically, but also physiologically regarding whether it generally causes apoptosis, or cell growth. It seems that some tissues respond to increased cGMP as a result of ANP or BNP by growth, like pheochromocytoma cells, whereas others respond by cell death, like cardiac myocytes and pulmonary endothelial cells. It seems that ANP may be considered quite a noteworthy factor in cell growth, one which should not be overlooked in significance. It also indicates that much more detailed research should be done into what differences are present between the cells that ANP causes to undergo apoptosis, and those cells in which it stimulates cell growth. Particularly, study into the changes in intracellular biochemical substrates linked to phosphorylation by cGMP will give a better insight in solving this mystery.

3.11 Clearance of Natriuretic Peptides

If natriuretic peptides are released consistently during acute increases in blood volume, why doesn't their natriuretic effect propagate and build on the previous concentration? In other words, why don't we continuously loose large amounts of sodium in the urine? The answer lies in the removal of the peptides from the blood plasma and thus neutralisation of their bioavailability [11]. This clearance is done by membrane endopeptidase and NPR-C [8], which have a similar affinity for all 3 peptide types. As discussed previously; NPR-C is different from the other two natriuretic peptide receptors in that it is not guanylate cyclase linked, and does not have a ATP-regulated module ('ARM') [32] or a kinase-like domain (KLD) associated with it, as a result, it is characteristically structurally shorter than the other two receptors.

This leads to some interesting intracellular signalling properties. When addressing these, we must first ask the question; how does NPR-C clear these peptides? It is generally agreed now that NPR-C works by internalizing the peptide ligand bound to its extracellular receptor, before degrading the peptide intracellulary by lysosomal enzyme hydrolysis [54]. After this cycle is complete, the NPR-C receptor returns to the surface of the cell to await another unsuspecting natriuretic peptide. ANP binding fails to inhibit new NPR-C synthesis; thus, down-regulation of NPR-C is not due to the previously discussed agonist-mediated negative feedback inhibition of the receptor as is the case in NPR-A and NPR-B[54]. It suggests that the down regulation of this receptor is due to the recycling of the receptors by internalisation [23,54]. This recycling seems to occur once every 1 hour.

4. CONCLUSIONS AND OUTLOOK

It seems apparent that the glycine rich ATP-regulated module (ARM) is vital for conveying signal from the extracellular receptor binding natriuretic peptide ligand to the intracellular guanylate cyclase. ATP is responsible for the allosteric changes resulting in the activation of guanylate cyclase, thus increasing cGMP production, as well being responsible for the receptor allosteric change causing the ligand to dissociate. cGMP is a crucial second messenger for both NPR A and NPR B when it is not linked to protein kinase G. PP5 is responsible for the negative feedback loop deactivating the guanylate cyclase C receptor. It is internalisation, and not desensitisation which is the mechanism used by NPR-C to remove these peptide ligands from the circulation. Protein kinase G's effects on ion transporters are responsible for the natriuretic

and vascular tone effects, and action on mRNA transcription results in RAAS inhibition.

This article has dealt with the recent advances in the mechanisms of action of natriuretic peptides at a cellular level as well as explored the cell proliferative effects of these peptides. However, this is a field which is rapidly progressing and expanding. As newer and more precise detection technologies develop, more and more intricate intracellular signalling pathways and messenger substrates are elucidated. However in light of this, this article has focused on the most recent developments in the field and has attempted to piece together the signalling pathways of these peptides during natriuresis, vasodilatation and other attributed effects. It is clear that although natriuretic peptides offer significant pharmacological potential, this field is complex and remains poorly understood. Evidently, there is still a need for more research on this topic in order to strengthen understanding and to develop pharmacological target sites for treatment of medical conditions.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Yap LB, et al. Natriuretic peptides, respiratory disease, and the right heart. Chest, 2004;126(4):1330-6.
2. Woodard GE, Rosado JA, Natriuretic peptides in vascular physiology and pathology. Int Rev Cell Mol Biol. 2008; 268:59-93.
3. Okolicany J, et al. Bilateral acute heart atrial auriectomy reduced diuresis and natriuresis following hypertonic sodium load in anaesthetized dogs. Physiol Bohemoslov. 1989;38(2):179-87.
4. Ponec J, Lichardus B. On the role of increased blood pressure in the mechanism of volume natriuresis and polyuria in rats. Physiol Bohemoslov.1974. 23(3):261-9.
5. Anand-Srivastava MB. Natriuretic peptide receptor-C signaling and regulation. Peptides. 2005;26(6):1044-59.
6. Das BB, R Solinger. Role of natriuretic peptide family in cardiovascular medicine. Cardiovasc Hematol Agents Med Chem. 2009;7(1):29-42.
7. Rubattu S, Volpe M. The atrial natriuretic peptide: a changing view. J Hypertens. 2001;19(11):1923-31.
8. Sagnella GA. The heart as an endocrine organ. Biologist (London). 2002;49(6):275-9.
9. Levin ER, Gardner DG, WK. Samson, Natriuretic peptides. N Engl J Med. 1998; 339(5):321-8.
10. Del Ry S, Cabiati M. Clerico A. Natriuretic peptide system and the heart. Front Horm Res. 2014;43:134-43.
11. Potthast R, Potter LR. Phosphorylation-dependent regulation of the guanylyl cyclase-linked natriuretic peptide receptors. Peptides. 2005;26(6):1001-8.
12. Shi SJ, et al. Natriuretic peptide receptor A mediates renal sodium excretory responses to blood volume expansion. Am J Physiol Renal Physiol. 2003;285(4): F694-702.
13. Hirose S, Hagiwara H, Takei Y. Comparative molecular biology of natriuretic peptide receptors. Can J Physiol Pharmacol. 2001;79(8):665-72.
14. Ito T, et al. Inhibitory effect of natriuretic peptides on aldosterone synthase gene expression in cultured neonatal rat cardiocytes. Circulation;2003;107(6):807-10.
15. Shimoda LA, Welsh LE, Pearse DB, Inhibition of inwardly rectifying K(+) channels by cGMP in pulmonary vascular endothelial cells. Am J Physiol Lung Cell Mol Physiol. 2002;283(2):L297-304.
16. Elesgaray R, et al. Signaling cascade that mediates endothelial nitric oxide synthase activation induced by atrial natriuretic peptide. Regul Pept. 2008;151(1-3):130-4.
17. Calderone A. et al. Nitric oxide, atrial natriuretic peptide, and cyclic GMP inhibit the growth-promoting effects of norepinephrine in cardiac myocytes and fibroblasts. J Clin Invest. 1998;101(4):812-8.
18. Zhu B, Strada S, Stevens T. Cyclic GMP-specific phosphodiesterase 5 regulates growth and apoptosis in pulmonary endothelial cells. Am J Physiol Lung Cell Mol Physiol. 2005;289(2):L196-206.

19. Tamura N, et al., Cardiac fibrosis in mice lacking brain natriuretic peptide. Proc Natl Acad Sci U S A. 2000;97(8):4239-44.
20. Ma L, Xiang X. Atrial natriuretic peptide/natriuretic peptide receptor A (ANP/NPRA) signaling pathway: a potential therapeutic target for allergic asthma. Med Hypotheses. 2011;77(5): 832-3.
21. Yao X, et al. A protein kinase G-sensitive channel mediates flow-induced Ca(2+) entry into vascular endothelial cells. FASEB J. 2000;14(7):932-8.
22. Yasoda A, et al., Natriuretic peptide regulation of endochondral ossification. Evidence for possible roles of the C-type natriuretic peptide/guanylyl cyclase-B pathway. J Biol Chem. 1998. 273(19): 11695-700.
23. Nussenzveig DR, Lewicki JA, Maack T, Cellular mechanisms of the clearance function of type C receptors of atrial natriuretic factor. J Biol Chem. 1990; 265(34):20952-8.
24. Hartmann M, et al. Alternative splicing of the guanylyl cyclase-A receptor modulates atrial natriuretic peptide signaling. J Biol Chem. 2008;283(42):28313-20.
25. Nesher M, et al. Interaction of atrial natriuretic peptide and ouabain in the myocardium. Can J Physiol Pharmacol, 2012;90(10):1386-93.
26. Nesher M, et al. Physiological roles of endogenous ouabain in normal rats. Am J Physiol Heart Circ Physiol, 2009;297(6): H2026-34.
27. Liu LP, et al. Ouabain stimulates atrial natriuretic peptide secretion via the endothelin-1/ET(B) receptor-mediated pathway in beating rabbit atria. Life Sci, 2012;90(19-20):793-8.
28. Wu ZJ, et al. [Recent advances in natriuretic peptide family genes and cardiovascular diseases]. Yi Chuan;34(2): 127-33.
29. Kuhn M. Structure, regulation, and function of mammalian membrane guanylyl cyclase receptors, with a focus on guanylyl cyclase-A. Circ Res. 2003;93(8):700-9.
30. Forte LR, et al. Mechanisms of guanylin action via cyclic GMP in the kidney. Annu Rev Physiol. 2000;62:673-95.
31. Fonteles MC, Do Nascimento NR. Guanylin peptide family: history, interactions with ANP, and new pharmacological perspectives. Can J Physiol Pharmacol. 2011;89(8):575-85.
32. Duda T, et al. ATP-regulated module (ARM) of the atrial natriuretic factor receptor guanylate cyclase. Peptides, 2005;26(6):969-84.
33. Goraczniak RM. Duda T, Sharma RK. A structural motif that defines the ATP-regulatory module of guanylate cyclase in atrial natriuretic factor signalling. Biochem J. 1992;282(Pt 2):533-7.
34. Robertson BE, et al. cGMP-dependent protein kinase activates Ca-activated K channels in cerebral artery smooth muscle cells. Am J Physiol. 1993;265(1 Pt 1): C299-303.
35. Stockand JD, Sansom SC. Mechanism of activation by cGMP-dependent protein kinase of large Ca(2+)-activated K+ channels in mesangial cells. Am J Physiol, 1996;271(5 Pt 1):C1669-77.
36. Marin-Grez M, Fleming JT, Steinhausen M. Atrial natriuretic peptide causes pre-glomerular vasodilatation and post-glomerular vasoconstriction in rat kidney. Nature. 1986;324(6096):473-6.
37. Beltowski J, G. Wojcicka. Regulation of renal tubular sodium transport by cardiac natriuretic peptides: two decades of research. Med Sci Monit, 2002;8(2):RA39-52.
38. Lichardus B. Natriuretic hormones in volume natriuresis. Physiol Res;1991. 40(2):161-8.
39. Sonnenberg H, et al. Atrial natriuretic factor inhibits sodium transport in medullary collecting duct. Am J Physiol, 1986;250(6 Pt 2):F963-6.
40. Zeidel ML. Renal actions of atrial natriuretic peptide: regulation of collecting duct sodium and water transport. Annu Rev Physiol. 1990;52:747-59.
41. Holschermann H, et al. Dual role of cGMP in modulation of macromolecule permeability of aortic endothelial cells. Am J Physiol. 1997;272(1 Pt 2):H91-8.
42. Bouley R, et al. Nitric oxide and atrial natriuretic factor stimulate cGMP-dependent membrane insertion of aquaporin 2 in renal epithelial cells. J Clin Invest, 2000;106(9):1115-26.
43. Munoz MC, et al. Downregulation of the ACE2/Ang-(1-7)/Mas axis in transgenic mice overexpressing GH. J Endocrinol. 2014;221(2):215-27.
44. Ogawa, Y, et al. Natriuretic peptide receptor guanylyl cyclase-A protects

podocytes from aldosterone-induced glomerular injury. J Am Soc Nephrol. 2012. 23(7):1198-209.
45. Johnston CI, et al. Interaction between atrial natriuretic peptide and the renin angiotensin aldosterone system. Endogenous antagonists. Am J Med. 1989;87(6B):24S-28S.
46. Demerath T, et al. Natriuretic peptides buffer renin-dependent hypertension. Am J Physiol Renal Physiol. 2014;306(12): F1489-98.
47. Song W, Wang H, Q. Wu. Atrial natriuretic peptide in cardiovascular biology and disease (NPPA). Gene; 2015.
48. Kudo T, Baird A. Inhibition of aldosterone production in the adrenal glomerulosa by atrial natriuretic factor. Nature. 1984; 312(5996):756-7.
49. Sanghi S, et al. Activation of protein kinase A by atrial natriuretic peptide in neonatal rat cardiac fibroblasts: role in regulation of the local renin-angiotensin system. Regul Pept. 2005;132(1-3):1-8.
50. Dhingra H, Roongsritong C,. Kurtzman NA. Brain natriuretic peptide: Role in cardiovascular and volume homeostasis. Semin Nephrol. 2002;22(5):423-37.
51. Lelievre V, et al. Proliferative actions of natriuretic peptides on neuroblastoma cells. Involvement of guanylyl cyclase and non-guanylyl cyclase pathways. J Biol Chem. 2001;276(47):43668-76.
52. Wu CF, Bishopric NH, Pratt RE. Atrial natriuretic peptide induces apoptosis in neonatal rat cardiac myocytes. J Biol Chem. 1997;272(23):14860-6.
53. Fiscus RR, Tu AW, Chew SB. Natriuretic peptides inhibit apoptosis and prolong the survival of serum-deprived PC12 cells. Neuroreport. 2001;12(2):185-9.
54. Levin ER. Natriuretic peptide C-receptor: more than a clearance receptor. Am J Physiol. 1993;264(4 Pt 1):E483-9.

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