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Signalling by cGMP-dependent protein kinases

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Abstract

The second messenger cGMP is a major intracellular mediator of the vaso-active agents nitric oxide and natriuretic peptides. The principal targets of cGMP are (i) phosphodiesterases, resulting in interference with the cAMP-signalling pathway, (ii) cGMP-gated cation channels, and (iii) cGMP-dependent protein kinases (cGKs). Only two mammalian isotypes of cGK have been described so far: type I cGK, consisting of an α and a β isoform, presumably splice variants of a single gene, and identified as the most prominent cGK isotype in the cardio-vascular system; and type II cGK, expressed mainly in the intestine, the kidney and the brain. High levels of cGK I are found in vascular smooth muscle cells, endothelial cells and platelets. In these cells, cGK I is thought to counteract the increase in contraction provoked by Ca-mobilizing agonists, to reduce endothelial permeability and to inhibit platelet aggregation, respectively. Relatively low levels of cGK I are found in cardiomyocytes. In this cell type, cGK is implicated in the negative inotropic effect of cGMP, presumably through modulation of Ca channels and by diminishing the Ca-sensitivity of contractile proteins. (Mol Cell Biochem **157**: 23–30, 1996)

Key words: cyclic GMP, nitric oxide, natriuretic peptides, guanylyl cyclase, cardiomyocytes, endothelium

Introduction

Cyclic GMP (cGMP) was discovered in the early 1960s as the result of a search for analogs of the second messenger cyclic AMP (cAMP) [1]. In contrast to cAMP, however, cGMP remained rather obscure because initially no clear physiological function of cGMP could be established. This was largely due to the lack of selective agonists of guanylyl cyclases, the enzymes which convert GTP into cGMP. The interest in cGMP signalling changed with the discovery in the 1970s that NO-donating vasodilators, like nitroglycerin and nitroprusside relax smooth muscle by activating a soluble form of guanylyl cyclase [2]. Furthermore, cGMP was found to be the intracellular mediator of severe secretory diarrhoea provoked by heat-stable enterotoxins (STs) secreted by certain enteropathogenic bacteria [3]. The subsequent discovery of endogenous activators of the cGMP-signalling pathway, i.e. natriuretic peptides, guanylin and, most importantly, nitric oxide (NO), led to the recent appreciation of the importance of cGMP in the (patho)physiological regulation of numerous cellular processes [4, 5].

In this article we will briefly review the synthesis and function of cGMP in the cardio-vascular system and the molecular mechanisms of cGMP-signalling. We will focus on the role of cGMP-dependent protein kinases (cGK) in mediating the effects of cGMP, although other intracellular cGMP receptors, including ion channels and phosphodiesterases, may also play a role and may contribute to the complexity of signal transduction by cGMP.

Regulation of cGMP synthesis and biological functions of cGMP

Natriuretic peptides and guanylin

The fist natriuretic peptide discovered was purified from an atrial extract and was therefore named atrial natriuretic peptide (ANP) [6, 7]. ANP is a low molecular weight peptide derived from a larger precursor protein (ANF) synthesized predominantly in atrial cells. It is released upon a volume load of the heart and has profound hypotensive activity [8]. The blood pressure lowering effect of ANP is a consequence of vascular smooth muscle relaxation and an increased natriuresis in the kidneys, as a result of direct effects of ANP on smooth muscle and kidney cells as well as indirect effects through interference with the sympathetic neuronal system,

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the renin-angiotensin system and the release of aldosterone and vasopressin [8, 9].

Besides ANP two other natriuretic peptides were identified, BNP and CNP, which likewise have hypotensive actions [10, 11]. Being isolated originally from brain tissue, BNP and CNP are now known to be more ubiquitously expressed [9]. As discussed below natriuretic peptides exert most of their effects through stimulation of membrane-bound guanylyl cyclases and an increase in cGMP levels. However a minor part of their biological actions may be mediated by the so-called natriuretic peptide clearance receptor, NPR-C [12], which is not coupled to guanylyl cyclase, but might signal through interaction with adenylyl cyclase or phospholipase C or by lowering the concentration of natriuretic peptides in the circulation [9].

Recently, another low-molecular weight peptide, named guanylin, was isolated from intestinal mucosa, and was found to elevate cGMP levels in intestinal epithelial cells [13]. Guanylin was shown to stimulate the intestine-specific membrane-bound guanylyl cyclase (GC-C) identified previously as a target for microbial heat-stable enterotoxins [9, 13]. Until recently, guanylin is considered primarily as a physiological regulator of intestinal water and salt transport [13,14]. However the presence of proguanylin in serum, and the discovery of uroguanylin, a guanylin-related peptide in urine hint at a more general role of this class of cGMP-linked hormones in mammalian physiology [14].

Nitric oxide

A short-lived endothelium-derived relaxing factor (EDRF), which is produced in endothelial cells in response to Ca-mobilizing stimuli like acetyl choline and shear stress, was subsequently identified as the free radical NO, a known stimulator of soluble guanylyl cyclase [15, 16]. NO is generated from L-arginine by the enzyme NO-synthase (NOS) [5]. Two general classes of NOS can be distinguished, a constitutive (cNOS) form, which is stimulatable by Ca/calmodulin, and an inducible form (iNOS), whose expression is upregulated by cytokines and endotoxins. cNOS is expressed in a wide variety of cell types throughout the cardiovascular system, and its activity can be modulated by various vaso-active hormones [5, 17]. Conceivably therefore, this isoenzyme is responsible for the physiological activation of the NO-cGMP signalling pathway in the cardio-vascular system. In contrast, massive amount of NO produced by iNOS after its induction in white blood cells by cytokines is thought to play a role as a defence mechanism against pathogens by virtue of its cytotoxicity as a free radical. In some pathological cases, however, iNOS can be induced in cells of the cardio-vascular system cells by endotoxins and cytokines. The overflow of NO generated by it may cause a septic shock by hyper-activation of the vascular NO-cGMP system causing a sometimes lethal hypotension [17–19]. The dramatic role of iNOS in septic shock was demonstrated by the beneficial effects of NOS inhibitors in patients [18], and by observations in transgenic mice lacking iNOS, which were more susceptible to certain pathogens, but resistant to endotoxin-induced shock [19].

Evidence has been obtained recently that another gaseous molecule, carbon monoxide (CO), generated by heme oxygenase, is also able to activate soluble GC, and to function as a cGMP-linked neuro-hormonal agent signalling through cGMP. The observation that vascular smooth muscle cells can generate CO in response to specific stimuli, suggests a role for a CO-cGMP pathway in addition to the NO-cGMP pathway in cardiovascular homeostasis [20].

Guanylyl cyclases

Guanylyl cyclases (GC) can be divided into two general groups, soluble GCs and membrane GCs (See Fig. 1) [9, 21]. Soluble GCs (GC-S) are expressed in almost all cell types in the cardiovascular system, including cardiomyocytes, vascular smooth muscle cells, endothelial cells and platelets [9]. GC-S functions as a heterodimer composed of an α and a β subunit. At least two α and two β isoforms are identified by molecular cloning [9], but the physiological relevance of the various isoforms is as yet unknown. Soluble GCs contain a prosthetic heme group, which functions as receptor for NO and CO, the putative physiological activators [21].

Membrane GCs combine both ligand binding and catalytic activity in a single poly-peptide chain which consists of a N-terminal extracellular receptor domain, a transmembrane segment, a domain homologous to protein kinases and a C-terminal catalytic domain [9, 21]. The enzyme probably functions as a homotetra or- trimer stabilized by interactions between the receptor domains [21, 22]. After binding of the ligand (natriuretic peptides or guanylin) a conformational shift is induced resulting in an interaction between two of the catalytic domains (internal dimerization), which in the absence of ligand is probably prevented by the kinase homology domain [21, 22]. Six isoforms have been cloned so far [9, 21, 23]. GC-A and GC-B (also termed NPR-A and NRP-B) were shown to function as natriuretic peptide receptors, and preferentially bind ANP/BNP and CNP respectively (See Fig. 1). Both GC-A and GC-B are distributed widely in the body [9], but the relative contribution of the ANP/BNP-GC-A route versus the CNP-GC-B pathway to cardiovascular homeostasis is not fully elucidated. The first system seems more important, functioning as a classical endocrine pathway; ANP and BNP are derived predominantly from the heart and affect distant targets like blood vessels and kidneys [8, 9]. The second system might act more locally as CNP was detected in endothelial cells, and was shown to relax vascular smooth muscle preparations by stimulating GC-B [9, 24].

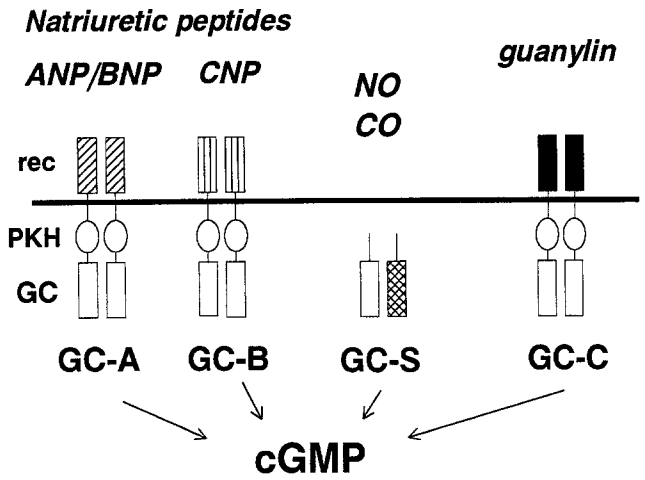

Fig. 1. Guanylyl cyclases and their ligands. Soluble guanylyl cyclases (GC-S) are heterodimers and contain a prosthetic heme group functioning as a receptor for nitric oxide (NO) and carbon monoxide (CO). The membrane GCs (GC-A, GC-B and GC-C) consist of two to four monomers, which each consists of an extracellular receptor domain (rec) and an intracellular protein kinase homology (PKH) and guanylyl cyclase (GC) domain.

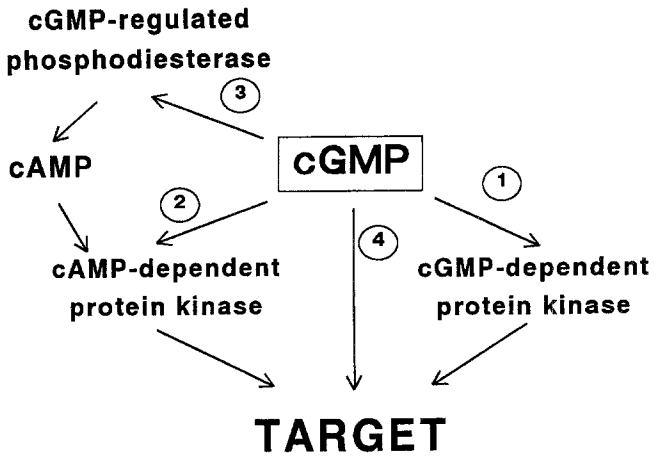

Fig. 2. Potential pathways by which cGMP may exert its physiological effects.

GC-C was identified as a receptor for guanylin and heat-stable enterotoxin and localized predominantly in intestinal epithelium [9, 25]. Conceivably, the (uro)guanylin-GC-C pathway may have a small effect on blood volume by regulating water absorption in intestine. GC-D is found in olfactory tissue, whereas GC-E and GC-F seem retina-specific [23]. For these three GCs no ligands have been found, but their enzyme activity is regulated by internal Ca [21, 23].

General mechanisms of cGMP function

Apart from its natriuretic and vasodilative effects mentioned above, cGMP was shown to inhibit platelet aggregation, to reduce the permeability of endothelial layers and to exert a negative inotropic effect on cardiomyocytes [5, 26, 27]. In general cGMP seems to counteract the effects of vasoconstricting and platelet aggregating hormones e.g. endothelin, angiotensin II, adrenaline and thrombin, and thus to protect the organism against hypertension and excessive sympathetic activation of the heart. Furthermore cGMP was shown to inhibit the proliferation of smooth muscle cells [28].

cGMP may exert its regulatory functions by interacting with various cGMP receptor proteins as summarized in Fig. 2.

1. In most cell types, including those of the cardio-vascular system the effects of cGMP are mediated mainly by a specific cGMP-dependent protein kinase (cGK). We will discuss cGK and its mechanism of action in more detail in a later session.
2. When accumulated in relatively high concentrations (> 5 μM), cGMP is able to cross-activate the cAMP-dependent protein kinase. For example, in colonic T84 cells, which do not contain detectable amounts of cGK, cGMP was shown to mediate the heat-stable enterotoxin-provoked Cl secretion by stimulating cAK [29]. Sofar, this mechanism is less well documented in the cardio-vascular system, but is suggested to be involved in the anti-proliferative effects of cGMP [30]. The opposite mechanism, i.e the cross-activation of cGK by cAMP, is thought to account at least partially for the relaxing effects of cAMP in smooth muscle [31].
3. A more common pathway by which cGMP utilizes the cAMP pathway is initiated by binding of cGMP to specific classes of phosphodiesterases (PDE), the enzymes responsible for the breakdown of cAMP and cGMP. Both a cGMP-stimulatable PDE (type III) and cGMP-inhibitable PDE (type II) are present in various cells, where they can cause a decrease or an increase of cAMP levels respectively in response to cGMP [26, 32].
4. In some tissues cGMP is known to regulate ion channels by direct allosteric interaction. The gating by cGMP of cation channels is well documented in the visual and olfactory system [26, 32]. A cGMP-gated channel was recently detected in heart and also in kidney, where it may contribute to the cGMP-mediated natriuresis [33, 34].

cGMP-dependent protein kinases

General properties

cGMP-dependent protein kinases (cGKs) belong to the large superfamily of protein kinases [26, 32, 35, 36]. These enzymes regulate the activity of numerous proteins by catalyzing the transfer of the γ-phosphoryl group of ATP, to the hydroxyl group of serine, threonine, or tyrosine residues of an acceptor substrate protein. Within the protein kinase superfamily cGK is most closely related to the cAMP-dependent protein kinases

(cAKs). Similar to cAK, cGK phosphorylates serine and threonine residues. The canonical cAK phosphorylation site Arg-Arg-X-Ser is also used by cGK, however differences between cAK and cGK in the affinity for various substrate peptides are observed [37, 38]. As discussed above, cGK and cAK are also related that both can be activated by cAMP as well as cGMP, albeit with different Ka values. Various analogues of cGMP can also interact with cGKs and were used to map the cGMP binding sites [26, 36]. The potent and lipophilic cGK-activators 8-Br-cGMP and 8-parachlorophenylthio-cGMP (8-pCPT-cGMP) are widely used in intact cell studies to investigate the involvement of cGK in physiological processes, since they are membrane permeant, relatively resistant against PDEs and do not crossreact with cAK except at very high concentrations [39]. Similarly, the recently developed cGK antagonists Rp-8-Br-cGMPS and Rp-8-pCPT-cGMPS can be used as selective inhibitors of cGKs in intact cells [40].

Isotypes and tissue distribution

Two isoenzymes of cGK have been identified in mammals [26, 32, 35, 36]. Both isotypes have been cloned [41–44], and were shown to exhibit a sequence homology of more than 50%, and a similar structural organization. The *in vitro* substrate specificity of both cGKs for various substrates seems also very similar [25], but an interesting functional difference was noted in their capacity to activate the cystic fibrosis transmembrane conductance regulator CFTR-Cl channel [45]. This channel is expressed predominantly in epithelial cells of several organ systems, including the lung, pancreas, liver and intestine, but has been detected also in non-epithelial cells including cardiomyocytes [46], and lymphocytes [47]. Non-functional, mislocated, or absent CFTR-Cl channels are the primary cause of the genetic disease cystic fibrosis. The channel is universally activated by cAMP and cAK in all tissues, but may additionally be activated by cGMP in some tissues, either through cross-activation of cAK (lymphocytes, and T84 colonocytes; [29, 47]), or through the cGMP-cGK II pathway (intestine: [48]). A specific role of type II cGK in CFTR-channel activation was supported by a recent patch clamp study showing that type II, but not type I cGK was able to activate CFTR-Cl channels in excised membrane patches [45].

Furthermore, cGK I and II also differ in cellular and subcellular distribution. Type I cGK is predominantly a cytosolic protein, but may be targeted to specific anchor proteins of the cytoskeleton, e.g. vimentin [49]. In contrast cGK II is tightly bound to the plasma membrane by both hydrophobic interaction and by its association with the cytoskeleton [50]. N-terminal myristoylation is likely to play an important role in the membrane-binding of cGK II (Vaandrager *et al.*, unpublished observation). At the tissue level, type I is more generally expressed than type II. Notably, high levels of cGK I have been found in platelets, tracheal, gastro-intestinal and vascular smooth muscle cells, Purkinje cells in the cerebellum [26, 32, 35, 36], and recently also in aortic and pulmonary artery endothelial cells, but not in umbilical vein endothelial cells [51]. Furthermore, low levels of cGK I were observed in cardiomyocytes [52]. Type II cGK is highly expressed in intestinal epithelial cells [44, 48], but mRNA for cGK II was also found in brain and kidney [43, 44]. These localization studies suggest that type I is the primary isotype mediating the cGK effects in the cardio-vascular system, whereas type II is involved in ion transport regulation in the intestine and perhaps in kidney and brain.

Recently, two isoforms of type I have been distinguished, designated Iα and Iβ [26, 32, 35, 36]. These isoforms differ only in the first 89 (Iα) or 104 (Iβ) amino acids, and presumably represent splice variants. Although both isoforms have identical cGMP-binding domains (see below), cGK Iα has an approximately ten fold lower Ka for cGMP (0.1 μM) compared to cGK Iβ (1.3 μM). However the membrane permeant cGMP-analogue β-phenyl-1-N^2-etheno-cGMP (PET-cGMP) can activate both isoforms with a similar, relatively low Ka. Both cGK I isoforms are present in vascular smooth muscle, whereas type Iα was found predominantly in lung, heart and cerebellum [53]. It has been suggested that the low-affinity cGK Iβ is expressed in vascular muscle to dampen the physiological effects of the large short-term increases in cGMP provoked by the NO-GC-S system [53], or alternatively that it mediates the cAMP-induced relaxation in this tissue, since it has a relatively low preference for cGMP over cAMP [32].

Molecular structure

Mammalian cGKs are dimers with a monomeric mass of 76, 78, and 86 kDa respectively for the Iα, Iβ and II isozymes [26, 43, 44]. Although type II was originally described as a monomer [50], recent studies showed that recombinant rat cGK II exists in a dimeric state under physiological conditions (Vaandrager, unpublished results). By analysis of their primary structure, similar functional domains can be recognized in cGK I and II, as depicted in Fig. 3.

A N-terminal leucine zipper motif is likely to be responsible for the dimerization of both isotypes. In cGK Iα, but not in cGK Iβ and II, the dimer is stabilized by an interchain disulfide bridge. Conceivably, the dimers are oriented in parallel, facilitating interactions between the C-terminal domains. A pseudo-substrate region located in close proximity to the autophosphorylation sites in the N-terminal domain may serve to inhibit the catalytic activity in the basal state. This auto-inhibitory site is thought to interact with the substrate-binding site on the catalytic domain, thus preventing it from binding to exogenous substrates.

Two cGMP binding sites are present per monomer of cGK. In type Iα cGK, high affinity binding to site 1, located more distally to the N-terminus, seems to depend on cooperative interaction between site 1 and the low affinity site 2. The cGMP binding domains are structurally related to the cAMP

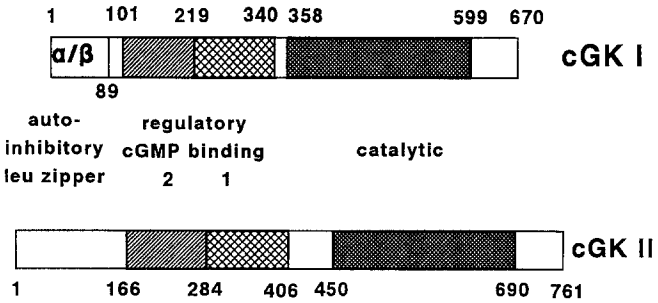

Fig. 3. Domain structure of cGMP-dependent protein kinases. Amino acid numbers at the boundaries of the various domains are taken by comparison of the domain structures of cGK Iα and GK II isotypes from refs [36, 43, 44]. The primary sequence of cGK Iβ is identical to that of cGK Iα except for the first 89 amino acids which are replaced by a different N-terminus of 104 residues in cGK Iβ.

binding domains in the regulatory subunit of cAK and to the cyclic nucleotide binding site of the cGMP or cAMP activated ion channels, but not to the allosteric cGMP binding site in PDE II or III.

The catalytic C-terminal domain is the most conserved region between the type I and II cGK (66% homology). It is also displays a relatively high homology to the catalytic subunit of cAK. However, in cGK the regulatory and catalytic domains are covalently linked, whereas in cAK each domain is encoded by a different gene.

Functions of cGKs

Relaxation of smooth muscle cells

Smooth muscle contraction has been shown to depend on phosphorylation of the regulatory light chain of myosin, by a specific myosin light chain kinase (MLCK). Since MLCK is activated by Ca/calmodulin, smooth muscle contraction is initiated primarily by a rise in intracellular free Ca level, as provoked by many contractile agents [54]. The cGMP-induced reduction of intracellular Ca, observed in many studies is therefore considered an important mechanism of cGMP-mediated relaxation. A major role for cGK in the Ca-lowering action of cGMP in smooth muscle cells was deduced from studies using cGK-specific analogues, the finding of a correlation between cGK levels and the effect of cGMP on Ca levels, and from the ability of exogenous cGK I to reconstitute some of the cGMP effects in cGK-deficient cells [30, 32].

cGK is proposed to modulate intracellular Ca levels by affecting a variety of Ca-regulating processes [26, 30, 32, 35, 36], including: (i) Inhibition of the phospholipase-C (PL-C) mediated generation of the Ca-mobilizing messenger inositol 1,4,5 trisphosphate (IP_3); the target of cGK in this process is not clear but might be the G protein that couples the hormone receptor to PL-C; accordingly, an increased phosphorylation of Gαi was observed in 8-Br-cGMP-triggered CHO-cells expressing recombinant cGK Iα [55]; (ii) activation of Ca-ATPase activity in the plasma membrane as well in the sarcoplasmic reticulum (SR); phosphorylation of phospholamban might play a role in the cGK-mediated modulation of the Ca-ATPase in the SR; (iii) activation of Ca-activated K channels, causing a hyperpolarization of the cell membrane, which inhibits Ca influx through voltage-operated Ca channels (VOC). cGK was suggested to activate the K channels directly by phosphorylation [56] or indirectly by activating a phosphatase, which subsequently activates the channel [57]; (iv) direct inhibition of VOC; (v) stimulation of the Na/Ca exchanger; (vi) inhibition of IP_3 receptor activity involved in Ca mobilization from internal stores (see Fig. 4). The relative contribution of the processes mentioned above in the Ca-lowering effect of cGK may differ considerably dependent on smooth muscle type, species, and contractile stimulus.

Furthermore cGK was also shown to relax smooth muscle by decreasing the Ca-sensitivity of the contractile proteins, conceivably by stimulating dephosphorylation of MLC through activation of a phosphatase [30].

Inhibition of platelet activation

The cellular events leading to inhibition of platelet activation/aggregation by cGMP were shown to be mediated primarily by cGK [26]. This model was supported by studies of cGK-deficient platelets from patients with chronic myelocytic leukemia, showing an impaired response to NO and cGMP-analogues [58]. A major mechanism of cGK action, as discussed earlier for smooth muscle cells, is the inhibition of an agonist-provoked rise in intracellular Ca by a blockade of the PL-C/IP_3 pathway [26]. Interestingly, in the platelet the effects of cGK on Ca are mimicked by cAK, suggesting that both protein kinases share a common target. Indeed, a proline-rich, microfilament- and focal adhesion-associated protein termed VASP was shown to be phosphorylated *in vivo* by both cGK and cAK and may serve as a convergence point for the cAMP and cGMP pathway in platelets [59, 60]. The recent identification of VASP in a variety of other cell types, including cardiomyocytes, where it was found in association with the intercalating discs [60], and in endothelial cells [51], suggests a more general role of this protein in cyclic nucleotide-regulated processes.

Decrease in endothelial permeability

Vasoactive substances, including thrombin and histamine increase endothelial permeability, and in this way stimulate vascular leakage and edema. The decrease in endothelial barrier function is considered to result from contraction of endothelial cells by a mechanism similar to smooth muscle cell contraction, involving Ca-induced phosphorylation of MLC [61]. cGMP was shown to inhibit the agonist-induced increase in endothelial permeability by different mechanisms, depending on the tissue source [62]. In human umbilical vein

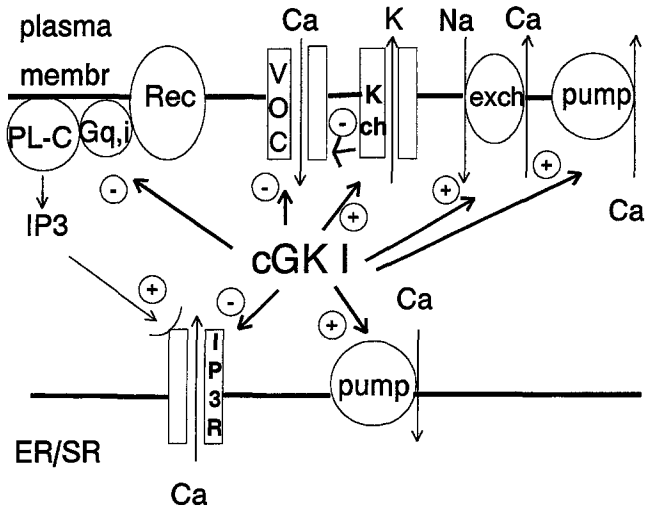

Fig. 4. Possible targets of cGK involved in intracellular Ca homeostasis in smooth muscle cells. cGK may decrease the level of intracellular Ca through modulation of: voltage operated Ca channels (VOC), receptor mediated activation of phospholipase C (PL-C), inositol 1,4,5 trisphosphate receptor Ca-channels (IP3 R), Ca ATPases (pump), Na/Ca exchangers (exch), and Ca activated potassium channels (K ch).

endothelial cells, showing no or very low expression of cGK I [51], cGMP signals through the cAMP/cAK-pathway by inhibiting PDE III [62]. In contrast, in cultured human aortic and microvascular endothelial cells, which were found to express relatively high levels of cGK I (150–500 ng/mg protein), the cGK-selective cGMP-analogues 8-pCPT-cGMP and 8-Br-cGMP were shown to block the thrombin-induced increase in permeability by inhibiting a rise in intracellular Ca [51, 62]. The focal-adhesion protein VASP (see above) was phosphorylated by cAK in umbilical vein endothelial cells and by cGK in aortic and microvascular endothelial cells, suggesting that it may play a role in the cyclic nucleotide-mediated modulation of permeability, probably through a mechanism different from inhibition of Ca-mobilization, as the latter was observed only in cGK containing cells [51].

Negative inotropic effect on cardiomyocytes

One of the main targets of cGMP-regulation of cardiac contractility is the Ca-current (I_{Ca}) mediated by the L-type Ca channel, which is responsible for the initiation of the intracellular Ca transient leading to cardiac contraction [63]. The cAMP/cAK-mediated increase in I_{Ca} plays a key role in the positive inotropic effects of cAMP-raising agents [64]; in contrast, cGMP was shown to decrease I_{Ca} in frog cardiomyocytes by stimulation of PDE II resulting in lowering of cAMP levels. However, in rat cardiomyocytes, which contain low but measurable levels of cGK 1, the cGMP-triggered decrease in cAMP-stimulated I_{Ca} was shown to be mediated mainly by cGK, since it was mimicked by cGK-specific agonists and by intracellular perfusion with a constitutively active fragment of cGK I [52]. Other studies in rat cardiomyocytes indicated that cGK may also exert a negative inotropic effect by reducing the myofilament response to Ca [65], suggesting that cGK in cardiomyocytes, like in smooth muscle cells, affects multiple processes. Furthermore, cGK was reported to inhibit gap junction channels and Na-K-Cl cotransporters in cardiomyocytes [66, 67]. The latter process induces cell shrinkage, while the former may decrease electrical coupling and the exchange of nutrients between cells. However, the physiological consequences of both processes for cardiac contractility are as yet not clear.

Conclusion

As is evident from the data summarized in this review, the information in the field of cGMP and NO has grown exponentially in recent years. Both messengers appear to play a major, and often beneficial role in cardiovascular physiology as a result of their anti-hypertensive, anti-thrombolytic, and anti-proliferative action and their protective effect on endothelial barrier function. Further elucidation of the molecular mechanism involved in cGMP metabolism and its signalling function in the cardio-vascular is therefore likely to lead to new pharmacological and molecular biological approaches for the prevention and cure of cardiovascular diseases.

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