cGMP-dependent protein kinases in drug discovery

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Cyclic guanosine-3′,5′-monophosphate (cGMP)-dependent protein kinases (cGKs) are key enzymes of nitric oxide–cGMP and natriuretic peptide signalling cascades. These kinases mediate most of the effects of cGMP-elevating drugs, such as nitrates and phosphodiesterase inhibitors. cGKs modulate smooth muscle relaxation (e.g. the vasculature, gastrointestinal tract, bladder and penile), platelet aggregation, renin release, intestinal secretion, learning and memory. Furthermore, several cGK substrates have been identified. Isozyme-specific inhibitors and activators of cGK and its downstream substrates might act more specifically than upstream signalling activators, such as organic nitrates and phosphodiesterase inhibitors.

Modulation of nitric oxide (NO) and cyclic guanosine-3′,5′-monophosphate (cGMP) concentration is an established therapy for cardiovascular diseases, including the treatment of angina pectoris using NO-releasing nitrates and pulmonary hypertension with gaseous NO, as well as erectile dysfunction with phosphodiesterase (PDE) 5 inhibitors. NO–cGMP signalling is mainly mediated by cGMP-dependent protein kinases (cGKs), which regulate various physiological functions including smooth muscle tone, platelet aggregation, intestinal secretion and hippocampal and cerebellar learning (Figure 1). The diverse functions of cGK have been highlighted by analysis of conventional and conditional murine knockout models. Activators and inhibitors have been developed to interfere with cGK activity and function. In addition, cGK is involved in various signalling pathways with distinct physiological importance in different tissues (e.g. in mediating vascular and intestinal smooth muscle relaxation). Modulation of cGK, its isozymes and its organ-specific downstream signalling targets could open the way for more specific pharmacological treatment of diseases related to NO–cGMP signalling dysfunction.

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Application of NO–cGMP-elevating drugs

Drug therapy of erectile dysfunction and cardiovascular and pulmonary diseases involves the modulation of NO–cGMP signalling pathways. Classic compounds are the NO-releasing organic nitrates nitroglycerine, isosorbide-mono-nitrate and isosorbide-di-nitrate, which are used mainly to alleviate the symptoms of acute and chronic angina pectoris. These substances release NO and thereby relax vascular smooth muscle. However, continuous application eventually leads to nitrate tolerance, the development of which depends on the activity of mitochondrial aldehyde dehydrogenase [1,2]. The NO-donor molsidomine releases NO in an enzyme-independent manner and its continuous use is not associated with the development of nitrate tolerance.

Inhibitors of the cGMP-dependent cGMP-specific PDE5 (sildenafil, vardenafil, taladafil) are used for the treatment of erectile dysfunction [3]. These compounds raise the intracellular cGMP concentration and activate cGMP kinase, which results in relaxation of the penile corpus cavernosum, thus facilitating penile erection. These inhibitors could also be beneficial for the treatment of pulmonary hypertension

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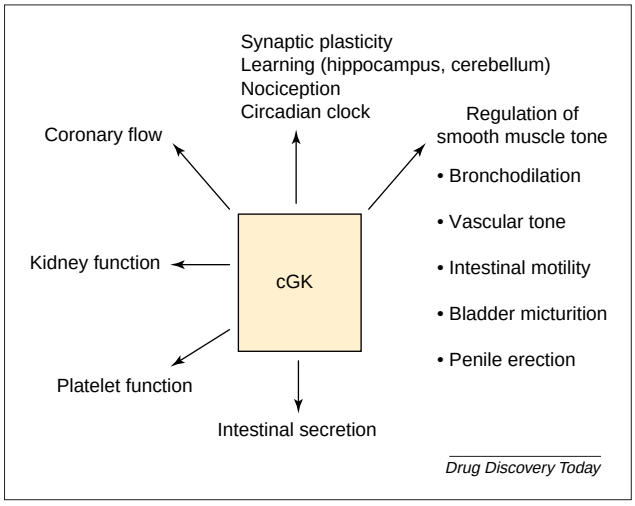

FIGURE 1

Physiological functions of cGK in diverse tissues. The cGK enzymes exert pleiotropic effects on a variety of organs and cells, underlining the physiological importance of cGK signalling.

[4] that is, at present, effectively treated by NO inhalation. In addition, PDE5 inhibition suppressed cardiac myocyte hypertrophy in heart cells from mice exposed to chronic pressure overload and could provide a new principle to treat cardiac hypertrophy and remodelling [5]. At higher concentrations, these substances can further increase cGMP levels by inhibiting Ca^{2+}/calmodulin-dependent cGMP-specific PDE1, which can lead to vasodilation (headache, flush) and gastrointestinal disturbances, and by precluding activity of the retinal photoreceptor-activated cGMP-specific PDE6, which can result in transient visual blurring [6,7].

Recently, activators of soluble guanylyl cyclase (sGC) have been introduced as a new approach to the modulation of NO–cGMP signalling pathways [8]. These drugs induce smooth muscle relaxation and inhibit platelet aggregation and could therefore reduce the risk of thrombosis. One of the most advanced sGC activators is the benzylindazole YC1 [3-(5′-hydroxymethyl-2′furyl)-1-benzylindazole] [9], which activates sGC independently of NO but is dependent on haem (the NO-binding moiety of sGC). Interestingly, a superadditive stimulation of sGC was observed on combination of YC1 and NO [10]. In addition to activation of sGC, YC1 can also inhibit PDE5, which explains the unexpectedly large increase of intracellular cGMP produced on administration of YC1. Bay412272 is a YC1 analogue that also activates sGC in a NO-independent manner and enhances sGC stimulation in the presence of NO [11]: Bay412272 also inhibits PDE5 [12]. The recently developed NO-independent activator Bay418563 is highly specific for sGC [13] and is approximately 100-fold more potent than YC1 in activating sGC, yet it has no detectable PDE inhibitory activity. Bay418543 and NO act synergistically at a wide range of concentrations (0.01–10.00 μM). This compound relaxes arteries and veins at nM concentrations, inhibits platelet function (which could be useful in the treatment and prevention of thrombosis) [14] and induces penile erection *in vivo*. A novel class of sGC activators, which are anthranil acid derivatives (e.g. S3448, HMR1766), acts on haem-oxidized sGC [15]. HMR1766, for example, induces vasorelaxation, lowers mean arterial blood pressure and inhibits platelet aggregation, and could therefore represent a novel approach for the therapy of coronary heart disease.

cGK signalling and function

Major mediators of cGMP signalling are the cGKs cGKI and cGKII [16,17]. cGKs are serine/threonine specific kinases that phosphorylate proteins at the RKRKXST structural motif. These proteins exhibit common structural features: (i) an N-terminal domain containing a leucine–isoleucine (Leu–Ile) zipper, which is required for homodimerization and for intracellular localization; (ii) an autoinhibitory domain; (iii) a regulatory domain containing two allosteric cGMP-binding sites; (iv) an ATP-binding domain; and (v) a catalytic domain.

Two isoforms of cGKI exist, cGKIα and cGKIβ, which differ in their N-terminal Leu–Ile domains. cGKIα is activated at tenfold lower cGMP concentrations than cGKIβ. cGKIα and cGKIβ might exhibit different physiological functions because they interact with different substrates, vary in their subcellular localization [18,19] and are expressed in different tissues [20,21]. cGKIα is highly expressed in the cerebellum, dorsal root ganglia and lung, whereas cGKIβ is found predominantly in smooth muscle, platelets, the hippocampus and the olfactory bulb. cGKII is localized at the plasma membrane through its myristoylated Gly2 residue and is expressed in the intestinal mucosa, kidney, chondrocytes and several brain nuclei.

cGKI in smooth muscle tissues

The NO–cGMP–cGKI signal relaxes all smooth muscle types. The mechanisms of cGKI that induce relaxation of smooth muscle are well characterized. In principle, relaxation requires Ca^{2+}-dependent and/or -independent mechanisms [22] (Figure 2). An important Ca^{2+}-dependent mechanism of cGKI is the inhibition of inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3]-dependent calcium release. This inhibition is mediated by phosphorylation of the Ins(1,4,5)P_3-receptor-associated cGMP kinase substrate (IRAG), a substrate of the β-isoform of cGKI [18,23]. Deletion of the Ins(1,4,5)P_3-receptor-binding site of IRAG in mice stops the inhibition of intracellular calcium release by cGKI and hinders the cGMP-dependent relaxation of vascular and colonic smooth muscle [24]. Another Ca^{2+}-dependent mechanism is the activation of the big calcium-activated potassium channel (BK_{Ca}) through phosphorylation by cGKI, leading to hyperpolarization and reduced calcium influx [17,25]. In addition, cGKI might reduce the synthesis of Ins(1,4,5)P_3 via the regulator of G-protein signalling 2 (RGS2) protein and potentially phospholipase Cβ3 (PLCβ3) [26–28]. Ca^{2+}-independent relaxation by cGKI

involves phosphorylation of the myosin-binding subunit [e.g. myosin phosphatase targeting subunit 1 (MYPT1)] [29]. Furthermore, cGKI might phosphorylate, and thereby inhibit, the small GTP-binding protein Rho, leading to an increased activity of myosin light chain phosphatase (MLCP) [30]. cGKI also induces a feedback mechanism (which lowers the intracellular cGMP concentration) by the phosphorylation and activation of PDE5 [31].

Other substrates of cGKI that might be involved in smooth muscle relaxation and other smooth muscle functions have been identified (Table 1). The phosphorylation of phospholamban is known to stimulate the sarcoendoplasmic reticulum pump Ca^{2+} ATPase (SERCA), which decreases intracellular calcium levels. However, it has been shown that ablation of phospholamban does not alter cyclic-nucleotide-mediated relaxation [32,33]. The reduction of the intracellular calcium concentration might also be mediated by phosphorylation, and thus inhibition, of the capacitative transient receptor potential channel 3 (a calcium channel) [34]. Furthermore, the cGKI-dependent phosphorylation of telokin, a C-terminal fragment of MLCK, inhibits MLCK activity and might lead to Ca^{2+}-desensitization [35]. Vasodilator-stimulated phosphoprotein (VASP) is abundant in smooth muscle tissues and contains two phosphorylation sites for cyclic-nucleotide-dependent protein kinases: one phosphoacceptor site, Ser239, is specific for cGKI [36]. VASP is involved in focal adhesion sites, but is dispensable for cyclic-nucleotide-mediated smooth muscle relaxation [37]. The cysteine rich protein 2 (CRP2) is phosphorylated by cGKI and cGKII and is found in smooth muscle and enteric neurons [38]. This protein, which contains a LIM-domain (a common zinc finger ion motif shared by the homeodomain proteins Lin11, Isl-1 and Mec-3), might have a role in smooth muscle differentiation.

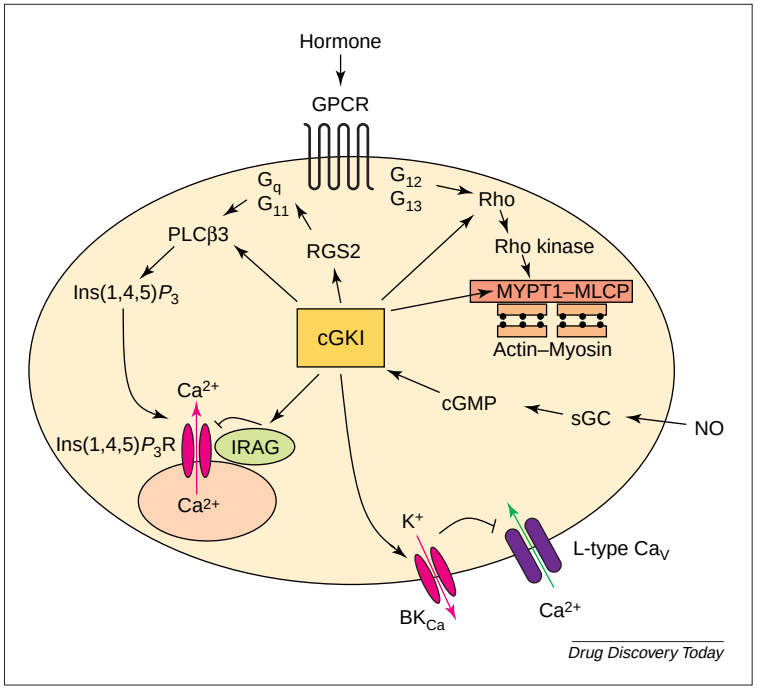

FIGURE 2

Mechanisms affected by cGKI. Hormones induce smooth muscle contraction via the G-proteins G_q and G_{11} (raise intracellular calcium) or via G_{12} and G_{13} (activate calcium-sensitizing mechanisms). NO–cGMP signalling activates cGKI, which then inhibits Ca^{2+}-elevating and/or Ca^{2+}-sensitizing mechanisms. Abbreviations: Ca_V, voltage-activated calcium channel; GPCR, G-protein-coupled receptor; RGS, regulators of G-protein signalling.

Many studies have shown that cGKI is essential for the function of smooth muscle tissues. In the vascular system, cGKI relaxes aortic rings and small arteries. Accordingly, a deficiency of cGKI might be involved in the development of hypertension [39]. Furthermore, cGKI might be important in the vascular remodelling that induces angiogenesis and the development of atherosclerosis [40]. The importance of cGKI for the function of the gastrointestinal tract was shown by deleting cGKI in mice, which induced gastrointestinal dysfunction, including dilation of the stomach and caecum, pyloric contraction and severely disturbed gastrointestinal motility [41]. A similar phenotype was observed in mice with a mutation in the cGKI substrate, IRAG [24]. Deletion of cGKI in mice reduced rhythmic bladder contractility and increased bladder volume, showing that cGKI is also involved in micturition. Moreover, hyperactive voiding and diminished NO–cGMP-dependent relaxation of the urinary duct were observed on deletion of cGKI [42]. Interestingly, deletion of the BK_{Ca} channel also altered micturition and could therefore be a main target of cGKI in bladder smooth muscle [43]. The importance of cGMP for the induction of penile erection is underlined by the finding that inhibition of PDE5 leads to relaxation of the corpus cavernosum [44]. These effects might be mediated by cGKI because male cGKI-null mutants failed to relax the corpus cavernosum on NO–cGMP activation and exhibited strongly reduced reproduction [45].

cGKI in platelets

NO–cGMP–cGKI inhibits platelet aggregation and granule secretion. Correspondingly, cGKI deletion in mice abolishes the antiaggregation activity of cGMP and enhances thrombosis in an ischemia–reperfusion model system [46]. However, there is evidence that cGKI is initially able to promote aggregation of platelets and then subsequently inhibit their aggregatory response [47]. The reduction of intracellular calcium concentration could be the main mechanism for the cGKI-dependent inhibition of platelet function [48]. There is evidence that cGKI decreases intracellular calcium release by the phosphorylation of IRAG, which then inhibits the Ins(1,4,5)P_3 receptor [17,18]. In addition, cGKI might phosphorylate the thromboxane receptor α (TPα) in platelets and thereby lead to TPα desensitization [49]. Other proteins that are phosphorylated by cGKI and could therefore modulate platelet function are VASP, heat shock protein 27 (Hsp27) and PDE5. The NO–cGMP-stimulated phosphorylation of VASP is important in preventing the adhesion of platelets to the vascular wall. [50]. Furthermore, phosphorylation of Hsp27 by

TABLE 1

Substrates of cGK

Substrate	Molecular weight	cGK isoform	Tissue or cells	Function of phosphorylation	Refs
Established cGK substrates (with probable function of phosphorylation)					
BK_{Ca}	130	cGKI	Smooth muscle	Hyperpolarization	[25]
G-substrate	32	cGKI	Cerebellum	Potential protein phosphatase inhibitor	[58]
Hsp27	27	cGKI	Platelets	Decrease of actin polymerisation *in vitro*	[51]
Ins(1,4,5)P_3 receptor type I	230	cGKI	Cerebellum	Stimulation of calcium release from intracellular stores	[57]
IRAG	125	cGKIβ	Smooth muscle	Reduced calcium release from intracellular stores	[18,23,24]
MYPT1	130	cGKIα	Smooth muscle	Calcium desensitization	[29]
PDE5	100	cGKI	Smooth muscle and platelets	Enhanced cGMP degradation	[31]
Phospholamban	6	cGKI	Vascular smooth muscle	Enhanced calcium uptake by the calcium ATPase SERCA, potentially no effect on relaxation?	[32,33]
RGS2	24	cGKI	Smooth muscle	Inhibition of Ins(1,4,5)P_3 generation	[26,27]
RhoA	22	cGKI	Smooth muscle and hippocampus	Reduced MLC phosphorylation and vesicle trafficking	[30,54]
Sox9	56	cGKII	Chondrocytes	Bone development	[64]
Telokin	17	cGKI	Smooth muscle	Inhibition of MLCK activity	[35]
VASP	46 50	cGKI cGKII	Smooth muscle, platelets and hippocampus	Regulation of the actin cytoskeleton and vesicle trafficking	[36,37,50,54]
Potential cGK substrates (in heterologous systems or with unidentified function of phosphorylation)					
CFTR	200	cGKII	IEC-CF7 cells (rat intestinal cell line)	Stimulation of chloride channel	[61]
CRP2	23	cGKI and cGKII	Intestinal smooth muscle and enteric neurons	Unknown	[38]
PLCβ3	140	cGKI	Aortic smooth muscle cells and COS	Potential inhibition of phospholipase C activity	[28]
Septin 3	40	cGKI	Brain	Potential vesicle trafficking	[55]
TRPC3	97	cGKI	HEK293	Inhibition of store operated calcium influx	[34]
TPIα	40	cGKI	HEK293	Desensitization of TPIα signalling	[49]

Abbreviations: TP, thromboxane receptor; TRPC3, transient receptor potential channel 3.

cGKI might be involved in platelet aggregation through the regulation of microfilament organization [51].

cGKI in the nervous system

The sites of highest expression of cGKI in the central nervous system (CNS) are the hippocampus, cerebellum and the olfactory bulb [52]. cGKIβ expressed in the hippocampus might be involved in long-term potentiation (LTP), and could therefore be important for spatial learning and memory. Hippocampus-specific cGKI knockout mice showed reduced LTP but no defect in spatial learning and memory tests [53]. Therefore, cGKI might be involved in more subtle types of learning in the hippocampus. Vesicle trafficking regulated by VASP and RhoA (and possibly by Septin 3) mediates hippocampal cGKI signalling [54,55].

Cerebellar Purkinje cells, which are important for motor learning by long-term depression (LTD), contain high concentrations of cGKIα. Purkinje-cell-specific cGKI-deletion abolishes cerebellar LTD, suggesting that cerebellar activity is regulated by cGKI [56]. These cGKI mutants showed an altered vestibulo-ocular reflex, whereas the general motor coordination remained unchanged. Phosphorylation of the Ins(1,4,5)P_3 receptor type I in the cerebellum stimulates intracellular calcium release and might therefore regulate neuronal activity [57]. Furthermore, the G-substrate, which is an inhibitor of protein phosphatase 1 and 2, is phosphorylated and thus activated by cGKI in cerebellar Purkinje cells, and might contribute to the role of NO–cGMP in LTD [58].

There are several indications that NO–cGMP–cGKI signalling is involved in nociception. The inhibition of cGKI in the spinal cord reduced hyperalgesia induced by formalin [59]. Furthermore, cGKI-deficient mice showed reduced nociception in inflammation assays [60]. The cGKI signalling pathway leading to nociception has yet to be fully elucidated, but could involve substance P, which is downregulated in spinal cord neurons and the fibres of cGKI-deficient mice.

TABLE 2

K_a and specificity of cGKIα and cGKII activation

Activators	cGKIα K_a (μM)	Specificity[a]	CGKII K_a (μM)
cGMP	0.110	ND	0.8000
8-Br-cGMP	0.010–0.026	100	0.0250
PET-cGMP	0.016–0.026	200	4.7000
8-Br-PET-cGMP	0.013	ND	1.6000
8-pCPT-cGMP	0.050	10–2000	0.0035–0.0800

[a]Specificity is defined as the ratio of K_a of cGKIα to K_a of cGKII.
Abbreviation: ND, not determined.

Functions of cGKII

Investigations using cGKII-deficient mice showed that cGKII modulates intestinal secretion, bone growth, renin secretion and circadian rhythmicity. cGKII stimulates chloride and water secretion in the small intestine, probably by phosphorylation of the cystic fibrosis transmembrane conductance regulator (CFTR), and leads to Na^+-absorption [61]. Interestingly, cGKII-deficient mice are resistant to *Escherichia coli* heat-stable enterotoxin (STa)-induced diarrhoea [62]. Mice with a cGKII deletion are dwarfs with altered endochondral ossification at the endochondral plate [62]. Studies using animals overexpressing C-type natriuretic peptide (CNP) show that cGKII is essential for normal endochondral bone development [63]. Deletion of cGKII in rat led to an expanded growth plate, impaired bone healing and the accumulation of postmitotic, but nonhypertrophic, cells. These effects are related to enhanced signalling of the transcription factor Sox9, which impairs hypertrophic differentiation of chondrocytes [64]. In the kidney, cGKII inhibits renin secretion, possibly by altering exocytosis of renin from juxtaglomerular cells [65]. cGKII is expressed in the zona glomerulosa and might be related to aldosterone homeostasis. Although overexpression of cGKII in zona glomerulosa cells enhanced aldosterone production, cGKII expression is upregulated by activation of the aldosterone system by a low salt diet containing 0.02% NaCl [66]. However, the deletion of cGKII does not alter blood pressure and aldosterone secretion, which could be explained by the effect of cGMP on cAMP-specific PDEs because the cGMP-stimulated PDE2 decreases cAMP concentrations and aldosterone production in adrenal glomerulosa cells [67]. Furthermore, cGKII is involved in regulation of the circadian clock, which is located in the suprachiasmatic nucleus (SCN), and influences a phase shift related to the expression of the clock genes *mPer1* and *mPer2* [68] that is required for night-to-day clock progression [69].

Activators and inhibitors of cGMP kinase

Several strategies have been used to develop cGK-specific activators or inhibitors that interfere with the binding sites for cGMP, ATP or cGK substrate peptides. Membrane-permeable analogues of cGMP interact with cGK at the cGMP binding sites and can be used as tools for the investigation of physiological functions and signalling of cGK [70,71] (Table 2). The widely used analogue, 8-bromo-cGMP, activates cGKI and cGKII (K_a of 0.010–0.026 μM) but is sensitive to hydrolysis by PDEs. The analogues β-phenyl-1,N^2-etheno (PET)-cGMP and 8-Br-PET-cGMP are specific cGKI activators at low concentrations (K_a of 0.016–0.026 μM and 0.013 μM, respectively) but can also activate cGKII in the μM range. These PET analogues are also sensitive to PDE hydrolysis. By contrast, the analogue 8-*para*-chlorophenylthio-cGMP (8-pCPT-cGMP) is resistant to hydrolysis by PDE and is selective for activation of cGKI (K_a of 0.0500 μM) and cGKII (K_a of 0.0035–0.0800 μM). 8-pCPT-cGMP is the most potent cGKII activator known to date. All these cGMP analogues crossactivate the cAMP-dependent protein kinase at high concentrations. Furthermore, at high concentrations, these compounds interfere with other cellular cGMP receptors by activating cyclic-nucleotide-gated ion channels (e.g. 8-Br-cGMP has a K_a of 1.6 μM) [72] and inhibiting PDE3 (8-Br-cGMP has a K_i of 8.0 μM) [73].

The (Rp)-diastereomers of cGMP, which contain a thiophosphate group in the cyclic phosphate moiety, are competitive *in vivo* inhibitors of cGKI and cGKII: these inhibitors are membrane-permeable and are resistant to degradation by PDEs [(Rp)-8-Br-PET-cGMP-S has a K_i of 0.035 μM (cGKI), 0.900 μM (cGKII); (Rp)-8-Br-pCPT-cGMP-S has a K_i of 0.500 μM (cGKI) 0.230–0.500 μM (cGKII) (Table 3)]. (Rp)-8-Br-PET-cGMP-S is the most specific cGKI inhibitor known to date [74,75]. The staurosporine analogue KT5823 inhibits cGK *in vitro* and selectively inactivates ATP binding (Table 3) [76]. Several studies show that there is a lack of *in vivo* inhibition of cGK in several cell types, including platelets [77]. A peptide library was screened for peptide–cGKI interaction using the SPOT method (peptide array on paper) to identify selective inhibitors that compete with the peptide binding site [78]. This peptide-based assay enabled the identification of several highly specific *in vitro* cGKI inhibitor peptides, for example, W7, W21 and W45 (Table 3) [79]. The cellular internalization of W45 occurs via the N-terminal fusion of membrane-translocation signal sequences from HIV tyrosine aminotransferase protein (fusion peptide DT-2) or from *Drosophila antennapedia* homeodomain (fusion peptide DT-3). The K_i values of DT-2 and DT-3 for inhibition of the purified cGKIα enzyme were comparable to that of the cGMP analogue (Rp)-8-Br-PET-cGMP-S [K_i of 0.0125 μM (DT-2) and 0.0250 μM (DT-3)] (Table 3). The peptides DT-2 and DT-3 inhibit vasodilation, and might be useful tools for the analysis of cGKI-specific functions [80,81].

To improve the development of cGKI activators and inhibitors, a HTS assay, based on fluorescence resonance energy transfer (FRET), was developed [82]. In this assay, the enzymatic phosphorylation of a VASP-derived peptide, which is biotinylated and binds to allophycocyanin-streptavidin, can be detected by a phospho-VASP-specific

TABLE 3

K_i and specificity of cGKIα and cGKII inhibition *in vitro*

Inhibitors	cGKIα K_i (μM)	Specificity[a]	CGKII K_i (μM)	Refs
(Rp)-8-Br-PET-cGMP-S	0.0350	300	0.90	[74]
(Rp)-8-pCPT-cGMP-S	0.5000	15–30	0.23–0.50	[75]
KT5823	0.2340	>42	ND	[79]
W7	15.0000	46	ND	[80]
W21	7.5000	100	ND	[80]
W45	0.8000	680	ND	[80]
DT-2	0.0125	19700	ND	[81]
DT-3	0.0250	1320	ND	[81]

[a]Specificity is defined as the ratio of K_i of cGKIα to K_i of cGKII.
Abbreviation: ND, not determined.

antibody and a Europium-labelled secondary (anti-immunoglobulin) antibody. Energy transfer from europium to allophycocyanin can be detected only when the peptide has been phosphorylated and all four components of the assay interact appropriately. The activation and inhibition constants obtained with this assay are comparable to the values obtained with conventional radioactive cGK kinase assays that measure the transfer of radioactive phosphate from γ[^{32}P]-ATP to substrates. This sensitive assay can be used for screening new compounds using purified enzymes and cell extracts such as platelet lysates.

Possible applications of cGMP kinase and its targets in drug development

The well known NO–cGMP-elevating drugs are associated with adverse effects, such as the generation of toxic NO radicals during treatment with NO donors, or the additional inhibition of PDE1 or PDE6 with inhibitors of PDE5. Therefore, specific activators and inhibitors of cGKI and its targets could probably improve the specificity and availability of treatment for several diseases related to NO–cGMP signalling (e.g. asthma, thrombosis, angina pectoris, penile erectile dysfunction, gastrointestinal dysmotility, graft transplantation and diarrhoea). Until now, studies with cGMP analogues were mostly performed with animal models. Inhaled 8-Br-cGMP lowers pulmonary hypertension in a time- and dose-dependent manner [83] and might be used for the treatment of asthma. The activators 8-Br-cGMP and 8-pCPT-cGMP showed beneficial effects on graft survival after liver transplantation [84] and lung transplantation [85]. Lower pulmonary vascular resistance, increased blood flow, improved arterial oxygenation, decreased neutrophil infiltration and improved recipient survival has been observed. The application of 8-Br-cGMP improves posttransplant lung edema and is superior to the clinically used prostaglandin E_1 [86]. However, all known cGK activators and inhibitors also show cGK-independent effects [87,88]. Therefore, it could be important to interfere more specifically with cGK signalling pathways using cGKIα-, cGKIβ- or cGKII-specific activators or inhibitors. Selectivity could be achieved by manipulation of the different localization signals of cGK enzymes or specific interference with their distinct target proteins. Selective activation of cGKIα and cGKIβ signalling could be beneficial for cerebellar and hippocampal learning, respectively. Inhibitors of cGKIα could reduce nociception and therefore have application in the treatment of pain [89]. Furthermore, inhibition of cGKI might be useful for the prevention of cardiovascular diseases and atherosclerosis. The specific activation of cGKI or its signalling pathways could also be beneficial for the treatment of intestinal dysmotility disorders. Alternatively, the specific inactivation of cGKII might prevent diarrhoea (the deletion of cGKII in mice prevented STa-induced diarrhoea – this is particularly significant given that STa causes traveller's diarrhoea and is responsible for 50% of infant mortality in developing countries) [62]. In addition, it has been demonstrated that cGKs are important for habituation in insects [90] and that the deletion of cGKII is specifically associated with increased alcohol consumption [91]. Therefore, interference with cGKII signalling in the CNS could be a possible approach to the treatment of alcoholism.

Conclusion

Recent studies have highlighted the importance of cGKs in the cardiovascular system, the gastrointestinal tract, kidney and bladder, penile erection, bone growth and the nervous system. Furthermore, the analysis of cGK signalling cascades has extended the understanding of tissue-specific cGK functions. Several activators and inhibitors of cGK that are specific *in vitro* have been developed based on structural and functional analyses. However, many of them lack specificity *in vivo*. Therefore, the analysis of specific cGK signalling pathways and the development of specific modulators of cGKI, and its isoforms, or cGKII is still an important issue. These cGK-specific drugs could be useful for the treatment of diseases related to the cardiovascular system, gastrointestinal motility, asthma, penile erection and addiction.

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