Cyclic GMP–Dependent Protein Kinases and the Cardiovascular System
Insights From Genetically Modified Mice

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Abstract—Signaling cascades initiated by nitric oxide (NO) and natriuretic peptides (NPs) play an important role in the maintenance of cardiovascular homeostasis. It is currently accepted that many effects of these endogenous signaling molecules are mediated via stimulation of guanylyl cyclases and intracellular production of the second messenger cGMP. Indeed, cGMP-elevating drugs like glyceryl trinitrate have been used for more than 100 years to treat cardiovascular diseases. However, the molecular mechanisms of NO/NP signaling downstream of cGMP are not completely understood. Recent in vitro and in vivo evidence identifies cGMP-dependent protein kinases (cGKs) as major mediators of cGMP signaling in the cardiovascular system. In particular, the analysis of conventional and conditional knockout mice indicates that cGKs are critically involved in regulating vascular remodeling and thrombosis. Thus, cGKs may represent novel drug targets for the treatment of human cardiovascular disorders. (Circ Res. 2003;93:907-916.)

Key Words: smooth muscle relaxation ■ cGMP ■ nitric oxide ■ atherosclerosis ■ cardiac hypertrophy
The N-terminus (cGKI) and type II (cGKII), have been identified in mammals. The N-terminus of cGKI, cGKII, and other cGMP effectors are available. Only selective, but not highly specific agonists and inhibitors of cGKI, cGKII, and other cGMP effectors are available.

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Indeed, the widely used “cGK inhibitor” KT5823, which inhibits cGK activity in vitro, may not inhibit, or may even stimulate the enzyme in certain intact cells. Second, primary cells that endogenously express cGKs may readily loose cGK expression and/or other signaling components on passaging.

Cyclic GMP–Dependent Protein Kinases

Genes and Proteins

cGKs are serine/threonine kinases that are present in a variety of eukaryotes ranging from the unicellular organism Paramecium to humans. Two cGK genes, coding for cGK type I (cGKI) and type II (cGKII), have been identified in mammals. The N-terminus (≈50 amino acids 1 to 100) of cGKI is encoded by two alternatively used exons resulting in the production of two cGKI isoforms, cGKIα and cGKIβ. cGKIβ is activated at ≈10-fold higher cGMP concentrations than cGKIα.

The pharmacological analysis of cultured cells and the identification of cGK substrate proteins suggested multiple, and sometimes contradictory, cellular functions and mechanisms of cGK-mediated signaling. These in vitro studies have been extensively reviewed and will be briefly discussed later with respect to cardiovascular cell types. However, our understanding of the significance of cGKs as mediators of NO/NP/cGMP signaling in vivo is only at the beginning. The analysis of which cellular functions are dependent on cGKs is complex for several reasons. First, not only cGKs, but several other receptors for cGMP (Figure 1A) have to be considered as potential mediators of cGMP effects. Only selective, but not highly specific agonists and inhibitors of cGKI, cGKII, and other cGMP effectors are available.

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Third, many studies were performed with transfected cells that overexpressed cGKs at levels that may not represent physiological conditions.

**cGK Knockout Mice**

To study the (patho)physiological roles of cGKs in vivo, knockout mice were generated that “chronically” lack cGKI, cGKII, or both, in all cells (so-called conventional knockout mice or null mutants). cGKI null mutants have a decreased life span (50% of these mice die before 5 to 6 weeks of age), disturbed platelet adhesion and activation, and impaired guidance of sensory axons during embryogenesis. The short life expectancy of cGKI null mutants precludes the investigation of adult mice and the performance of long-term experiments like the analysis of atherosclerosis. Furthermore, these animals, in which the cGKI gene is inactivated in the germ line and thus in every cell throughout ontogeny, develop multiple defects, and it is difficult to distinguish whether a given phenotype reflects a primary/cell-autonomous requirement of cGKI in the affected cell type or arises secondary to defects in other cell types reflecting a non–cell-autonomous requirement of the cGKI gene in the affected cells. To overcome these limitations, a mouse line has been generated that allows for the time- and/or cell-specific (so-called conditional) inactivation of the cGKI gene in selected cell types using the Cre/lox site-specific recombination system. In contrast to conventional cGKI knockout mice, conditional mouse mutants that lack cGKI selectively in cardiomyocytes, SMCs, or distinct regions of the CNS are fully viable and can be studied throughout adulthood. Hippocampus- and Purkinje cell-specific cGKI mutants have deficits in synaptic plasticity in the CNS and motor learning. Conventional and conditional cGKI knockout mice show several cardiovascular phenotypes (Table), which are discussed below. cGKII knockout mice have a normal life span, decreased longitudinal bone growth, decreased intestinal chloride secretion, decreased cGMP-mediated inhibition of sodium reabsorption, loss of cGMP-induced inhibition of renin secretion, and a mild defect in circadian rhythmicity.

**cGKI and Vascular Function**

**Vasorelaxation and Blood Pressure**

NO and ANP stimulate cGMP synthesis in vascular SMCs and relax small arteries and arterioles resulting in a decreased blood pressure. Targeted inactivation of the genes encoding endothelial NOS, ANP, or the ANP receptor, GC-A, causes hypertension (see review). Juvenile (4- to 5-week-old) cGKI knockout mice show impaired cGMP-dependent relaxation of large and small arteries and have an elevated blood pressure, suggesting that the anti-hypertensive effects of NO and ANP are at least partially mediated by cGKI. Interestingly, cGKI null mutants show normal arteriolar dilations in response to acetylcholine in vivo. These findings support the concept that acetylcholine-induced vasorelaxation is mediated, at least in part, by mechanisms not involving NO, cGMP, and cGKI but the endothelium-derived hyperpolarizing factor (EDHF). Blood pressure may be regulated also by cGKII via inhibition of renin secretion (see following sections).

Potential in vivo targets for cGKI in vascular SMCs are the Ca2+-activated K+ (BKCa) channel and IRAG, proteins involved in the modulation of extracellular Ca2+ entry and intracellular Ca2+ release, respectively. Phosphorylation of these two proteins is thought to reduce the cytosolic Ca2+ concentration, thereby, leading to vasorelaxation (Figure 2). An alternative substrate for cGKI may be phospholamban, which modulates the activity of the Ca2+-ATPase of the endoplasmic reticulum. However, it was reported that phospholamban plays only a minor role, if any, in cyclic nucleotide-mediated vasorelaxation. It cannot be excluded that the Ca2+-ATPase is directly activated by cGMP-dependent phosphorylation, which would also decrease cytosolic Ca2+ levels. Vascular SMCs isolated from wild-type mice endogenously express both cGKIα and cGKIβ. NO/
cGMP inhibits noradrenaline-induced Ca\(^{2+}\) transients in wild-type but not in cGKI-deficient vascular SMCs. Interestingly, the defective Ca\(^{2+}\) regulation in cGKI-deficient cells can be rescued by transfection of the cGKI\(\alpha\) isoform but not the I\(\beta\) isoform. These results suggest that cGKI\(\alpha\) relaxes smooth muscle by decreasing the cytosolic Ca\(^{2+}\) level. The role of cGKI\(\beta\) in SMCs is unclear at present, but may be more related to the cGKI effects on smooth muscle proliferation, differentiation, and gene expression (see following section). The results described above do not exclude the possibility that cGKI decreases vessel tone by additional mechanisms resulting in dephosphorylation of the myosin light chains without affecting the cytosolic Ca\(^{2+}\) level (Figure 2). These mechanisms could involve the activation of myosin phosphatase, inhibition of RhoA signaling, or phosphorylation of the myosin-binding protein, telokin.

The interpretation of the pathophysiology of cGKI knockout animals is complicated by the finding that 7-week-old and older cGKI null mutants have a normal or only slightly elevated blood pressure, indicating that the lack of cGKI can be bypassed in older animals. Similarly, deletion of the BK\(_{\alpha}\) channel, one of the known targets of cGKI in vascular smooth muscle, only marginally affects the blood pressure of adult animals. These results suggest that mice with defects in cGKI signaling develop mechanisms to compensate for lost gene functions, or, alternatively, that the physiological significance of this pathway for blood pressure regulation is age-dependent. However, cGKI null mutants develop multiple phenotypes with increasing age including infections and inflammation, which are known to induce massive NO synthesis. High concentrations of NO can increase cGMP levels to extreme values in vascular smooth muscle. Furthermore, cGMP levels may be elevated in cGKI-deficient animals as a result of less cGMP degradation, because normally phosphorylation by cGKI enhances the activity of the cGMP-hydrolyzing PDE. Thus, it is tempting to speculate that the apparent “normalization” of blood pressure in older cGKI null mutants is due to cross-activation of cAK by high cGMP levels that are potentially generated in these mice. Taken together, the analysis of cGKI null mutants supports the notion that vasorelaxation via the cGMP/cGKI pathway contributes but is not essential to the regulation of basal blood pressure. This view was recently supported by the finding that mice lacking the ANP receptor, GC-A, in vascular smooth muscle have normal blood pressure under basal conditions.

**Vascular Remodeling**

In addition to vasodilation, NO/NP/cGMP signaling is involved in the development of vasculoproliferative disorders, such as restenosis and atherosclerosis. The analysis of transgenic mice showed that NO can both promote and inhibit pathological vascular remodeling (see review). This finding could explain why NO-generating drugs have not been reported to limit the progression of atherosclerosis in humans. The opposing actions of NO might depend on the magnitude and spatiotemporal profile of its production in a specific pathophysiological setting and are likely mediated through different cellular and molecular mechanisms. A key process in vascular remodeling is the phenotypic modulation of vascular SMCs from contractile to proliferative/differentiated cells. It has been reported that NO and cGMP can both promote and inhibit the proliferation of cultured SMCs (see reviews). The reason for these contradictory findings and their (patho)physiological significance is not clear. Different results may be related to the use of primary versus subcultured cells. As discussed, repeated passaging might lead to downregulation of cGKI expression and/or alterations in other signaling components and proliferative responses. cGKI is expressed in SMCs of the media and neointima, although some studies but not others found a transient reduction of its expression after vascular injury. Adenoviral gene transfer of the constitutively active kinase domain of cGKI reduced neointima formation after vascular injury in rats and pigs, whereas gene transfer of wild-type cGKI\(\beta\) was ineffective. The relevance of endogenous cGKI to restenosis has not been investigated yet.

The specific role of smooth muscle cGKI in vascular remodeling was recently studied in hypercholesteremic ApoE-deficient mice, a mouse model of atherosclerosis. Postnatal SMC-selective ablation of cGKI resulted in decreased atherosclerotic plaque formation in the aorta of ApoE-deficient mice. In the same study, the fate of SMCs was followed by a genetic cell marking technique. Interestingly, the development of SMC-derived plaque cells was strongly impaired in cGKI mutant mice. These findings support the notion that endogenous smooth muscle cGKI promotes the development of SMC-derived plaque cells and atherosclerotic lesions in the intact animal. The in vivo results were corroborated by the analysis of primary aortic SMCs isolated from wild type and cGKI knockout mice. Treatment of wild-type cells (which endogenously express cGKI) with a membrane-permeable cGMP analogue led to cells showing enhanced phosphatidylinositol 3-kinase (PI3K)/Akt kinase signaling and proliferation, increased levels of vascular cell adhesion molecule-1 and peroxisome proliferator-activated receptor \(\gamma\), and a decreased level of plasminogen activator inhibitor-1 (PAI-1), all potentially proatherogenic properties. These cGMP effects were apparently mediated by cGKI because they were not observed in cGKI-deficient cells. Thus, smooth muscle cGKI promotes the development of atherogenic SMCs in vivo and in vitro, and could contribute...
to the proatherogenic but not to the antiatherogenic effect of NO. NO/NP/cGMP signaling is also involved in angiogenesis, another process that involves vascular remodeling. Ischemia-induced angiogenesis was significantly potentiated in transgenic mice overexpressing cGKIA and attenuated in cGKI null mutants, indicating that cGKI is critical for neovascularization in vivo. Taken together, these studies suggest that cGKI-dependent pathways promote a variety of vasculoproliferative processes under pathological conditions (Figure 2).

What could be the molecular mechanism(s) for the proatherogenic and proangiogenic effect of cGKI? An emerging theme of cGK-mediated signaling is the regulation of gene expression and cell growth. In transfected nonvascular cells, activation of cGKI can result in translocation of the enzyme into the nucleus and stimulation of the fos promoter. However, many studies failed to detect nuclear cGKI in various cell types including vascular SMCs, leading to the speculation that cGKI may be retained in the cytoplasm by cell-specific anchor proteins. Several lines of evidence indicate that cytosolic cGKI activates mitogen-activated protein kinase (MAPK) and PI3K/Akt kinase pathways in vascular SMCs and endothelial cells. Thus, the cGMP/cGKI system might modulate gene expression and promote vascular cell proliferation via cross-talk with MAPK and/or PI3K/Akt kinase signaling (Figure 2). cGKI-dependent pathways regulate the expression of several proteins that are involved in the pathogenesis of vascular disorders. For example, both cGMP and cGKI suppress the expression of PAI-1. PAI-1 is secreted by vascular SMCs and has an atheroprotective effect, in part by inhibiting the accumulation of macrophages in plaques. The basal expression of the small G-protein RhoA, which has been implicated in excessive proliferation associated with atherosclerosis, is upregulated by cGKI in vascular SMCs. However, cGKI also inhibits RhoA-dependent effects on smooth muscle contraction and gene expression. The net effect of cGKI on RhoA signaling is not clear. Phenotypic changes of vascular SMCs might also be mediated by phosphorylation of the vasodilator-stimulated phosphoprotein (VASP), a well-known cGKI substrate. VASP and its homologue Ena have been identified as regulators of cell shape and motility.

**cGKI and Platelet Function**

High concentrations of cGKI were detected in human platelets at a time when the heterogeneity of cGMP effector systems was only partially recognized and when physiological stimulators of cGMP such as NPs and NO were just being discovered. Today, we know that human platelets generate cGMP only by the soluble, NO-activated guanylyl cyclase, degrade cGMP via PDE 2 and 5, and contain cGMP effector systems consisting of cGKIβ and cGMP-regulated PDEs [PDE 5, which hydrolyzes cGMP, and PDEs that reduce (PDE 2) and enhance (PDE 3) cAMP levels]. It is now also well established that the most important in vivo physiological platelet inhibitors are endothelium-derived factors and their mediators, i.e., NO/cGMP, prostacyclin/cAMP, and CD39/ATPase (ATP diphosphohydrolase), which inactivates the platelet agonist ADP. The NO/cGMP and prostacyclin/cAMP inhibitory pathways have multiple synergistic interactions with respect to cyclic nucleotide generation/degradation and protein phosphorylation in platelets. There is still a lack of in vivo evidence in volunteers and/or patients, of direct platelet inhibition by NO-generating drugs such as glyceryl trinitrate and other nitrates that activate sGC. However, recent preclinical studies with an NO-independent sGC activator suggest that elevation of platelet cGMP in vivo is in fact associated with platelet inhibition.

Therapeutically used platelet inhibitors are aspirin (a cyclooxygenase inhibitor), glycoprotein IIb/IIIa inhibitors, ADP receptor P2Y12 inhibitors, and adenosine uptake/PDE 5 inhibitors such as dipyridamole, which increase cGMP. As discussed later, cGKI may mediate inhibitory effects on platelets via several of these same targets. Experiments with both cGKI-deficient human and murine platelets demonstrated that cGKI mediates many aspects of NO/cGMP inhibition of platelet activation.

Importantly, a prominent role of cGKI in the inhibition of platelet adhesion/activation in vivo during ischemia/reperfusion of the microcirculation was conclusively demonstrated by intravitral video microscopy analyses comparing cGKI-deficient versus wild-type murine platelets perfused back into mice. These experiments clearly showed that platelet cGKI, but not endothelial or smooth muscle cGKI, is essential to prevent intravascular adhesion and aggregation of platelets after ischemia. Furthermore, NO/cGMP/cGKI causes phosphorylation of platelet VASP, which closely correlates with inhibition of platelet activation both in vitro and in vivo, with fibrinogen receptor (integrin GPlib/IIIa) inhibition, and with inhibition of both VASP binding to F-actin and VASP localization to focal adhesions/integrins. Besides VASP, other platelet substrates phosphorylated by cGKI (see review) include the IP3 receptor, heat shock protein 27, the LIM and SH3 protein (LASP), and the small GTPase Rap 1b.

NO/cGMP signaling via cGKI is also known to inhibit platelet Gq/Gi-coupled receptor responses, and in particular the platelet ADP receptor P2Y12. Thus, one consequence of the impaired NO/cGMP signaling found in cardiovascular diseases characterized by endothelial dysfunction, could be enhanced signaling via purinergic Gq/Gi-coupled receptors (P2Y1 and P2Y12) leading to enhanced platelet activation. The molecular mechanisms of inhibition of G-protein–coupled responses by cGKI have yet to be elucidated. Furthermore, evidence indicates that cGKI mediates at least some major antiplatelet effects of dipyridamole, which in combination with low-dose aspirin (Aggrenox) is very effective in preventing recurrent stroke. Therapeutically relevant concentrations of dipyridamole, an inhibitor of cAMP and cGMP hydrolysis, were shown to selectively enhance antiplatelet effects and cGKI substrate phosphorylation stimulated by the NO donor, sodium nitroprusside. In vitro and in vivo experiments, including ones with cGKI null mice, have shown that cGMP/cGKI mediated inhibition of platelet aggregation. Although dipyridamole also elevates adenosine and stimulates the release of endothelial prostacyclin (agents which elevate cAMP), cAMP/cAK signaling was not enhanced by therapeutically relevant concentrations of dipyridamole.
cGKI also contributes to negative feedback or to cycling in signaling systems it transduces. Recent results indicated that cGKI phosphorylation of PDE 5 and activation of cGMP degradation may contribute to desensitization of the platelet NO/cGMP response and to the contraction-relaxation cycle in smooth muscle.\textsuperscript{23,54} cGKI has also been suggested by some studies to even promote transient platelet activation under certain conditions.\textsuperscript{23} In response to the platelet agonist von Willebrand factor, a biphasic cGMP/cGKI-mediated response was observed, consisting of rapid platelet activation (perhaps involving VASP phosphorylation),\textsuperscript{23} followed by a more sustained, long-lasting platelet inhibition (perhaps involving VASP activation),\textsuperscript{23} clearly, further elucidation of the functional properties of cGKI and its substrates in platelets will be required to more precisely define the molecular mechanisms of cGKI-mediated platelet responses and to identify novel targets for intervention in platelet-dependent pathology.

**cGKI and Cardiac Function**

Previous studies suggested that NO/cGMP contribute to the regulation of cardiac function and remodeling.\textsuperscript{116} The combined analysis of conventional and cardiomyocyte-specific cGKI knockout mice demonstrated that cGKI mediates the negative inotropic effect of NO/cGMP in the juvenile as well as in the adult murine heart.\textsuperscript{25} However, the NO/cGMP/cGKI pathway does not appear to be involved in the negative inotropic action of acetylcholine.\textsuperscript{25,117,118} The development of cardiac hypertrophy and congestive heart failure is associated with the expression of several fetal genes such as ANP and BNP.\textsuperscript{119} Mice lacking ANP or the ANP receptor, GC-A, develop pressure-independent cardiac hypertrophy.\textsuperscript{120–124} The hypotrophic response of cultured neonatal rat ventricular myocytes to α\textsubscript{1}-adrenergic stimulation is suppressed by ANP, NO, or cGMP.\textsuperscript{125} These results indicate that stimulation of cGMP synthesis by ANP/GC-A or NO inhibits cardiomyocyte hypertrophy. Whether or not the antihypertrophic effect of cGMP is mediated by cGKI is presently unclear. Adenoviral overexpression of cGKI inhibits myocyte hypertrophy in vitro,\textsuperscript{126} at least in part via inhibition of the calcineurin-NFAT pathway.\textsuperscript{127} However, neither global nor cardiomyocyte-specific ablation of cGKI affects the development of cardiac hypertrophy under basal or hypertrophy-inducing conditions in vivo (L.J. De Windt and R.F., unpublished data, 2003).

**cGKs and Kidney Function**

Whereas elevation of blood pressure is counteracted by ANP, cGKs and Kidney Function also contribute to negative feedback or to cycling in signaling systems it transduces. Recent results indicated that cGKI phosphorylation of PDE 5 and activation of cGMP degradation may contribute to desensitization of the platelet NO/cGMP response and to the contraction-relaxation cycle in smooth muscle.\textsuperscript{53,54} cGKI has also been suggested by some studies to even promote transient platelet activation under certain conditions.\textsuperscript{23} In response to the platelet agonist von Willebrand factor, a biphasic cGMP/cGKI-mediated response was observed, consisting of rapid platelet activation (perhaps involving VASP phosphorylation),\textsuperscript{23} followed by a more sustained, long-lasting platelet inhibition (perhaps involving VASP activation),\textsuperscript{23} clearly, further elucidation of the functional properties of cGKI and its substrates in platelets will be required to more precisely define the molecular mechanisms of cGKI-mediated platelet responses and to identify novel targets for intervention in platelet-dependent pathology.

**cGKI is a candidate for ANP signaling in the kidney**

Mechanisms of ANP effects in the kidney include afferent arteriole dilation and efferent arteriole constriction to increase glomerular capillary hydraulic pressure and glomerular filtration rate, as well as inhibition of angiotensin II–stimulated reabsorption of NaCl and water in the proximal tubule, and inhibition of renin release and thus angiotensin II formation.\textsuperscript{131} There is also evidence that NO has biphasic effects that, besides inhibition of renin release, include inhibition of PDE 3 to increase cAMP, which stimulates renin release.\textsuperscript{132} cGKI is expressed at several sites in the kidney, including juxtaglomerular cells and proximal tubules.\textsuperscript{133} Stimulation of endogenous cGK or adenoviral overexpression of cGKI or cGKII in juxtaglomerular cells suppresses renin release.\textsuperscript{134} However, a physiological role for primarily cGKI, rather than cGKI, is suggested by the fact that only cGKI shows strong colocalization with renin in juxtaglomerular cells.\textsuperscript{134} Indeed, inhibition of renin release is abolished in cGKI null mice, but not in cGKI null mice.\textsuperscript{34} Thus, cGKI may mediate ANP inhibition of NaCl and water reabsorption by inhibiting renin release and, therefore, angiotensin II formation. In contrast to cGKI, cGKII is present in kidney vasculature, mesangial cells, and contractile interstitial cells,\textsuperscript{135} suggesting cGKI as a candidate mediator of ANP stimulation of the glomerular filtration rate. Furthermore, cGKs could be involved in ANP/NO/cGMP inhibition of the epithelial Na\textsuperscript{+}/H\textsuperscript{+} exchanger, NHE3, which plays a major role in Na\textsuperscript{+} and HCO\textsubscript{3}\textsuperscript{-} absorption in the proximal tubule as well as in intestinal epithelial cells.\textsuperscript{136,137} Whether the impaired Na\textsuperscript{+} absorption observed in the intestine of cGKI null mice\textsuperscript{33} applies also to the proximal tubule requires further investigation. Other candidate proteins that could be modulated by cGK in the proximal tubule include cGMP-inhibited K\textsuperscript{+} channels,\textsuperscript{138} Na\textsuperscript{+}/K\textsuperscript{+}-ATPase in the basolateral membrane,\textsuperscript{139} and cystic fibrosis transmembrane conductance regulator (CFTR) Cl\textsuperscript{−} channels.\textsuperscript{140} However, the CFTR channel is unlikely to play an important role in Cl\textsuperscript{−} transport in the kidney, comparable to its key role in Cl\textsuperscript{−} secretion in the intestine,\textsuperscript{2,31,33} because proximal tubule function is normal in CFTR mutant mice.\textsuperscript{140} Interestingly, disruption of the mouse gene encoding the protein phosphatase 1 inhibitor, DARPP-32, a cGK substrate, causes loss of both ANP-induced natriuresis and inhibition of Na\textsuperscript{+}/K\textsuperscript{+}-ATPase activity, and results in increased arterial blood pressure.\textsuperscript{139} This finding raises the possibility that cGK effects on protein phosphorylation might also be indirect via regulation of protein phosphatase activity. A similar mechanism was recently suggested for cGKI modulation of synaptic plasticity and learning in the cerebellum.\textsuperscript{30} cGKII mRNA and protein have also been detected in the apical and basolateral membranes of epithelial cells in the rat ascending thin limb,\textsuperscript{133} and in rabbit connecting tubules and cortical collecting ducts.\textsuperscript{141} However, firm evidence for a role of cGKII in the regulation of Na\textsuperscript{+} or Cl\textsuperscript{−} transport in these segments is presently lacking.\textsuperscript{141} Clearly, microperfusion studies in cGKII null mice are needed to identify possible effects of cGKII signaling on ion transport in each nephron segment separately. The apparent absence of gross abnormalities in salt or water retention in cGKII-deficient mice does
not exclude a prominent role of cGKII in renal handling of salt and water, because such abnormalities might be compensated by intraintrarenal homeostatic mechanisms involving tubuloglomerular feedback and adaptations in renal plasma flow. An example of unmasking such compensatory mechanisms was recently demonstrated in NHE3-deficient mice with transgenic rescue of small intestinal NHE3 expression.\textsuperscript{142}

**Therapeutic Potential of cGK-Mediated Cardiovascular Signaling**

The phenotypes of conventional and conditional mouse mutants (Table) identify key roles of cGKI in the cardiovascular system, particularly in modulating SMC, cardiomyocyte, and platelet properties. cGKII might influence hemodynamic parameters via regulation of renin release and ion transport in the kidney. The general view that vascular signaling via cGKI has antihypertensive and antiproliferative effects, and thus should play a beneficial role in cardiovascular diseases, has been tempered by the results obtained with cGKI-deficient mouse models. These studies indicate that cGKI has effects on, but is probably not essential for, the regulation of basal blood pressure, however, is critically involved in vasculoproliferative processes like atherosclerosis and neovascularization, and in platelet responses. Additional studies with mice carrying cell type–specific cGKI mutations, for example, in endothelial and immune cells, will be necessary to fully explore the role of cGKI as a component of pathological vascular remodeling.

The therapeutic potential of cGMP-elevating drugs has been documented by the clinical success of NO-generating drugs for the treatment of angina pectoris and congestive heart failure, and more recently by PDE 5 inhibitors for the treatment of erectile dysfunction and pulmonary hypertension. However, the novel findings with transgenic mice discussed here raise concerns that in addition to their short-term beneficial effects, cGMP-elevating drugs may have undesired long-term effects that could perhaps even promote atherosclerosis and its complications. Indeed, it has been reported that long-term nitrate therapy in chronic coronary artery disease is associated with a significantly increased mortality risk.\textsuperscript{143} It is tempting to speculate that cGKI mediates, at least in part, potentially deleterious cardiovascular effects of endogenous NO/cGMP and cGMP-elevating drugs. Pharmacological inhibition of smooth muscle cGKI might be a novel therapeutic option to treat atherosclerosis. It is anticipated that cGKI\textsubscript{\textalpha} and cGKI\textsubscript{\textbeta} serve different functions such as vasorelaxation and modulation of phenotypic changes. The development of isoform-specific cGKI mouse mutants and isoform-specific cGKI inhibitors and activators should help to further dissect cGKI signaling and its therapeutic potential for cardiovascular diseases.

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