[Frontiers in Bioscience 10, 1279-1289 May 1, 2005]

INSIGHTS INTO cGMP SIGNALLING DERIVED FROM cGMP KINASE KNOCKOUT MICE

Jens Schlossmann, Robert Feil and Franz Hofmann

Institute for Pharmacology and Toxicology, Technical University Munich, Biedersteiner Strasse 29, 80802 Munich, Germany

TABLE OF CONTENTS

1. ABSTRACT

cGMP signalling plays an important physiological role in diverse organs including the vasculature, the GI-tract and the nervous system. Furthermore, cGMP-elevating substances such as glyceryl trinitrate are important drugs used in cardiovascular diseases. Physiologically, cGMP synthesis is induced by nitric oxide (NO) and natriuretic peptides through the stimulation of guanylyl cyclases. Major mediators of cGMP signalling are the cGMP-dependent protein kinases type I and II (cGKI and cGKII). The functional significance of each kinase type in diverse organs was determined using total and tissue-specific cGKI- and cGKII-deficient mice. These studies established that cGKI plays a major role in the regulation of the cardiovascular and the gastrointestinal system, hippocampal and cerebellar learning and pain perception. cGKII is involved in intestinal water secretion, bone growth and circardian rhythmicity. The cGK mutant mice are important tools to obtain detailed insights into cGMP-mediated signalling pathways in health and disease.

2. CYCLIC GMP

Cyclic guanosine-3`,5`-monophosphate (cGMP) is a second messenger molecule which plays a physiological role in diverse organs comprising the cardiovascular system, the gastrointestinal tract, the immune system, and the brain. cGMP is generated by soluble and particulate guanylyl cyclases that are activated by nitric oxide (NO) and by atrial, brain or C-type natriuretic peptides (ANP, BNP and CNP), respectively. cGMP exerts its physiological effects through at least three classes of cGMP effectors: cGMP-dependent protein kinases (cGK), cyclic nucleotide-gated ion channels and cGMP-regulated phosphodiesterases (PDE) which hydrolyze cGMP and/or cAMP. In addition, cGMP is able to activate cAMP-dependent protein kinase either at high cGMP concentrations or by inhibiting cAMP-specific PDE which rise the cAMP concentration. cGMP is finally degraded by cGMP-hydrolyzing PDE which regulate the duration and the amplitude of the cGMP signal.

3. cGKs: GENES AND PROTEINS

3.1. cGKI and cGKII in mammals

In mammals two different genes encode cGMP-dependent protein kinases, cGKI and cGKII. The cGKs belong to a subfamily of serine/threonin kinases with common structural features. The protein has three functional domains: an N-terminal domain, a regulatory domain and a catalytic domain. The catalytic domain transfers the gamma-phosphate of ATP to the serine/threonine residue of the substrate protein. The

regulatory domain contains two allosteric cGMP binding sites. The N-terminal domain contains a leucine/isoleucine zipper which is required for i) homodimerization ii) inhibition of the catalytic center which is released after cGMP binding to the regulatory domain iii) autophosphorylation-dependent activation of the enzyme at low cGMP concentrations and iv) subcellular targeting of the enzymes (1-5).

cGKI is a cytosolic enzyme, whereas cGKII is targeted to the plasma membrane by myristoylation of the Gly2 residue (6). Alternative splicing of the exons encoding the N-terminal domain of cGKI leads to expression of two different isoforms, cGKIalpha and cGKIbeta. The cGKI isozymes differ from each other in their cGMP dependence, substrate specificity and tissue distribution. The cGKIalpha enzyme is activated at 10-fold lower cGMP concentrations than the cGKIbeta enzyme (1). cGKIalpha and cGKIbeta might regulate different physiological functions, because they bind distinct substrate proteins through their different N-terminal domains and may localize to different subcellular compartments. cGKIalpha and cGKIbeta are also localized in different tissues. cGKIalpha is highly expressed in the cerebellum, in dorsal root ganglia and in smooth muscle tissues. cGKIbeta is mainly found in smooth muscle, in platelets, in the hippocampus and in the olfactory bulb (7-10). Lower levels of cGKI are present in fibroblasts, in renal cells, in the spinal cord and in leukocytes. cGKII is expressed in the intestinal mucosa, kidney, chondrocytes, lung and several brain nuclei (11). The broad expression pattern of the cGKs indicates a pleiotropic function of these enzymes. The function of cGKI and cGKII was analysed in detail using total knockout mice which lack cGKI or cGKII in all cells and tissue-specific mutants in which the particular cGK gene was inactivated in selected cell types using the Cre/lox site-specific recombination system (12, 13). The functional consequences of the enzyme deletions will be described in sections 3.3 and 4. More general applicable results will be described in section 3.2.

3.2. cGK signalling

The cGKI signalling pathway is best understood in smooth muscle, where cGKI activation leads to a decreased vascular tone (14-16). Relaxation is induced by Ca^{2+}-dependent and Ca^{2+}-independent mechanisms. Ca^{2+}-dependent mechanisms include reduction of $[Ca^{2+}]_i$ by interference with inositol 1,4,5-trisphosphate ($InsP_3$)-induced calcium release from intracellular stores and membrane hyperpolarization which reduces Ca^{2+}-influx. Ca^{2+}-independent mechanisms center around the inhibition of Rho signalling leading to an increased activity of myosin light chain phosphatase. Ca^{2+}-dependent mechanisms might be mediated by the $InsP_3$-receptor associated cGMP kinase substrate (IRAG), a substrate for cGKIbeta. Its phosphorylation inhibits $InsP_3$-induced calcium transients (3, 17). Indeed, recent evidence obtained with mice in which IRAG was mutated suggests that cGKI-dependent relaxation of hormone-induced tension is mainly caused by the Ca^{2+}-dependent cGKI/IRAG pathway (Schlossmann and Hofmann, unpublished data). These results also indicate that identification and analysis of the cGKI substrates are very important issues to understand the precise cGKI signalling events. Another Ca^{2+}-dependent mechanism of cGKI is the activation of maxi potassium channel (BK_{Ca}) (18, 19). Activated BK_{Ca} channels hyperpolarize the cell membrane and close L-type calcium channels that provide extracellular calcium for contraction (20, 21). Interestingly, deletion of the BK_{Ca} channel only marginally affected the blood pressure in adult animals (22). Several groups showed that phospholamban is phosphorylated in smooth muscle by cGKI (23, 24). This reaction should increase Ca^{2+}-uptake into the intracellular stores and ,thereby, induce relaxation. However, phospholamban-deficient mice showed unaltered cyclic nucleotide-induced aortic relaxation (25). The Ca^{2+}-desensitizing mechanisms which induce smooth muscle relaxation involve the myosin phosphatase and RhoA (14). Myosin phosphatase might be activated by cGKIalpha via phosphorylation of the myosin binding subunit (26, 27). Nevertheless, a direct effect of cGKI on myosin phosphatase activity was not observed up to now. The activation of myosin phosphatase could also result from the phosphorylation and thereby inactivation of RhoA by cGKI (28).

The best known signalling mechanism for cGKII so far is the phosphorylation of the cystic fibrosis transmembrane conductance regulator (CFTR) ion channel. Only the membrane-bound cGKII phosphorylates CFTR. This phosphorylation stimulates chloride and water secretion (29). Several other targets exist for cGKII, but the underlying signalling mechanisms have yet to be resolved.

3.3. cGK knockout mice

cGK-deficient mice are useful tools to determine the specific physiological functions of cGKs. The cGKI null mutants have a reduced life-span (50% of the mice die before 5 to 6 weeks of age). These mice show multiple defects including impaired smooth muscle relaxation, disturbed gastrointestinal motility (30) and enhanced platelet adhesion (31). Tissue-specific cGKI knockout mice were generated for cardiac and smooth muscle as well as for different regions of the central nervous system (hippocampus and cerebellum) which allowed one to examine the cell type-specific functions of cGKI. The cGKII null mutants have a normal life-span. They also show multiple phenotypes including dwarfism, decreased intestinal chloride and water secretion, an altered renin secretion and an alcohol preference (32-35). A variety of functional studies were performed with these cGKI- and cGKII-deficient mice (see below and summary in Table 1).

4. cGK: FUNCTION

4.1. cGKI in the cardiovascular system

cGKI plays an important role in cardiovascular physiology and pathophysiology. For instance, cGKI affects the contractility and phenotype of vascular smooth muscle cells, platelet function and cardiac contractility.

4.1.1. Vascular smooth muscle

NO and ANP stimulate the synthesis of cGMP which relaxes small arteries and thereby decreases blood

Table 1. Phenotypes of Transgenic Mice That Lack or Overexpress cGMP-dependent Protein Kinases

GENE	MOUSE MODEL	ORGAN	PHENOTYPES	REFERENCES
cGKI	Null mutation	Vascular system	Impaired NO/cGMP-dependent vasorelaxation	19, 30, 39
			Age-dependent hypertension	30
			Impaired ischemia-induced angiogenesis	45
		Platelets	Enhanced platelet activation	31
			Impaired fibrinogen-receptor-mediated platelet activation (*in vitro*)	31
		Heart	Blunted cardiac negative inotropic response to cGMP	57
		GI-tract	Defective NO/cGMP-dependent relaxation	62, 63
			Severe motility defects	30
			Pylorus stenosis	30
		Bladder	Decreased relaxation and rhythmic activity	71
		Nervous system	Normal synaptic plasticity in the hippocampal CA1-region	9
			Guidance defect of sensory axons	8
			Reduced nociception	89
		Penis	Lack of penile erection	92
		Immune system	Enhanced neutrophil chemotaxis and granule secretion	95
	Smooth muscle-specific mutation	Vascular system	Decreased atherosclerosis on ApoE-/- background	44
		Intestine	Impaired relaxation of intestine *in vitro* but no motility defects	64
	Cardiomyocyte-specific mutation	Heart	Blunted cardiac negative inotropic response to cGMP	57
	Hippocampus-specific mutation	Hippocampus	Normal LTP in young animals, reduced LTP in adult animals in CA1-region	76
	Purkinje cell-specific mutation	Cerebellum	Impaired cerebellar LTD and learning	79
	Overexpression		Increased ischemia-induced angiogenesis	45
cGKII	Null mutation	Small intestine	Decreased anion and water secretion response to STa	32
		Kidney	Increased renin secretion	34
		Bone	Retarded bone growth, dwarfism	32, 33
		Nervous system	Normal synpatic plasticity in hippocampal CA1-region	9
			Alcohol preference	35
			Altered circadian rhythmicity	82

pressure. Accordingly, the deletion of endothelial NO synthase (eNOS), ANP or its receptor, GC-A, in mice caused high blood pressure (36-38). In line with this, cGKI knockout mice exhibited impaired NO/cGMP-dependent relaxation of small arteries and aortic rings (19, 30). Furthermore, young cGKI knockout mice (about 4-5 weeks) developed hypertension Therefore, the antihypertensive effects of nitrates and ANP might be mediated at least partially by cGKI. However, older cGKI knockout animals (about 7 weeks) developed normotension. This might be caused by compensatory mechanisms in the mice. Interestingly, cGKI null mutants showed normal arteriolar dilations in response to acetylcholine *in vivo* (39). This supports the view that not only nitric oxide (EDRF) via cGMP and cGKI but also the endothelium-derived hyperpolarization factor (EDHF) is involved in acetylcholine-induced vasorelaxation.

cGKI relaxes the vascular smooth muscle tone through Ca^{2+}-dependent and Ca^{2+}-independent mechanisms as described in section 3.2. The inhibitory effect of cGMP on noradrenaline-induced calcium transients in vascular smooth muscle cells was abolished in cGKI-deficient mice. Reconstitution of cultured cGKI-deficient vascular smooth muscle cells with cGKIalpha but not with cGKIbeta restored the inhibitory effect of cGMP on noradrenaline-induced calcium transients (40). These results suggest that cGKIalpha might be the predominant cGKI isoform inhibiting intracellular calcium transients in vascular smooth muscle. However, the role of the cGKIalpha and cGKIbeta isoforms in smooth muscle relaxation *in vivo* remains to be analysed. In this respect it is interesting to note that mice expressing a mutated form of IRAG, a substrate for cGKIbeta but not cGKIalpha, show reduced NO/cGMP-dependent inhibition of hormone-induced calcium transients and smooth muscle contraction (Schlossmann and Hofmann, unpublished data). It is, therefore, likely that the cGKIbeta/IRAG pathway contributes to smooth muscle relaxation *in vivo.* In the future it will be important to determine whether cGKIalpha and/or cGKIbeta restores the defective cGMP-dependent vascular relaxation *in vivo* in cGKI-deficient mice.

In smooth muscle, the cGMP level is regulated by phosphodiesterases, mainly by PDE5. cGKI-specific phosphorylation of PDE5 contributes to the activation of the enzyme and lowers thereby cGMP (41). Therefore, it is possible that cGMP levels might be enhanced in cGKI-deficient mice. The potentially elevated cGMP concentration might be involved in normalization of the

blood pressure of older cGKI-deficient mice via cross-activation of PKA (19).

Hitherto it is controversial whether NO/cGMP/cGKI signalling enhances and/or limits vascular remodelling. The analysis of transgenic mice showed that NO is able to promote and also to inhibit vascular remodelling (42). Furthermore, NO as well as cGMP can exhibit both antiproliferative and proliferative effects on smooth muscle cells (43). Thus, at least part of the NO effects on vascular remodelling might be mediated by cGMP and cGKI. Indeed, recent results obtained with cGKI mutant mice indicate that cGKI promotes the phenotypic modulation and proliferation of vascular smooth muscle cells. Smooth muscle-specific cGKI knockout mice showed an impaired development of smooth muscle-derived plaque cells and a reduced atherosclerotic lesion area on an ApoE knockout background indicating that smooth muscle cGKI contributed to the proatherogenic effect of (44). In line with this observation is a report that natriuretic peptides and cGKI improved vascular regeneration and angiogenesis in an ischemia model (45).

4.1.2. Platelets

Nitric oxide and cGMP are important inhibitors of platelet functions including aggregation and granule secretion. There is evidence that these effects are mainly mediated by cGKI as deletion of cGKI in mice enhanced platelet aggregation and serotonin secretion (31). Furthermore, the activation of the fibrinogen receptor was not inhibited in cGKI knockout mice. *In vivo*, cGKI-deficient platelets showed increased adhesion and aggregation after ischemia/reperfusion.

The main mechanism of cGKI-dependent inhibition of platelet function is probably the reduction of agonist-induced increases in $[Ca^{2+}]_i$. Thus, cGKI-deficient platelets from patients with chronic myelocytic leukemia lacked cGMP-mediated regulation of intracellular calcium (46). Activation of cGKI inhibited the calcium release from $InsP_3$-sensitive stores in platelets and megakaryozytes (47, 48). Although the $InsP_3$-receptor ($InsP_3R$) is phosphorylated by cyclic nucleotide-dependent kinases in platelets (49, 50). other evidence suggests that the decreased intracellular calcium release is caused by phosphorylation of the cGKI substrate IRAG (51). Other substrates which could be involved in the cGKI-dependent regulation of platelet function include the cGKI-substrate VASP and the phosphodiesterase PDE5. The deletion of VASP lead only to a minor phenotype and affected only slightly the inhibition of platelet aggregation by cyclic nucleotides (47, 52). VASP is of physiological importance because nitric oxide did not inhibit the adhesion of VASP-deficient platelets to the injured vascular wall. Furthermore, the concerted action of endothelial and platelet VASP prevented thrombotic events (53). As in smooth muscle cells (see above), the activation of PDE5 by cGMP provides a negative feedback mechanism for the cGMP response. The NO-induced cGMP levels did not differ in platelets from wild type and cGKI knockout mice suggesting that platelet PDE5 is directly activated by cGMP and not via phosphorylation by cGKI (54).

4.1.3. Heart

NO and ANP have been reported to regulate cardiac function (55, 56). Therefore, conventional and cardiomyocyte-specific cGKI knockout mice were analysed in respect to cardiac phenotypes. The cardiomyocyte-specific cGKI knockout mice were fully viable without any grossly altered phenotype (57). Deletion of cGKI attenuated the inhibitory effect of cGMP on the beta-adrenergic stimulated positive inotropy. In contrast, the negative inotropic effect of muscarinic agonists was not influenced in the cGKI-deficient mice. A possible cardiac cGKI target might be the $Ca_V1.2$ L-type calcium channel which was directly inhibited by cGKI overexpressed in cardiomyocytes (58).

4.2. cGKI and cGKII in the gastrointestinal tract

NO has been suggested to function in the regulation of intestinal function, because the intestine is innervated by NANC (non-adrenergic non-cholinergic) neurons which are part of the enteric nervous system and release NO upon excitation. This might be the reason that deletion of the nNOS lead to severe gastrointestinal defects including deficient motility, gastric stasis and pylorus stenosis (59, 60). cGKI is expressed in all gastrointestinal smooth muscle tissues including esophagus, gastric smooth muscle, the intestine, the colon and caecum and in the enteric nervous system (10, 30, 61). cGKI null mutants exhibited a severe gastrointestinal phenotype including stomach dilation, pyloric contraction, pylorus hypertrophy, poststenotic dilation of the duodenum, dilation of the caecum and contraction of the ileocaecal region (62). As consequence, the gastrointestinal motility was severely disturbed in these animals (30). There is some evidence that the gastrointestinal phenotype might result from a defect in smooth muscle relaxation. In cGKI-deficient mice it was observed that both the Ca^{2+}-dependent and the Ca^{2+}-independent component of intestinal relaxation was impaired (63). Furthermore, the contractility of the gastric fundus induced by electrical field stimulation was disturbed in cGKI-deficient mice. The anatomy of the enteric nervous system was not altered, NO synthase and the vasoactive intestinal peptide had a similar immunostaining pattern in wild type and cGKI-deficient animals (62). Interestingly, the induction of a smooth muscle-specific cGKI knockout in adult mice did not lead to altered gastrointestinal motility *in vivo*, although NO/cGMP-dependent relaxation of intestinal smooth muscle was strongly reduced *in vitro* (64). Thus, it is likely that the gastrointestinal defect in the constitutive cGKI-deficient mice could be the consequence of a defect in the interplay between the enteric nervous system (where cGKI is also expressed, (61)) and the smooth muscle cells.

Guanylin and the *Escherichia coli* heat stable toxin STa increase water secretion in the small intestine through activation of the guanylyl cyclase C (GC-C) causing diarrhea. cGKII is expressed in the secretory epithelium of the small intestine. cGKII-mediated phosphorylation of the CFTR stimulates chloride and water secretion (29). Furthermore, cGKII leads to Na^+-absorption

in the small intestine (65). cGKII-deficient mice were resistant to *Escherichia coli* STa (32).

4.3. cGKI and cGKII in kidney and bladder

The kidney regulates blood pressure by renin secretion from juxtaglomerular cells and subsequent activation of the renin-angiotensin-aldosterone system (RAAS). The effect of NO on kidney function is controversial as both stimulatory and inhibitory effects on renin secretion were observed. Studies using eNOS-deficient mice showed that eNOS-derived NO enhances renin release and inhibits renal perfusion pressure (66, 67). cGMP signalling might affect the RAAS as cGMP-analogs inhibited renin secretion in isolated kidney or juxtaglomerular cells (68, 69). Furthermore, disruption of the ANP gene caused salt-sensitive hypertension (37). In contrast, the deletion of the ANP-receptor GC-A resulted in salt-resistant hypertension (38). These effects might be partly mediated by cGKII which is associated with renin storage granules in juxtaglomerular cells (70). After feeding mice with different salt diets, the renin mRNA levels in kidneys from cGKII-, but not from cGKI-deficient mice, were enhanced. Furthermore, renin secretion from juxtaglomerular cells was increased in the cGKII-deficient mice. Therefore, cGKII exerts a general negative control on the renin system and might be involved in the exocytosis of renin in juxtaglomerular cells. The inhibition of renin secretion by cGKII could in principal affect blood pressure. However, cGKII-deletion did not change blood pressure, maybe because of a dual role of cGMP, e.g. activating cGKII and inhibiting cAMP degradation (34).

cGKI deletion did not change the bladder morphology or weight, but reduced rhythmic contractility of the bladder and increased bladder volume. Furthermore, the deletion of cGKI diminished NO/cGMP-dependent relaxation of the urinary duct smooth muscle–and resulted in hyperactive voiding. Forskolin-induced relaxation was also impaired in the urethra from cGKI knockout mice which could indicate a crosstalk between cAMP and cGKI (71).

4.4. cGKI and cGKII in the nervous system

4.4.1. Synaptic plasticity and learning

Long term potentiation (LTP) is a form of synaptic plasticity characterized by an activity-dependent long-lasting enhancement of synaptic transmission. LTP can be induced in the CA1-region of the hippocampus and could be important for spatial learning and memory. It was suggested that NO is generated postsynaptically and acts a retrograde messenger inducing LTP via activation of the presynaptic soluble guanlylyl cyclase and, finally, cGK (72-75). However, LTP in the hippocampal CA1-region was normal in total cGK knockout mice lacking cGKI, cGKII or both kinases (9). Nevertheless, the pleiotropic defects in the total knockout mice and the fact that cGKI knockout mice have a reduced life-span could have affected the LTP regulation in these animals. Therefore, hippocampus-specific knockout mice were used to study the effect of cGKI deletion on LTP more precisely. These hippocampus-specific cGKI knockout mice showed normal LTP in young animals (4-6 weeks), but reduced LTP in older animals (12 weeks) (76). However, the hippocampus-specific cGKI knockout mice performed normal in two tests for hippocampus-dependent learning, a discriminatory water maze and contextual fear conditioning. Therefore, the role of hippocampal cGKI for spatial learning and memory is not clear. cGKI might affect more subtle types of learning in the hippocampus.

Cerebellar long term depression (LTD) is an activity-dependent attenuation of synaptic transmission at the parallel-fiber-Purkinje cell synapse which is involved in motor learning. Cerebellar Purkinje cells contain high concentrations of cGKIalpha. In these cells, cGKI phosphorylates the G-substrate that then inhibits protein phosphatase 1 (77) and/or phosphatase 2A (78). Purkinje cell-specific deletion of the cGKI gene abolished cerebellar LTD suggesting that cerebellar output is regulated by cGKI (79). These LTD-deficient cGKI mutants did not exhibit general defects in motor performance, but showed a diminished adaptation of the vestibulo-ocular reflex, a simple form of cerebellum-dependent motor learning. In conclusion, cGKI in Purkinje cells is dispensable for general motor coordination, but is required for LTD and specific forms of motor learning.

4.4.2. Behaviour and rhythm

The deletion of NO-synthases in mice changed strongly the behaviour leading to enhanced aggressiveness and stereotyped behaviour in nNOS-deficient mice or reduced aggressiveness in eNOS knockout mice (80, 81). Hippocampus- and Purkinje cell-specific cGKI mutants as well as cGKII-deficient mice did not show gross behavioural phenotypes. However, cGKII knockout mice exhibited a slightly enhanced fear perception in the elevated maze and an abnormal alcohol preference during a first encounter but not in later test sessions (35).

The master circadian clock which determines many rhythmic processes is located in the suprachiasmatic nuclei (SCN) of the hypothalamus. Circadian rhythm depends on several clock genes including Period 1 and 2. cGKII is expressed in the hypothalamus including the SCN. cGKII knockout mice displayed a normal circadian rhythm but were defective in resetting the circadian clock. The basis for this phenotype could be the opposing effect of cGKII on Period 1 and 2 expression. The light induction of Period 2 is strongly reduced during the early night phase, but induction of Period 1 is enhanced in cGKII knockout mice (82).

4.4.3. Development

For the development of the nervous system a correct pathfinding of axons is essential. Pathfinding in various areas of the developing brain is affected by cGMP (83). For example, the semaphorin-induced growth cone collapse can be antagonized by cGMP (84, 85). Hence, cGK could be involved in the axon guidance process. cGKIalpha is expressed in sensory axons during developmental stages. Branching of sensory nerve fibers in the entry zone of the dorsal root is defective in cGKI-deficient embryos leading to a decreased sensory and nociceptive transmission in newborn mice (8).

Furthermore, activation of cGKI in embryonic dorsal root ganglia counteracts semaphorin 3A-induced growth cone collapse.

4.4.4. Nociception

Several observations lead to the hypothesis that the NO/cGMP/cGKI pathway is critical for nociception. NO donors resulted in hyperalgesia and inhibitors of NO synthases caused antinociception (86). cGMP was critical for long-term sensitization of sensory neurons (87). Furthermore, inhibition of cGKI in the spinal cord reduced formalin-induced hyperalgesia (88). cGKI-deficient mice showed reduced nociception in formalin assays and zymosane-induced paw inflammation. However, acute thermal nociception was unaltered in these animals (89). These nociceptive effects of cGKI might be mediated, at least in part, by substance P, because its expression was reduced in spinal cord neurons and fibers of cGKI-deficient mice.

4.5. cGKI and cGKII in other organs

4.5.1. Bone growth

Deletion of cGKII in mice resulted in dwarfs which developed short bones. In these mice, bones derived by membranous ossification evolve normally while bones derived by endochondral ossification were shortened. The dwarfism is caused by a defect in the endochondral ossification at the endochondral plate (32, 90). Endochondral ossification is stimulated by CNP. cGKII is downstream of the CNP receptor GC-B (33). The growth defect induced by the deletion of cGKII was not rescued by overexpression of CNP indicating that cGKII is absolutely required for normal endochondral bone development.

4.5.2. Penile erection

Penile erection is mediated by NANC neurons that release NO upon excitation (91). Downstream signalling of NO is mediated through cGKI. Male cGKI null mutants failed to relax the corpus cavernosum after activation of the NO/cGMP signalling pathway and exhibited a dramatically reduced reproduction. The reduced fertility in these mice was not caused by a faulty spermatogenesis, because sperms of cGKI-deficient mice were able to fertilize eggs (92).

4.5.3. Immune system

The influence of NO and cGMP on the immune system is controversial, since high concentration of NO donors and cGMP analogues inhibited neutrophil chemotaxis and granule secretion, whereas lower concentrations facilitated this response (93, 94). Studies with cGKI-deficient neutrophils showed enhanced neutrophil chemotaxis and granule secretion (95). Hence, cGKI might inhibit the migration of neutrophils and the secretion of granules. The underlying signalling mechanisms need to be further analysed.

5. CONCLUSION AND PERSPECTIVES

The analysis of conventional and tissue-specific cGKI- and cGKII-knockout mice has advanced our understanding of the functional significance of these protein kinases as mediators of cGMP signalling in health and disease. These studies highlighted the importance of cGKs in the vascular system, platelets and neutrophils, the gastrointestinal tract, kidney and bladder, penile erection, bone growth, and the nervous system. Nevertheless, we are still only at the beginning to understand these signalling pathway, because only a few *in vivo* substrates of the cGKs are known. Our knowledge on many systems is marginal. We need a better understanding of the interaction of platelets with the vessel wall and the endothelium, the changes induced by atherosclerosis in vascular smooth muscle cells and macrophages, the interplay between the gastrointestinal smooth muscle and the enteric nervous system, and the diverse effects of cGKs in the nervous system. Furthermore, the pathophysiological consequences of cGK defects and the underlying mechanisms have to be resolved in more detail. Moreover, we lack informations on the substrates and roles of the cGK isozymes Ialpha and Ibeta. Studies in these areas will provide a deeper understanding of the physiology of cGKs and their potential as future therapeutic targets.

6. ACKNOWLEDGEMENTS

This work was supported by grants from the Deutsche Forschungsgemeinschaft, Graduate Program 438, Wilhelm Sander-Stiftung, VolkswagenStiftung, and Fonds der Chemischen Industrie.

7. REFERENCES

1. Pfeifer, A., P. Ruth, W. Dostmann, M. Sausbier, P. Klatt and F. Hofmann: Structure and function of cGMP-dependent protein kinases. *Rev Physiol Biochem Pharmacol* 135, 105-149 (1999)

2. Ruth, P., A. Pfeifer, S. Kamm, P. Klatt, W. R. Dostmann and F. Hofmann: Identification of the amino acid sequences responsible for high affinity activation of cGMP kinase Ialpha. *J Biol Chem* 272, 10522-10528 (1997)

3. Ammendola, A., A. Geiselhöringer, F. Hofmann and J. Schlossmann: Molecular determinants of the interaction between the inositol 1,4,5-trisphosphate receptor-associated cGMP kinase substrate (IRAG) and cGMP kinase Ibeta. *J Biol Chem* 276, 24153-24159 (2001)

4. Surks, H. K., N. Mochizuki, Y. Kasai, S. P. Georgescu, K. M. Tang, M. Ito, T. M. Lincoln and M. E. Mendelsohn: Regulation of myosin phosphatase by a specific interaction with cGMP-dependent protein kinase I. *Science* 286**,** 1583-1587 (1999)

5. Vaandrager, A. B., M. Edixhoven, A. G. Bot, M. A. Kroos, T. Jarchau, S. Lohmann, H. G. Genieser and H. R. de Jonge: Endogenous type II cGMP-dependent protein kinase exists as a dimer in membranes and can be functionally distinguished from the type I isoforms. *J Biol Chem* 272, 11816-11823 (1997)

6. Vaandrager, A. B., E. M. Ehlert, T. Jarchau, S. M. Lohmann and H. R. de Jonge: N-terminal myristoylation is

required for membrane localization of cGMP-dependent protein kinase type II. *J Biol Chem* 271, 7025-7029 (1996)

7. Keilbach, A., P. Ruth and F. Hofmann: Detection of cGMP dependent protein kinase isozymes by specific antibodies. *Eur J Biochem* 208, 467-473 (1992)

8. Schmidt, H., M. Werner, P. A. Heppenstall, M. Henning, M. I. More, S. Kuhbandner, G. R. Lewin, F. Hofmann, R. Feil and F. G. Rathjen: cGMP-mediated signaling via cGKIalpha is required for the guidance and connectivity of sensory axons. *J Cell Biol* 159, 489-498 (2002)

9. Kleppisch, T., A. Pfeifer, P. Klatt, P. Ruth, A. Montkowski, R. Fassler and F. Hofmann: Long-term potentiation in the hippocampal CA1 region of mice lacking cGMP-dependent kinases is normal and susceptible to inhibition of nitric oxide synthase. *J Neurosci* 19, 48-55 (1999)

10. Geiselhöringer, A., M. Antl, F. Hofmann and J. Schlossmann: Localization and assembly of IRAG. *Naunyn-Schmiedeberg`s Arch of Pharmacol* 367, R47 (2003) Abstract

11. Lohmann, S. M., A. B. Vaandrager, A. Smolenski, U. Walter and H. R. De Jonge: Distinct and specific functions of cGMP-dependent protein kinases. *Trends Biochem Sci* 22, 307-312 (1997)

12. Metzger, D and R. Feil: Engineering the mouse genome by site-specific recombination. *Curr Opin Biotechnol* 10470-10476 (1999)

13. Tronche F., E. Casanova, M. Turiault, I. Sahly and C. Kellendonk: When reverse genetics meets physiology: the use of site-specific recombinases in mice. *FEBS Lett* 529, 116-121 (2002)

14. Lincoln, T. M., N. Dey and H. Sellak: Invited review: cGMP-dependent protein kinase signaling mechanisms in smooth muscle: from the regulation of tone to gene expression. *J Appl Physiol* 91, 1421-1430 (2001)

15. Hofmann, F., A. Ammendola and J. Schlossmann: Rising behind NO: cGMP-dependent protein kinases. *J Cell Sci* 113, 1671-1676 (2000)

16. Schlossmann, J., R. Feil and F. Hofmann: Signaling through NO and cGMP-dependent protein kinases. *Ann Med* 35, 21-27 (2003)

17. Schlossmann, J., A. Ammendola, K. Ashman, X. Zong, A. Huber, G. Neubauer, G. X. Wang, H. D. Allescher, M. Korth, M. Wilm, F. Hofmann and P. Ruth: Regulation of intracellular calcium by a signalling complex of IRAG, IP3 receptor and cGMP kinase Ibeta. *Nature* 404, 197-201 (2000)

18. Alioua, A., Y. Tanaka, M. Wallner, F. Hofmann, P. Ruth, P. Meera and L. Toro: The large conductance, voltage-dependent, and calcium-sensitive K+ channel, Hslo, is a target of cGMP-dependent protein kinase phosphorylation *in vivo. J Biol Chem* 273, 32950-32956 (1998)

19. Sausbier, M., R. Schubert, V. Voigt, C. Hirneiss, A. Pfeifer, M. Korth, T. Kleppisch, P. Ruth and F. Hofmann: Mechanisms of NO/cGMP-dependent vasorelaxation. *Circ Res* 87, 825-830 (2000)

20. Moosmang, S., V. Schulla, A. Welling, R. Feil, S. Feil, J. W. Wegener, F. Hofmann and N. Klugbauer: Dominant role of smooth muscle L-type calcium channel Cav1.2 for blood pressure regulation. *Embo J* 22, 6027-6034 (2003)

21. Wegener, J. W., V. Schulla, T.-S. Lee, A. Koller, S. Feil, R. Feil, T. Kleppisch, N. Klugbauer, S. Moosmang, A. Welling and F. Hofmann: An essential role of $Ca_v1.2$ L-type calcium channel for urinary bladder function. *FASEB J* Epub ahead of print (2004)

22. Sausbier, M., H. Hu, C. Arntz, S. Feil, S. Kamm, H. Adelsberger, U. Sausbier, C. A. Sailer, R. Feil, F. Hofmann, M. Korth, M. J. Shipston, H. G. Knaus, D. P. Wolfer, C. M. Pedroarena, J. F. Storm and P. Ruth: Cerebellar ataxia and Purkinje cell dysfunction caused by Ca^{2+}-activated K^+ channel deficiency. *PNAS,* in press (2004)

23. Raeymaekers, L., F. Hofmann and R.Casteels: Cyclic GMP-dependent protein kinase phosphorylates phospholamban in isolated sarcoplasmic reticulum from cardiac and smooth muscle. *Biochem J* 252**,** 269-73 (1988)

24. Koller, A., J. Schlossmann, K. Ashman, S. Uttenweiler-Joseph, P. Ruth and F. Hofmann: Association of phospholamban with a cGMP kinase signaling complex. *Biochem Biophys Res Commun* 300, 155-160 (2003)

25. Lalli, M. J., S. Shimizu, R. L. Sutliff, E. G. Kranias and R. J. Paul: $[Ca^{2+}]i$ homeostasis and cyclic nucleotide relaxation in aorta of phospholamban-deficient mice. *Am J Physiol* 277, H963-970 (1999)

26. Surks, H. K., N. Mochizuki, Y. Kasai, S. P. Georgescu, K. M. Tang, M. Ito, T. M. Lincoln and M. E. Mendelsohn: Regulation of myosin phosphatase by a specific interaction with cGMP- dependent protein kinase Ialpha. *Science* 286, 1583-1587 (1999)

27. Surks, H. K. and M. E. Mendelsohn: Dimerization of cGMP-dependent protein kinase 1alpha and the myosin-binding subunit of myosin phosphatase: role of leucine zipper domains. *Cell Signal* 15, 937-944 (2003)

28. Ellerbroek, S. M., K. Wennerberg and K. Burridge: Serine phosphorylation negatively regulates RhoA *in vivo. J Biol Chem* 278, 19023-19031 (2003)

29. Vaandrager, A. B., A. Smolenski, B. C. Tilly, A. B. Houtsmuller, E. M. Ehlert, A. G. Bot, M. Edixhoven, W. E. Boomaars, S. M. Lohmann and H. R. de Jonge: Membrane

targeting of cGMP-dependent protein kinase is required for cystic fibrosis transmembrane conductance regulator Cl-channel activation. *Proc Natl Acad Sci USA* 95, 1466-1471 (1998)

30. Pfeifer, A., P. Klatt, S. Massberg, L. Ny, M. Sausbier, C. Hirneiss, G. X. Wang, M. Korth, A. Aszodi, K. E. Andersson, F. Krombach, A. Mayerhofer, P. Ruth, R. Fassler and F. Hofmann: Defective smooth muscle regulation in cGMP kinase I-deficient mice. *Embo J* 17, 3045-3051 (1998)

31. Massberg, S., M. Sausbier, P. Klatt, M. Bauer, A. Pfeifer, W. Siess, R. Fassler, P. Ruth, F. Krombach and F. Hofmann: Increased adhesion and aggregation of platelets lacking cyclic guanosine 3',5'-monophosphate kinase I. *J Exp Med* 189**,** 1255-1264 (1999)

32. Pfeifer, A., A. Aszodi, U. Seidler, P. Ruth, F. Hofmann and R. Fassler: Intestinal secretory defects and dwarfism in mice lacking cGMP-dependent protein kinase II. *Science* 274, 2082-2086 (1996)

33. Miyazawa, T., Y. Ogawa, H. Chusho, A. Yasoda, N. Tamura, Y. Komatsu, A. Pfeifer, F. Hofmann and K. Nakao: Cyclic GMP-dependent protein kinase II plays a critical role in C-type natriuretic peptide-mediated endochondral ossification. *Endocrinology* 143, 3604-10 (2002)

34. Wagner, C., A. Pfeifer, P. Ruth, F. Hofmann and A. Kurtz: Role of cGMP-kinase II in the control of renin secretion and renin expression. *J Clin Invest* 102, 1576-1582 (1998)

35. Werner, C., I. Sillaber, R. Spanagel and F. Hofmann: Reduced ethanol sensitivity and enhanced ethanol consumption in cGMP-kinase 2 deficient mice. *Naunyn-Schmiedeberg`s Arch. of Pharmacol* 365, R105 (2001) Abstract

36. Shesely, E. G., N. Maeda, H. S. Kim, K. M. Desai, J. H. Krege, V. E. Laubach, P. A. Sherman, W. C. Sessa and O. Smithies: Elevated blood pressures in mice lacking endothelial nitric oxide synthase. *Proc Natl Acad Sci USA* 93, 13176-13181 (1996)

37. John, S. W., J. H. Krege, P. M. Oliver, J. R. Hagaman, J. B. Hodgin, S. C. Pang, T. G. Flynn and O. Smithies: Genetic decreases in atrial natriuretic peptide and salt-sensitive hypertension. *Science* 267, 679-81 (1995)

38. Oliver, P. M., J. E. Fox, R. Kim, H. A. Rockman, H. S. Kim, R. L. Reddick, K. N. Pandey, S. L. Milgram, O. Smithies and N. Maeda: Hypertension, cardiac hypertrophy, and sudden death in mice lacking natriuretic peptide receptor A. *Proc Natl Acad Sci USA* 94, 14730-14735 (1997)

39. Koeppen, K., R. Feil, S. Feil, F. Hofmann, U. Pohl and C. deWit: NO-, but not Ach-induced arteriolar dilations are impaired in cGMP-dependent protein kinase k.o. mice. *Pflugers Arch* 445, 19 (2003) Abstract

40. Feil, R., N. Gappa, M. Rutz, J. Schlossmann, C. R. Rose, A. Konnerth, S. Brummer, S. Kuhbandner and F. Hofmann: Functional reconstitution of vascular smooth muscle cells with cGMP-dependent protein kinase I isoforms. *Circ Res* 90, 1080-1086 (2002)

41. Rybalkin, S. D., I. G. Rybalkina, R. Feil, F. Hofmann and J. A. Beavo: Regulation of cGMP-specific phosphodiesterase (PDE5) phosphorylation in smooth muscle cells. *J Biol Chem* 277, 3310-3317 (2002)

42. Hofmann, F., M. Biel, R. Feil and T. Kleppisch: Mouse Models of NO/Natriuretic peptides/cGMP Kinase Signaling. In: Handb. Exp. Pharm. Eds: S. Offermanns, L. Hein, Springer-Verlag, Berlin Heidelberg, Germany, 159, 95-130 (2004)

43. Feil, R., S. M. Lohmann, H. de Jonge, U. Walter and F. Hofmann: Cyclic GMP-dependent protein kinases and the cardiovascular system: insights from genetically modified mice. *Circ Res* 93, 907-916 (2003)

44. Wolfsgruber, W., S. Feil, S. Brummer, O. Kuppinger, F. Hofmann and R. Feil: A proatherogenic role for cGMP-dependent protein kinase in vascular smooth muscle cells. *Proc Natl Acad Sci USA* 100, 13519-13524 (2003)

45. Yamahara, K., H. Itoh, T. H. Chun, Y. Ogawa, J. Yamashita, N. Sawada, Y. Fukunaga, M. Sone, T. Yurugi-Kobayashi, K. Miyashita, H. Tsujimoto, H. Kook, R. Feil, D. L. Garbers, F. Hofmann and K. Nakao: Significance and therapeutic potential of the natriuretic peptides/cGMP/cGMP-dependent protein kinase pathway in vascular regeneration. *Proc Natl Acad Sci USA* 100, 3404-3409 (2003)

46. Eigenthaler, M., H. Ullrich, J. Geiger, K. Horstrup, P. Honig-Liedl, D. Wiebecke and U. Walter: Defective nitrovasodilator-stimulated protein phosphorylation and calcium regulation in cGMP-dependent protein kinase-deficient human platelets of chronic myelocytic leukemia. *J Biol Chem* 268, 13526-13531 (1993)

47. Aszodi, A., A. Pfeifer, M. Ahmad, M. Glauner, X. H. Zhou, L. Ny, K. E. Andersson, B. Kehrel, S. Offermanns and R. Fassler: The vasodilator-stimulated phosphoprotein (VASP) is involved in cGMP- and cAMP-mediated inhibition of agonist-induced platelet aggregation, but is dispensable for smooth muscle function. *Embo J* 18, 37-48. (1999)

48. Tertyshnikova, S., X. Yan and A. Fein: cGMP inhibits IP3-induced Ca2+ release in intact rat megakaryocytes via cGMP- and cAMP-dependent protein kinases. *J Physiol* 512, 89-96 (1998)

49. Cavallini, L., M. Coassin, A. Borean and A. Alexandre: Prostacyclin and sodium nitroprusside inhibit the activity of the platelet inositol 1,4,5-trisphosphate receptor and promote its phosphorylation.

J Biol Chem 271, 5545-5551 (1996)

50. El-Daher, S. S., Y. Patel, A. Siddiqua, S. Hassock, S. Edmunds, B. Maddison, G. Patel, D. Goulding, F. Lupu, R. J. Wojcikiewicz and K. S. Authi: Distinct localization and function of (1,4,5)IP(3) receptor subtypes and the (1,3,4,5)IP(4) receptor GAP1(IP4BP) in highly purified human platelet membranes. *Blood* 95, 3412-3422 (2000)

51. Antl, A., A. Geiselhöringer, F. Hofmann and J. Schlossmann: Phosphorylation of the cGMP kinase substrate IRAG in platelets. *cGMP2003* 1, 57 (2003) Abstract

52. Horstrup, K., B. Jablonka, P. Honig-Liedl, M. Just, K. Kochsiek and U. Walter: Phosphorylation of focal adhesion vasodilator-stimulated phosphoprotein at Ser157 in intact human platelets correlates with fibrinogen receptor inhibition. *Eur J Biochem* 225, 21-27 (1994)

53. Massberg, S., S. Gruner, I. Konrad, M. I. Garcia Arguinzonis, M. Eigenthaler, K. Hemler, J. Kersting, C. Schulz, I. Muller, F. Besta, B. Nieswandt, U. Heinzmann, U. Walter and M. Gawaz: Enhanced *in vivo* platelet adhesion in vasodilator-stimulated phosphoprotein (VASP)-deficient mice. *Blood* 103, 136-142 (2004)

54. Mullershausen, F., A. Friebe, R. Feil, W. J. Thompson, F. Hofmann and D. Koesling: Direct activation of PDE5 by cGMP: long-term effects within NO/cGMP signaling. *J Cell Biol* 160, 719-727 (2003)

55. Kelly, R. A. and T. W. Smith: Nitric oxide and nitrovasodilators: similarities., differences, and interactions. *Am J Cardiol* 77 (13), 2C-7C (1996)

56. Gyurko, R., P. Kuhlencordt, M. C. Fishman and P. L. Huang: Modulation of mouse cardiac function *in vivo* by eNOS and ANP. *Am J Physiol Heart Circ Physiol* 278, H971-981 (2000)

57. Wegener, J. W., H. Nawrath, W. Wolfsgruber, S. Kuhbandner, C. Werner, F. Hofmann and R. Feil: cGMP-dependent protein kinase I mediates the negative inotropic effect of cGMP in the murine myocardium. *Circ Res* 90, 18-20 (2002)

58. Schroder, F., G. Klein, B. Fiedler, M. Bastein, N. Schnasse, A. Hillmer, S. Ames, S. Gambaryan, H. Drexler, U. Walter, S. M. Lohmann and K. C. Wollert: Single L-type Ca(2+) channel regulation by cGMP-dependent protein kinase type I in adult cardiomyocytes from PKG I transgenic mice. *Cardiovasc Res* 60, 268-277 (2003)

59. Huang, P. L., T. M. Dawson, D. S. Bredt, S. H. Snyder and M. C. Fishman: Targeted disruption of the neuronal nitric oxide synthase gene. *Cell* 75, 1273-1286 (1993)

60. Mashimo, H., A. Kjellin and R. K. Goyal: Gastric stasis in neuronal nitric oxide synthase-deficient knockout mice. *Gastroenterology* 119, 766-773 (2000)

61. Salmhofer, H., W. L. Neuhuber, P. Ruth, A. Huber, M. Russwurm and H. D. Allescher: Pivotal role of the interstitial cells of Cajal in the nitric oxide signaling pathway of rat small intestine. Morphological evidence. *Cell Tissue Res* 305, 331-340 (2001)

62. Ny, L., A. Pfeifer, A. Aszodi, M. Ahmad, P. Alm, P. Hedlund, R. Fassler and K. E. Andersson: Impaired relaxation of stomach smooth muscle in mice lacking cyclic GMP-dependent protein kinase I. *Br J Pharmacol* 129, 395-401 (2000)

63. Bonnevier, J., R. Fassler, A. P. Somlyo, A. V. Somlyo and A. Arner: Modulation of Ca2+ sensitivity by cyclic nucleotides in smooth muscle from protein kinase G-deficient mice. *J Biol Chem* 279, 5146-5151 (2004)

64. Wörner, R., J. W. Wegener, W. Wolfsgruber, V. Goldis, S. Feil, R. Feil and F. Hofmann: Analysis of intestinal motility in conventional and smooth-muscle cGKI knockout mice. *Naunyn-Schmiedeberg`s Arch of Pharmacol* 368, R43 (2004) Abstract

65. Vaandrager, A. B., A. G. Bot, P. Ruth, A. Pfeifer, F. Hofmann and H. R. de Jonge: Differential role of cyclic GMP-dependent protein kinase II in ion transport in murine small intestine and colon. *Gastroenterology* 118, 108-114 (2000)

66. Wagner, C., A. Godecke, M. Ford, J. Schnermann, J. Schrader and A. Kurtz: Regulation of renin gene expression in kidneys of eNOS- and nNOS-deficient mice. *Pflugers Arch* 439, 567-572 (2000)

67. Ortiz, P. A. and J. L.Garvin: Cardiovascular and renal control in NOS-deficient mouse models. *Am J Physiol Regul Integr Comp Physiol* 284, R628-638 (2003)

68. Henrich, W. L., E. A. McAllister, P. B. Smith and W. B. Campbell: Guanosine 3',5'-cyclic monophosphate as a mediator of inhibition of renin release. *Am J Physiol* 255, F474-478 (1988)

69. Schricker, K and A. Kurtz: Liberators of NO exert a dual effect on renin secretion from isolated mouse renal juxtaglomerular cells. *Am J Physiol* 265, F180-186 (1993)

70. Gambaryan, S., C. Hausler, T. Markert, D. Pohler, T. Jarchau, U. Walter, W. Haase, A. Kurtz and S. M. Lohmann: Expression of type II cGMP-dependent protein kinase in rat kidney is regulated by dehydration and correlated with renin gene expression. *J Clin Invest* 98, 662-670 (1996)

71. Persson, K., R. K. Pandita, A. Aszodi, M. Ahmad, A. Pfeifer, R. Fassler and K. E. Andersson: Functional characteristics of urinary tract smooth muscles in mice lacking cGMP protein kinase type I. *Am J Physiol Regul Integr Comp Physiol* 279, R1112-1120 (2000)

72. Zhuo, M., Y. Hu, C. Schultz, E. R. Kandel and R. D. Hawkins: Role of guanylyl cyclase and cGMP-dependent

protein kinase in long-term potentiation. *Nature* 368, 635-639 (1994)

73. Arancio, O., V. Lev-Ram, R. Y. Tsien, E. R. Kandel and R. D. Hawkins: Nitric oxide acts as a retrograde messenger during long-term potentiation in cultured hippocampal neurons. *J Physiol Paris* 90, 321-322 (1996)

74. Arancio, O., I. Antonova, S. Gambaryan, S. M. Lohmann, J. S. Wood, D. S. Lawrence and R. D. Hawkins: Presynaptic role of cGMP-dependent protein kinase during long-lasting potentiation. *J Neurosci* 21, 143-149 (2001)

75. Monfort, P., M. D. Munoz, E. Kosenko and V. Felipo: Long-term potentiation in hippocampus involves sequential activation of soluble guanylate cyclase, cGMP-dependent protein kinase, and cGMP-degrading phosphodiesterase. *J Neurosci* 22, 10116-10122 (2002)

76. Kleppisch, T., W. Wolfsgruber, S. Feil, R. Allmann, C. T. Wotjak, S. Goebbels, K. A. Nave, F. Hofmann and R. Feil: Hippocampal cGMP-dependent protein kinase I supports an age- and protein synthesis-dependent component of long-term potentiation but is not essential for spatial reference and contextual memory. *J Neurosci* 23, 6005-6012 (2003)

77. Hall, K. U., S. P. Collins, D. M. Gamm, E. Massa, A. A. DePaoli-Roach and M. D. Uhler: Phosphorylation-dependent inhibition of protein phosphatase-1 by G-substrate. A Purkinje cell substrate of the cyclic GMP-dependent protein kinase. *J Biol Chem* 274, 3485-3495 (1999)

78. Endo, S., M. Suzuki, M. Sumi, A. C. Nairn, R. Morita, K. Yamakawa, P. Greengard and M. Ito: Molecular identification of human G-substrate, a possible downstream component of the cGMP-dependent protein kinase cascade in cerebellar Purkinje cells. *Proc Natl Acad Sci USA* 96, 2467-2472 (1999)

79. Feil, R., J. Hartmann, C. Luo, W. Wolfsgruber, K. Schilling, S. Feil, J. J. Barski, M. Meyer, A. Konnerth, C. I. De Zeeuw and F. Hofmann: Impairment of LTD and cerebellar learning by Purkinje cell-specific ablation of cGMP-dependent protein kinase I. *J Cell Biol* 163, 295-302 (2003)

80. Nelson, R. J., G. E. Demas, P. L. Huang, M. C. Fishman, V. L. Dawson, T. M. Dawson and S. H. Snyder: Behavioural abnormalities in male mice lacking neuronal nitric oxide synthase. *Nature* 378, 383-386 (1995)

81. Demas, G. E., L. J. Kriegsfeld, S. Blackshaw, P. Huang, S. C. Gammie, R. J. Nelson and S. H. Snyder: Elimination of aggressive behavior in male mice lacking endothelial nitric oxide synthase. *J Neurosci* 19, RC30 (1999)

82. Oster, H., C. Werner, M. C. Magnone, H. Mayser, R. Feil, M. W. Seeliger, F. Hofmann and U. Albrecht: cGMP-dependent protein kinase II modulates mPer1 and mPer2 gene induction and influences phase shifts of the circadian clock. *Curr Biol* 13, 725-733 (2003)

83. Song, H. J. and M. M. Poo: Signal transduction underlying growth cone guidance by diffusible factors. *Curr Opin Neurobiol* 9, 355-363 (1999)

84. Song, H., G. Ming, Z. He, M. Lehmann, L. McKerracher, M. Tessier-Lavigne and M. Poo: Conversion of neuronal growth cone responses from repulsion to attraction by cyclic nucleotides. *Science* 281, 1515-1518 (1998)

85. Polleux, F., T. Morrow and A. Ghosh: Semaphorin 3A is a chemoattractant for cortical apical dendrites. *Nature* 404, 567-73 (2000)

86. Aley, K. O., G. McCarter and J. D. Levine: Nitric oxide signaling in pain and nociceptor sensitization in the rat. *J Neurosci* 18, 7008-7014 (1998)

87. Lewin, M. R. and E. T. Walters: Cyclic GMP pathway is critical for inducing long-term sensitization of nociceptive sensory neurons. *Nat Neurosci* 2, 18-23 (1999)

88. Schmidtko, A., P. Ruth, G. Geisslinger and I. Tegeder: Inhibition of cyclic guanosine 5'-monophosphate-dependent protein kinase I (PKG-I) in lumbar spinal cord reduces formalin-induced hyperalgesia and PKG upregulation. *Nitric Oxide* 8, 89-94 (2003)

89. Tegeder, I., D. Del Turco, A. Schmidtko, M. Sausbier, R. Feil, F. Hofmann, T. Deller, P. Ruth and G. Geisslinger: Reduced inflammatory hyperalgesia with preservation of acute thermal nociception in mice lacking cGMP-dependent protein kinase I. *Proc Natl Acad Sci USA* 101, 3253-5257 (2004)

90. Talts, J. F., A. Pfeifer, F. Hofmann, E. B. Hunziker, X. H. Zhou, A. Aszodi and R. Fassler: Endochondral ossification is dependent on the mechanical properties of cartilage tissue and on intracellular signals in chondrocytes. *Ann NY Acad Sci* 857, 74-85 (1998)

91. Andersson, K. E. and G. Wagner: Physiology of penile erection. *Physiol Rev* 75, 191-236 (1995)

92. Hedlund, P., A. Aszodi, A. Pfeifer, P. Alm, F. Hofmann, M. Ahmad, R. Fassler and K. E. Andersson: Erectile dysfunction in cyclic GMP-dependent kinase I-deficient mice. *Proc Natl Acad Sci USA* 97, 2349-2354 (2000)

93. Bogdan, C: Nitric oxide and the immune response. *Nat Immunol* 2, 907-16 (2001)

94. Wanikiat, P., D. F. Woodward and R. A. Armstrong: Investigation of the role of nitric oxide and cyclic GMP in both the activation and inhibition of human neutrophils. *Br J Pharmacol* 122, 1135-1145 (1997)

95. Werner, C., K. B. Pryzwansky and F. Hofmann: Cyclic GMP kinase I affects murine neutrophil migration and

superoxide production *Naunyn-Schmiedeberg`s Arch of Pharmacol* 364, R81 (2000) Abstract

Abbreviations: ANP, BNP or CNP: atrial, brain or C-type natriuretic peptide, BK_{Ca}: maxi potassium channel, CFTR: cystic fibrosis transmembrane conductance regulator, cGMP: cyclic guanosine-3`,5`-monophosphate, cGK: cGMP-dependent protein kinase, cGKI: cGMP-dependent protein kinase type I, cGKII: cGMP-dependent protein kinase type II, EDHF: endothelium-derived hyperpolarization factor, eNOS: endothelial NO synthase, $InsP_3$: inositol 1,4,5-trisphosphate, GC: guanylyl cyclase, $InsP_3R$: $InsP_3$-receptor, IRAG: $InsP_3$-receptor associated cGMP kinase substrate, LTD: long term depression, LTP: long term potentiation, NANC: non-adrenergic non-cholinergic, NO: nitric oxide, PDE: phosphodiesterases, RAAS: renin-angiotensin-aldosterone system, SCN: suprachiasmatic nuclei, VASP: vasodilator-stimulated phosphoprotein

Key Words: cGMP, cGMP-dependent protein kinase, cGKI, cGKII, Signalling, Knockout mice, Nitric oxide, Natriuretic peptides, Review

Send correspondence to: Dr Jens Schlossmann, Institut für Pharmakologie und Toxikologie der Technischen Universität München, Biedersteiner Straße 29, 80802 München, Germany, Tel: 49-89-4140-3260, Fax: 49-89-4140-3261, E-mail: Schlossmann@ipt.med.tu-muenchen.de

http://www.bioscience.org/current/vol10.htm