cGMP-Dependent Protein Kinase I Mediates the Negative Inotropic Effect of cGMP in the Murine Myocardium

Jörg W. Wegener, Hermann Nawrath, Wiebke Wolfsgruber, Susanne Kühbandner, Claudia Werner, Franz Hofmann, Robert Feil

To study the role of cGMP-dependent protein kinase I (cGKI) for cardiac contractility, force of contraction (F_c) was studied in electrically driven heart muscle from wild-type (WT) mice and from conventional and conditional cGKI knockout mice. Both 8-Br-cGMP and 8-pCPT-cGMP reduced F_c in cardiac muscle from juvenile WT but not from juvenile cGKI-null mutants. Similarly, the cGMP analogues reduced F_c in forskolin-stimulated ventricular muscle from WT mice but not from cGKI-null mutants. In contrast, carbachol reduced F_c in both groups of animals. 8-Br-cGMP reduced F_c also in heart muscle from adult WT mice but not from adult cardiomyocyte-specific cGKI-knockout mice. These results demonstrate that cGKI mediates the negative inotropic effect of cGMP in the myocardium of juvenile and adult mice.

A cetylcholine and the muscarinic agonist carbachol (CCh) induce negative inotropy in human and rodent heart. The molecular basis for muscarinic inhibition of cardiac contractility is controversial. Activation of NO synthase III leading to an increase of the cGMP level has been implicated in the inhibitory effects of cGMP on L-type calcium current and contraction.

We have investigated the role of cGMP/cGKI signaling for cardiac contractility using myocardium from conventional and conditional cGKI-knockout mice. This study shows that cGKI mediates negative inotropic effects elicited by cGMP in the absence and presence of forskolin, an activator of the β-adrenergic/cAMP pathway but is not involved in inhibition of cardiac contractility by CCh.

Materials and Methods
The Materials and Methods section is available online in the data supplement at http://www.circresaha.org.

Results and Discussion
A conventional cGKI-null allele (cGKI) was obtained by replacing the 3′ region of exon 10 of the cGKI gene (which is essential for kinase activity) with a DNA cassette encoding CreER recombinase. The CreER recombinase was not expressed from the cGKI allele. A conditional cGKI allele (L2) was obtained by flanking exon 10 with loxp sites. Excision of exon 10 from the L2 allele by Cre-mediated recombination of the loxp sites produced an L− allele (Figures 1A and 1B). Heterozygous cGKI+/−, cGKI+/L2, cGKI+/−L2, and cGKI−/L2 mice as well as homozygous cGKI−/− mice expressed cGKI protein and were phenotypically normal. Homozygous cGKI−/− and cGKI−/−L2 mice did not express cGKI protein and were phenotypically indistinguishable from a cGKI-deficient mouse line reported previously (data not shown). These results indicate the successful generation of the modified cGKI alleles and that the foreign DNA introduced into the cGKI locus should not confound the analysis of cGKI-deficient mice.

To disrupt the cGKI gene specifically in cardiomyocytes, the MLC2a-Cre transgenic mouse line was used. These mice express Cre recombinase under the control of the atrial myosin regulatory light chain gene promoter and allow for the efficient deletion of loxp-flanked DNA in both atrial and ventricular myocytes. Mating of conditional cGKI mice with MLC2a-Cre mice produced offspring in which the level of cGKI protein was highly reduced in both atrial and ventricular tissue but not in other organs (Figure 1C). The lower level of cGKI protein that was detected in heart extracts of cardiomyocyte-specific mutants may represent cGKI protein expressed in the cardiac vasculature.

The effects of membrane-permeable cGMP analogues on myocardial contractility were first studied in cardiac muscle from juvenile (3- to 6-week-old) wild-type (WT) mice and conventional cGKI-null mutants (cGKI−/− mice). Both 8-Br-cGMP and 8-pCPT-cGMP reduced force of contraction (F_c) in atrial and ventricular preparations from WT mice but not from cGKI−/− mice (Figures 2A and 2B) indicating that these effects were mediated by cGKI. The NO donor DEA/NO (100 μmol/L) transiently (according to its time course of NO release) reduced F_c in preparations from WT but not from cGKI−/− mice (Figure 2C). The isozyme specificity was confirmed using cardiac muscles from cGKII-deficient mice.
were used because conventional cGKI cardiomyocyte-specific cGKI knockout mice (Figure 1C) of cGKI in the heart muscle of adult (4- to 6-month-old) mice, a minor role for this enzyme in adults. To study the function mice in which cGMP analogues reduced Fc to the same extent as in WT preparations (data not shown).

Forskolin increased Fc (in percent of control) in ventricular preparations from WT and cGKI−/− mice to 147±8% and 138±7%, respectively. In the presence of forskolin, cGMP analogues reduced Fc in ventricular muscle from WT but not from cGKI−/− mice, whereas CCh reduced Fc in both preparations (Figure 2E).

Recently, it has been shown that cGKI expression decreases during maturation in rabbit myocardium indicating a minor role for this enzyme in adults. To study the function of cGKI in the heart muscle of adult (4- to 6-month-old) mice, cardiomyocyte-specific cGKI knockout mice (Figure 1C) were used because conventional cGKI−/− mice show a high mortality rate with increasing age. Furthermore, the cardiomyocyte-specific knockout mouse allowed an analysis of whether the myocardial phenotype reflected a cell-autonomous function of cGKI in cardiomyocytes or was a secondary effect due to the loss of cGKI expression in other cell types. 8-Br-cGMP reduced Fc in preparations from adult control animals but not from adult cardiomyocyte-specific cGKI mutants (Figure 2E). It was noticed that the negative inotropic effect of cGMP was weaker in adult than in juvenile WT mice (compare Figures 2E and 2B). However, CCh still decreased Fc in adult cardiomyocyte-specific cGKI mutants (Figure 2F).
Taken together, these results indicate that the reduction of myocardial contractility by cGMP is mediated by activation of cGKI in both juvenile and adult murine myocardium. The mechanism behind the negative inotropic action of cGKI may include desensitization of contractile filaments and/or inhibition of calcium-channel activity. The results of this study are also in agreement with recent reports suggesting that the NO/cGMP/cGKI signaling pathway is not involved in the negative inotropic effect of muscarinic agonists. However, we cannot fully exclude that endogenous cGMP can stimulate cGKI-independent pathways that are not activated by the cGMP analogues and the NO donor used in the present study.

Acknowledgments

This work was supported by the Volkswagen Stiftung and the Deutsche Forschungsgemeinschaft. We thank Sabine Brunner and Johanna Rupp for technical assistance and K.R. Chien for the gift of MLC2a-Cre mice.

References


Key Words: contractility • mouse • gene targeting • Cre recombinase
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*Circ Res.* 2002;90:18-20; originally published online December 6, 2001;
doi: 10.1161/hh0102.103222

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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