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The G protein Gα11 is essential for hypertrophic signalling in diabetic myocardium

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ABSTRACT

Aims/hypothesis: Pathological cardiac hypertrophy is an early phenotype in both types 1 and 2 diabetes. The primary stimulus for hypertrophic growth in diabetes is yet unknown and may involve neurohumoral stimulation of Gq-coupled receptors as well as direct glucose-dependent mechanisms. To discriminate between these hypertrophic stimuli we analyzed hypertrophic signalling pathways in wildtype and $G\alpha_{11}$-knockout mice.
Methods: Experimental diabetes was induced in wildtype and knockout mice by intraperitoneal injection of streptozotocin. 8 weeks after induction of diabetes myocardial function and structure was assessed by echocardiography before sacrifice. To identify prohypertrophic signalling pathways expression and translocation of protein kinase C isoforms α, β_{II}, δ, ε and ζ were analyzed by immunohistochemical staining and immunoblot analysis after tissue fractionation. Changes in calcineurin signalling were identified by immunoblot analysis and functional assays. Expression levels of transcription factors GATA4 and NF-κB were quantified by real-time RT-PCR.
Results: Diabetic wildtype mice developed myocardial hypertrophy with preserved cardiac function. Calcineurin signalling was not different between the two groups. However, diabetic wildtype mice showed increased protein levels of PKC-α and PKC-ζ, translocation of PKC-α, -δ and -ε to cellular membranes and higher levels of NF-κB expression. In contrast, diabetic $G\alpha_{11}$-knockout mice showed no altered phenotype and no changes in NF-κB or PKC expression, although translocation of PKC-ε occurred as in wildtypes.
Conclusions: $G\alpha_{11}$ is essential for the development of cardiac hypertrophy in type 1-diabetes. Stimulation of hypertrophic signalling through PKC-α, PKC-δ, PKC-ζ, and NF-κB appears to be receptor-dependent, whereas PKC-ε is activated by hyperglycemia, independent of $G\alpha_{11}$.

1. Introduction

Figs. 2 and 3

Cardiovascular complications are the leading cause of death in patients suffering from diabetes mellitus. Both types 1 and 2 diabetes are associated with cardiomyopathy that share similar characteristics, including ventricular hypertrophy, decreased ventricular relaxation and a reduced peak filling rate [1–3]. Diabetic cardiomyopathy is distinct from ischemic cardiomyopathy because it is present in diabetic patients and animal models of diabetes in the absence of coronary artery disease. Changes in cell metabolism but also several receptor-mediated signalling pathways, especially those that couple to the Gq class of G-proteins have been implicated in development of myocardial hypertrophy early in the course of diabetic heart disease [4,5]. Virtually every cardiomyocyte receptor that couples to Gq stimulates cardiac or cardiomyocyte hypertrophy, the most important of which are the AT1 receptor for angiotensin II, the ET-A receptor for endothelin-1, and the α1-adrenergic receptors for norepinephrine and phenylephrine. There is evidence from large clinical trials suggesting excessive neurohumoral stimulation as a central mechanism in the pathogenesis of diabetic heart disease [6,7]. Increased circulating levels of angiotensin II and endothelin-1 have consistently been found in animal models and human diabetes [8], although the cardiac para- and/or autocrine formation of these neurohormones may be of primary importance in the development of myocardial hypertrophy.

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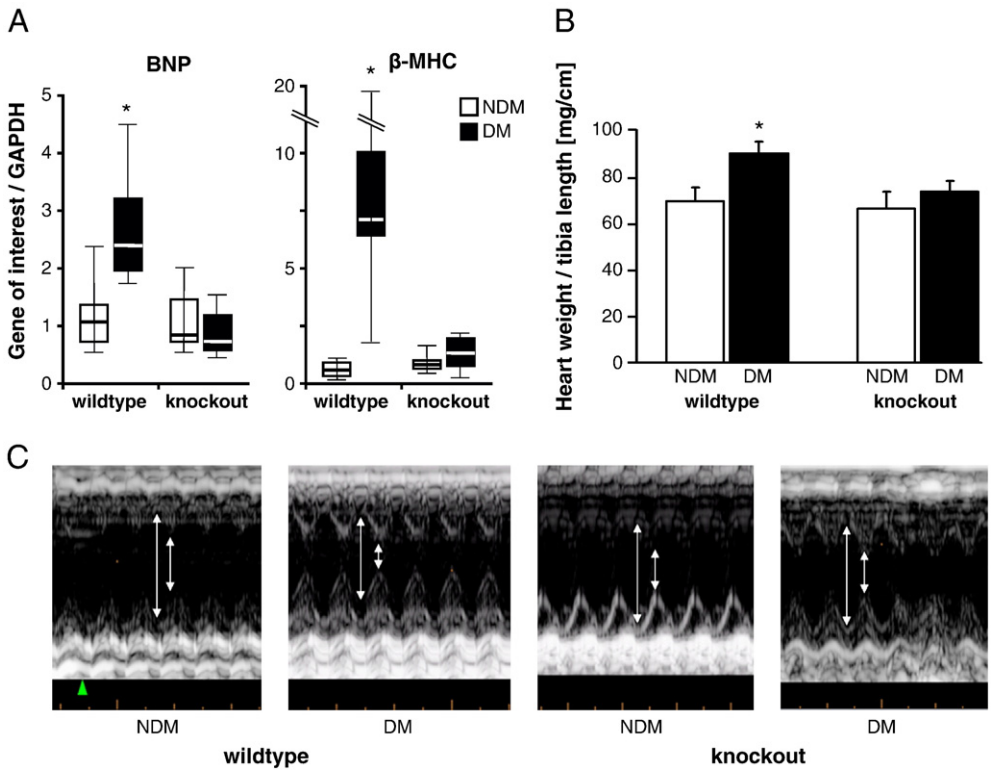

Fig. 1. Diabetes mellitus stimulates hypertrophic growth in wildtype, but not in $G\alpha_{11}$ knockout mice. After 8 weeks of diabetes, wildtype mice (WT) developed typical concentric left ventricular hypertrophy, whereas knockout mice (KO) revealed no altered phenotype under the same experimental conditions (A-C). (A) Box and whiskers plots showing the increased expression of b-type natriuretic peptide (BNP, left) and β-myosin heavy chain (β-MHC, right) as typical markers of myocardial hypertrophy. The boxes show the 25th and 75th percentile (interquartile) ranges. Median values are shown as a horizontal bar within each box. The whiskers mark levels outside the 25th and 75th percentiles. Panel B depicts the increase in the heart weight to tibia length ratio, which could be detected only in hearts from diabetic wildtype mice. (C) The echocardiographic M-Mode recordings present the increased thickness of the septum and the posterior myocardial wall in hearts from diabetic wildtype mice. The arrows indicate the diameter of the left ventricular cavity during contraction and relaxation, which is reduced in hearts from diabetic wildtype mice, accordingly. Summary data are presented in Table 2. DM: Diabetes mellitus; NDM: non-diabetic controls. * $p<0.05$.

G proteins are heterotrimeric, composed of an α- and obligate βγ heterodimeric subunits. Hormone catalyzed activation of G proteins releases Gα-GTP and Gβγ, which can regulate independent effector proteins. Of the four Gq class α-subunits found in mammals, only $G\alpha_q$ and $G\alpha_{11}$ are expressed in the heart. $G\alpha_q$ and $G\alpha_{11}$ have largely overlapping receptor- and effector-specificity and once activated by neurohormones these G alpha proteins stimulate phospholipase C (PLC)-β, resulting in hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2). The products of this reaction are inositol1,4,5-triphosphate (IP_3) and diacylglycerols (DAGs), both of which are known to activate strong signals of hypertrophic growth.

Gq/11 signaling releases critical mediators of pathologic, but not physiologic hypertrophy. IP_3 liberates intracellular Ca^{2+} stores, thus activating the protein phosphatase calcineurin and its target, the transcription factor nuclear factor of activated T-cells 3 (NFAT3). Evidence implicating this signalling pathway also in models of receptor-mediated hypertrophy came from the observation that stimulation of spontaneous Ca^{2+} transients by angiotensin II or norepinephrine activated calcineurin and nuclear NFAT translocation whereas AT1 receptor blockade in a diabetic rat model was effective to reduce calcineurin activity [9–11].

The other effector arm of the Gq/PLC-β signalling cascade is the activation of protein kinase C (PKC) through the synthesis of DAGs. In the heart, the five most functionally significant PKC family members are PKC-α and -β from the "conventional" group (calcium- and DAG-activated), PKC-δ and -ε as members of the "novel" group (DAG-activated, but no calcium required) and -ζ, which belongs to the "atypical" group of PKC since its activation is independent of both, DAG and calcium. Members of the PKC family share structural homology, however, their impact on cardiac morphology and function in heart disease ranges from strongly adverse to potentially cardioprotective, depending on the isoform [12]. Furthermore, the central pathway leading to PKC activation in diabetes is still a controversial issue. Besides neurohormonal mediators that activate the Gq/PLC-β pathway, PKC could directly be activated by hyperglycemia through DAG synthesis from the glycolytic intermediate dihydroxyacetone phosphate. Hyperglycemia may also activate PKC isoforms indirectly through both ligation of receptors for advanced glycation end-products (AGE) and increased activity of the polyol-pathway, presumably by increasing reactive oxygen species (for review see [4]). Taken together, these findings strongly implicate overreactivity of the PKC signalling pathways in the pathogenesis of diabetes-induced cardiac hypertrophy and failure.

The present study was therefore designed to investigate the role of $G\alpha_{11}$-mediated cell signalling for the development of myocardial hypertrophy in diabetes. Our model reproduces the primary characteristics of hypertrophic cardiomyopathy that is frequently observed in the early course of diabetes in man [1,2]. Furthermore we systematically analyzed the activation of specific PKC isoforms and transcription factors that have been implicated in the development of diabetic cardiomyopathy and aimed at discriminating the direct impact of hyperglycemia from receptor-mediated effects in stimulating PKC activity.

2. Methods

2.1. Animal experiments

Investigations were carried out in $G\alpha_{11}$ knockout mice and wildtype mice of the same line. The generation of knockout mice was described previously [13]. C57BL6 wild type mice were obtained from Charles River Laboratories (Sulzfeld, Germany). For the induction of diabetes knockout and wildtype mice of both sexes 8 weeks of age received an i.p. injection of streptozotocin (STZ, 130 mg/kg; Calbiochem, San