Diacylglycerol kinase ζ inhibits Gαq-induced atrial remodeling in transgenic mice

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BACKGROUND Our previous study showed that diacylglycerol kinase ζ (DGKζ), which degenerates diacylglycerol (DAG), inhibits ventricular structural remodeling and rescues activated G protein αq (Gαq)–induced heart failure. However, whether DGKζ inhibits atrial remodeling is still unknown.

OBJECTIVE This study aimed to elucidate the effects of DGKζ on atrial remodeling.

METHODS A transgenic mouse (Gαq-TG) with cardiac expression of activated Gαq and a double transgenic mouse (Gαq/DGKζ-TG) with cardiac overexpression of DGKζ and activated Gαq were created.

RESULTS During electrocardiogram (ECG) recording for 10 min, atrial fibrillation was observed in 5 of 11 anesthetized Gαq-TG mice but not in any wild-type (WT) and Gαq/DGKζ-TG mice ($P < .05$). All of the ECG parameters measured were prolonged in the Gαq-TG compared with WT mice. Interestingly, in Gαq/DGKζ-TG mice, although the PR and RR intervals were still prolonged, the P interval, QRS complex, and QT interval were not different from those in WT mice. In Langendorff-perfused hearts, the incidence of atrial tachyarrhythmia induced by rapid atrial pacing was greater in Gαq-TG hearts than in Gαq/DGKζ-TG hearts ($P < .05$). Action potential duration prolongation and impulse conduction slowing were observed in Gαq-TG atria compared with Gαq/DGKζ-TG atria. Dilatation of the left atrium with thrombus formation was observed in 9 Gαq-TG hearts but not in any Gαq/DGKζ-TG hearts. Moreover, the degree of extensive interstitial fibrosis in the left atrium was greater in Gαq-TG hearts than that in Gαq/DGKζ-TG hearts ($P < .05$).

CONCLUSION These results show that DGKζ inhibits Gαq-induced atrial remodeling and suggest that DGKζ is a novel therapeutic target for atrial fibrillation.

KEYWORDS Atrial fibrillation; Atrial remodeling; G protein coupled receptor; Mouse heart; Transgenic mouse; Diacylglycerol kinase; Optical mapping; Atrial arrhythmias.

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Introduction

Atrial fibrillation (AF) is the most common arrhythmia in clinical practice. AF is associated with a wide range of potential morbidities and mortality. Our understanding of AF pathophysiology has advanced significantly through an increased awareness of the role of atrial electrical and structural remodeling. Many forms of atrial remodeling promote the occurrence or maintenance of AF by acting on the fundamental arrhythmia mechanisms.[1] The Gαq protein-coupled receptor (GPCR) signaling pathway plays a critical role in the development of cardiac hypertrophy and congestive heart failure. GPCR agonists such as angiotensin II and endothelin-1 stimulate phospholipase C, leading to diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3) production. DAG activates protein kinase C, and IP3 causes intracellular Ca^{2+} release, both of which may promote remodeling in the heart.[1–4] One major route for terminating DAG signaling is thought to be its phosphorylation and inactivation by DAG kinase (DGK), producing phosphatidic acid.[5–8] We recently generated transgenic mice with cardiac-specific overexpression of DGKζ using an α-myosin heavy chain promoter and showed that DGKζ negatively regulated the hypertrophic signaling cascade and resultant ventricular remodeling in response to GPCR agonists.[9] In addition, Niizeki et al[10] have shown that DGKζ inhibits ventricular structural remodeling and rescues Gαq-induced heart failure. However, whether DGKζ inhibits atrial remodeling is still unknown. The objective of this study is to elucidate effects of DGKζ on atrial remodeling using a transgenic mouse with transient cardiac expression of activated Gαq[11] and a double transgenic mouse with cardiac-specific overexpression of both DGKζ and the activated Gαq.[10]

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Methods

The experimental protocol was approved by the institutional animal experiments committee and complied with the *Guide for Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health (publication 85-23, revised 1996).

Animals

A transgenic mouse (Gαq-TG mouse) with transient cardiac expression of activated G protein αq and a double transgenic mouse (Gαq/DGKζ-TG mouse) with cardiac-specific overexpression of both DGKζ and the activated Gαq were used in the present study.[10,11] The genotypes of the wild-type (WT), Gαq-TG, Gαq/DGKζ-TG mice were identified by polymerase chain reaction (PCR) with the use of tail genomic DNA as previously reported.

Electrocardiography (ECG)

Age-matched WT (n = 10), Gαq-TG (n = 11), and Gαq/DGKζ-TG (n = 13) female mice were anesthetized with sodium pentobarbital (30 mg/kg) applied intraperitoneally. ECG lead II was recorded and filtered (0.1 to 300 Hz), digitized with 12-bit precision at a sampling rate of 1,000 Hz per channel (Microstar Laboratories Inc., Bellevue, Washington), and transmitted into a microcomputer and saved to CD-ROM.

Langendorff-perfused mouse heart

After ECG lead II recording, all mice were treated with sodium heparin (500 USP units/kg intravenously). After a midline sternal incision, hearts were quickly excised and connected to a modified Langendorff apparatus. A polytetrafluoroethylene-coated silver unipolar electrode was used to stimulate the epicardial surface of the left atrial appendage at twice diastolic threshold current with a duration of 1 ms. Each preparation was perfused under constant flow conditions with oxygenated (95% oxygen, 5% CO_2) Tyrode solution containing in mM: NaCl, 141.0; KCl, 5.0; $CaCl_2$, 1.8; $NaHCO_3$, 25.0; $MgSO_4$, 1.0; NaH_2PO_4, 1.2; HEPES, 5; and dextrose, 5.0 (pH of 7.4 at 36°C ± 1°C). Perfusion pressure was measured with a pressure transducer (Nihon Kohden Co, Tokyo, Japan) and maintained within a pressure range (50 to 60 mm Hg) by adjusting flow. Preparations were stained with 5 μl voltage-sensitive dye, di-4-ANEPPS (Molecular Probes, Eugene, Oregon) dissolved in 0.19 ml ethanol at a concentration of 8 mM. Cardiac rhythm was monitored using 3 silver disk electrodes fixed to the chamber in positions corresponding to ECG limb leads II. The ECG signals were filtered (0.3 to 300 Hz), amplified (1000×), and displayed on a digital recorder. Perfusion pressure and flow were continuously monitored during each experiment. After each experiment, tissue viability was confirmed by 2 ml of 2,3,5-tetrazolium chloride (14 mg/ml) staining.

Optical mapping system

Beating and perfused hearts were immersed in a custom-built chamber specifically designed for optical recordings and filled with Tyrode solution. In every experiment, the mapping field was positioned at the left atrial free wall. The optical mapping system used in this study has been described in detail elsewhere.[12] Briefly, excitation light (510 nm) obtained from a 250-W quartz tungsten halogen lamp (Oriel Co. Stratford, Connecticut) was directed toward the heart using a liquid light guide. Fluoresced light from the heart was collected by a tandem lens assembly and directed to a long-pass filter (>630 nm) that passes light of longer wavelengths to a 16 × 16-element photodiode array. Signals recorded from each photodiode and ECG signals were multiplexed and digitized with 12-bit precision at a sampling rate of 3,000 Hz per channel (Microstar Laboratories Inc., Bellevue, Washington). An optical magnification of 3× was used, corresponding to a mapping field of 0.6 × 0.6 cm and 0.37-mm spatial resolution between recording pixels. To view, digitize, and store anatomical features, a mirror was temporarily inserted between the lenses of the tandem lens assembly to direct reflected light to a digital video camera (DCR-PC120 Sony Co. Tokyo, Japan).

Experimental protocol

First, after anesthesia ECG lead II was recorded for 10 min in all mice (Figure 1). Second, to measure action potential duration (APD) and conduction velocity (CV), optical action potentials were recorded for 10 s from the left atrial free walls at a basic cycle length of 150 ms in isolated Langendorff-perfused hearts (Figure 2). Third, rapid atrial pacing at a pacing cycle length of 80 to 100 ms for approximately 5 s from the left atrial appendage was performed to induce atrial tachyarrhythmia (AT). AT was defined as a rapid (cycle length <100 ms) regular or irregular rhythm persisting more than 5 beats. When rapid pacing induced AT that terminated spontaneously within 1 min, AT was reinitiated at the same cycle length. Each experiment ended after rapid pacing was performed 10 times or when rapid pacing induced AT lasting more than 1 min. Optical action potentials were recorded during the AT from the epicardial surface of left atrial free wall for 10 s.

Data analysis

In all anesthetized mice, P, PR, QRS complex, QT, and RR interval were measured from ECG lead II. High incidence of premature atrial contraction (high PAC) was defined as PAC occurring more than 10 beats/min. AF was defined as an irregularly irregular rhythm without P waves. In all Langendorff hearts, left atrial size was measured from the distance between upper and bottom end of the left atrium as described in Figure 3A. In all optical action potentials, automated algorithms were used to determine depolarization time relative to a single fiducial point (i.e., the stimulus). Depolarization time was defined as the point of maximum positive derivative in the action potential upstroke (dV/dt_{max}). Depolarization contour maps were computed for the entire mapping field. Repolarization time was defined as the time when repolarization reached a level of 80%. The APD was defined as the difference between repolarization