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Differential modulation of cardiac potassium channels by Grb adaptor proteins

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ABSTRACT

Scaffolding growth factor receptor-bound (Grb) adaptor proteins are components of macromolecular signaling complexes at the plasma membrane and thus are putative regulators of ion channel activity. The present study aimed to define the impact of Grb adaptor proteins on the function of cardiac K^+ channels. To this end channel proteins were coinjected with the adaptor proteins in *Xenopus* oocytes and channel activity analyzed with two-electrode voltage-clamp. It is shown that coexpression of Grb adaptor proteins can reduce current amplitudes of coexpressed channels. Grb7 and 10 significantly inhibited functional currents generated by hERG, Kv1.5 and Kv4.3 channels. Only Grb10 significantly inhibited KCNQ1/KCNE1 K^+ channels, and only Grb7 reduced Kir2.3 activity, whereas neither Grb protein significantly affected the closely related Kir2.1 and Kir2.2 channels. The present observations for the first time provide evidence for a selective and modulatory role of Grb adaptor proteins in the functional expression of cardiac K^+ channels.

Introduction

The Grb7 (growth factor receptor-bound protein 7) family represents a group of SH2 domain-containing cytosolic adaptor proteins, consisting of three members, Grb7, 10 and 14 [1,2]. Grb7 family proteins are differentially expressed in a variety of human tissues with highest expression of Grb10 in skeletal muscle and pancreas, and high expression in cardiac muscle and brain [3,4].

The physiological functions of the Grb7 family members are incompletely understood. However, it is likely that these proteins act as negative regulators in several physiological processes [5] and the presence of a Pleckstrin homology (PH) domain through its interaction with phospholipids may contribute to Grb's membrane targeting. Specifically, Grb10 has been shown to modulate the activity of plasma membrane-localized Kv1.3 channels [6].

The present study explored whether Grb7 or Grb10 participate in the regulation of cardiac K^+ channels. For this, cRNA encoding voltage-gated K^+ channels hERG, Kv1.5, Kv4.3, KCNQ1/KCNE1, and inward-rectifier K^+ channels Kir2.1, Kir2.2 and Kir2.3 channels, was injected into *Xenopus* oocytes either alone or together with cRNA encoding Grb7 or Grb10 and the activity of the respective K^+ channels was determined by two-electrode voltage-clamp. As a result, hERG and other Kv channel currents were strongly downregulated by coexpression of both Grb7 and Grb10, whereas KCNQ1/KCNE1 was only mildly downregulated by Grb10. Inhibitory effects of coexpressed Grb proteins on Kir2 channels in general were only weak, but reached statistical significance in case of Grb7 and coexpressed Kir2.3 currents. Interestingly, Grb7 was also able to stimulate Kir2.2 channels.

Materials and methods

Molecular biology. Template cDNAs encoding hKir2.x, hERG, hKv1.5, hKv4.3, hKCNQ1, hKCNE1 and hGrb were linearized with an appropriate restriction enzyme (New England Biolabs, MA, USA) and cRNA was synthesized from 1 μg of linearized DNA by *in vitro* transcription (mMessage mMachine T7 kit or mMessage mMachine SP6 kit, Ambion, Applied Biosystems, Germany). cRNA concentrations were evaluated by photospectrometry and transcript quality was verified by agarose gel electrophoresis.

Oocyte isolation. Ovarian lobes were harvested from *Xenopus laevis* frogs anesthetized with a 0.17% tricaine solution. Oocytes were treated for 120 min with collagenase (1 mg/ml, Worthington, type II) in ND96-Ca^{2+}-free solution containing (in mM): 96 NaCl, 2 KCl, 2 $MgCl_2$, 5 HEPES (*N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid) (pH 7.6) to remove follicle cells, and then stored at 17 °C in ND96 containing gentamycin (50 mg/L), theophylline

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(0.5 mM) and Na-pyruvate (2.5 mM). For characterization of cardiac K^+ channels, each oocyte was injected with 1 ng Kir2.x cRNA or 4 ng cRNA encoding the other channels followed by coinjections with 3 ng cRNA encoding Grb7 or 10.

Electrophysiology. A Turbo-TEC10CX amplifier was used to record currents at room temperature (at 22–23 °C) in oocytes 2–4 days after injection with cRNAs using standard two-electrode voltage-clamp technique (TEVC). The pipettes were filled with 3 M KCl and had resistances of 0.5–1.0 MΩ. For all experiments the TEC-integrator (set to 2–3 ms) was used to enable good clamp performance of currents and the clamp performance was always controlled via the TEC-PI-controller. Data were Bessel filtered at 500 Hz (Kir2.x) or 2 kHz (voltage-gated channels) and stored at a sample rate of 2–3 kHz. The standard recording solution ND96 for voltage-gated channels contained in mM: 96 NaCl, 2 KCl, 1.8 $CaCl_2$, 2 $MgCl_2$, 5 HEPES and was equilibrated to pH 7.5. The recording solution KD60 (in mM: 60 KCl, 38 NaCl, 1.8 $CaCl_2$, 2 $MgCl_2$, 5 HEPES and was equilibrated to pH 7.5) was used to record Kir2.x. Data acquisition was performed using an IBM compatible computer with Pentium 3 processor, a Digidata 1322 A/D interface and pClamp 8 software (Axon Instruments).

Data were analyzed utilizing Clampfit 8 (Axon Instruments) and Origin 6 (Microcal) software. Data are expressed as arithmetic means ± SEM and statistical analysis was made by ANOVA. $p < 0.05$ was considered as statistically significant. "*n*" denotes the number of independent experiments.

Results

Grb7 was originally identified by us as a binding protein of the carboxy-terminal tail of renal inward-rectifier K^+ channel ROMK (Kir1.1) in a yeast-two hybrid screen, and it reduced the amplitude of the coexpressed ROMK channel in *Xenopus* oocytes [7]. This result prompted us to evaluate the effects of Grb7 and Grb10 proteins on the related cardiac inward-rectifier channels of the Kir2 family. Kir2.x constructs were either expressed alone or together with the respective Grb protein in *Xenopus laevis* oocytes, and after 2 days voltage-clamp recordings were performed in high K^+ solution, KD60.

The Kir2.1 channel generated strongly inwardly rectifying K^+ currents that, in contrast to the related Kir1.1, were not significantly affected by any of the two coinjected Grb proteins (Fig. 1A). Grb10 apart from a slight tendency to reduce Kir2.2 currents (Fig. 1B), but which did not reach statistical significance, also had no effect on coexpressed Kir2.3 currents (Fig. 1C). In contrast, coexpression of Grb7 significantly decreased Kir2.3 currents and, surprisingly slightly increased the Kir2.2 currents (Fig. 1B). While Grb adaptor proteins were able to change current amplitudes, kinetics of coexpressed Kir channels were not markedly altered.

After demonstrating differential effects on several inward-rectifiers, in a second set of experiments, we studied the effects of Grb7 and Grb10 on outwardly rectifying cardiac channels hERG, Kv1.5, Kv4.3 and KCNQ1/KCNE1.

Coinjection of KCNQ1 and KCNE1 generated slowly activating, non inactivating channels, typical for the cardiac slow delayed rectifier I_{Ks} conductance. When additionally coexpressed, only Grb10 significantly decreased KCNQ1/KCNE1 currents. Coinjected K^+ channels with Grb, changes in current amplitude were not accompanied by obvious changes in kinetics. I_{Ks} currents contrastingly remained virtually unaffected by coexpression of Grb7 (Fig. 2).

Currents carried by the rapid delayed rectifier hERG were markedly and significantly reduced by both, Grb7 and Grb10 (Fig. 3A). At the same amount of injected cRNA inhibition of hERG currents reached about 50% with Grb7, but was almost complete with coexpressed Grb10. The hERG kinetics were not obviously modulated by the Grbs.

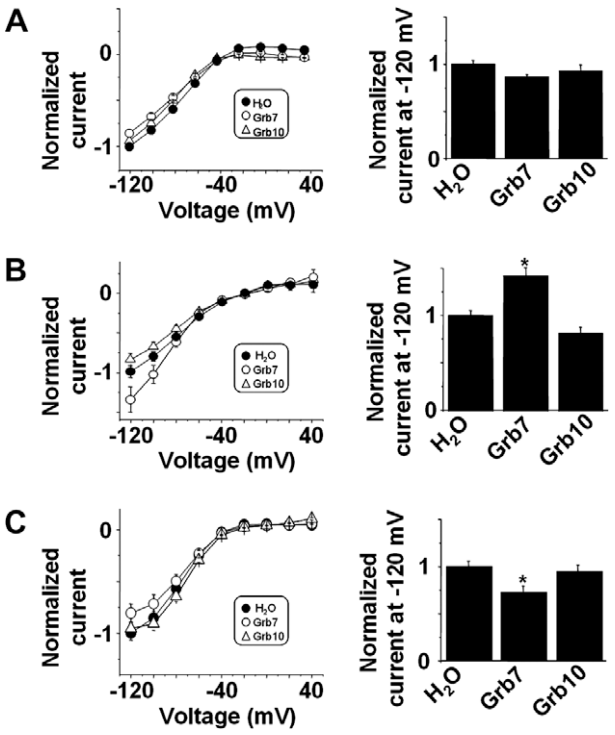

Fig. 1. Grb proteins differentially modulate coexpressed Kir2.x channels. *Xenopus* oocytes were injected with Kir2.1 (A), Kir2.2 (B) and Kir2.3 (C) either alone or in combination with Grb7 or Grb10. Kir2.x currents were measured 2 days after injection in KD60, and effects of coexpressed Grb proteins on Kir2 current amplitudes were analyzed at −120 mV. No difference in current amplitude of Kir2.1 channels could be observed in presence of any Grb protein, while Kir2.2 currents were significantly increased and Kir2.3 currents decreased by coexpressed Grb7 adaptor protein. Arithmetic means ± SEM. * indicates a statistically significant difference ($p < 0.05$); $n = 15–31$.

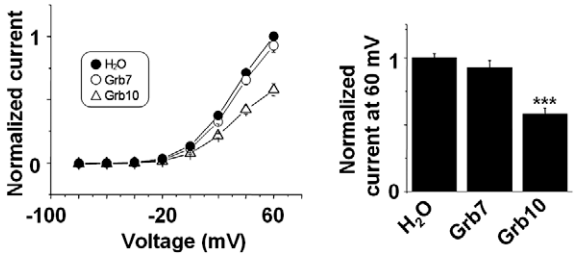

Fig. 2. Heteromeric KCNQ1/KCNE1 currents are decreased by Grb10 but not Grb7 coexpression. KCNQ1/KCNE1 K^+ channels were recorded 3 days after injection either with or without Grb7 or Grb10. Currents were measured after stepping the potential from −80 to +60 mV. A significant inhibition of currents was only observed in presence of Grb10. *** indicates a statistically significant difference ($p < 0.001$); $n = 25–30$.

Similar to hERG, the ultrarapid delayed rectifier Kv1.5 also was sensitive to Grb7 and 10 coexpression. Again, inhibition by Grb10 was stronger than by Grb7 (Fig. 3B).

Both, Grb7 and Grb10, also strongly reduced currents generated by the transient outwardly rectifying Kv4.3 channel, and inhibition was equally potent for both Grb proteins (Fig. 3C). Again, as was the case with coinjected I_{Ks} channels, changes in current amplitude were not accompanied by obvious changes in kinetics.

Discussion

The present study reveals that scaffolding proteins of the growth factor receptor-bound (Grb) protein family modulate the