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Original article

SHP2-mediated signaling cascade through gp130 is essential for LIF-dependent I_{CaL}, $[Ca^{2+}]_i$ transient, and APD increase in cardiomyocytes

Yoko Hagiwara [a], Shunichiro Miyoshi [a,*], Keiichi Fukuda [b], Nobuhiro Nishiyama [a], Yukinori Ikegami [a], Kojiro Tanimoto [a], Mitsushige Murata [a], Eiichi Takahashi [a], Kouji Shimoda [c], Toshio Hirano [d], Hideo Mitamura [e], Satoshi Ogawa [a]

[a] Division of Cardiology, Department of Medicine, Keio University School of Medicine, Japan
[b] Department of Regenerative Medicine and Advanced Cardiac Therapeutics, Keio University School of Medicine, Japan
[c] Laboratory Animal Center, Keio University School of Medicine, Japan
[d] Department of Molecular Oncology, Graduate School of Medicine, Osaka University, Japan
[e] Saiseikai Central Hospital, Japan

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Abstract

Leukemia inhibitory factor (LIF), a cardiac hypertrophic cytokine, increases L-type Ca^{2+} current (I_{CaL}) via ERK-dependent and PKA-independent phosphorylation of serine 1829 in the $Cav_{1.2}$ subunit. The signaling cascade through gp130 is involved in this augmentation. However, there are two major cascades downstream of gp130, i.e. JAK/STAT3 and SHP2/ERK. In this study, we attempted to clarify which of these two cascades plays a more important role. Knock-in mouse line, in which the SHP2 signal was disrupted ($gp130^{F759/F759}$ group), and wild-type mice (WT group) were used. A whole-cell patch clamp experiment was performed, and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$ transient) was monitored. The I_{CaL} density and $[Ca^{2+}]_i$ transient were measured from the untreated cells and the cells treated with LIF or IL-6 and soluble IL-6 receptor (IL-6+sIL-6r). Action potential duration (APD) was also recorded from the ventricle of each mouse, with or without LIF. Both LIF and IL-6+sIL-6r increased I_{CaL} density significantly in WT (+27.0%, $n=16$ $p<0.05$, and +32.2%, $n=15$, $p<0.05$, respectively), but not in $gp130^{F759/F759}$ (+9.4%, $n=16$, NS, and −6.1%, $n=13$, NS, respectively). Administration of LIF and IL-6+sIL-6r increased $[Ca^{2+}]_i$ transient significantly in WT (+18.8%, $n=13$, $p<0.05$, and +32.0%, $n=21$, $p<0.05$, respectively), but not in $gp130^{F759/F759}$ (−3.8%, $n=7$, NS, and −6.4%, $n=10$, NS, respectively). LIF prolonged APD_{80} significantly in WT (10.5±4.3%, $n=12$, $p<0.05$), but not in $gp130^{F759/F759}$ (−2.1±11.2%, $n=7$, NS). SHP2-mediated signaling cascade is essential for the LIF and IL-6+sIL-6r-dependent increase in I_{CaL}, $[Ca^{2+}]_i$ transient and APD.

Keywords: IL-6; Leukemia inhibitory factor (LIF); Ion channel; L-type Ca^{2+} current; Patch clamp; Fluo-4; SHP2

1. Introduction

Leukemia inhibitory factor (LIF) is a member of the IL-6 family of cytokines that induces a wide range of responses in a variety of cells [1]. LIF is known to have various effects on cell growth, differentiation, and function [2–4]. The receptors of the IL-6 family of cytokines have common subunits, gp130 [5,6]. The binding of the IL-6 family cytokines to their receptors activates Janus kinases (JAK1, JAK2, and TYK2) [7,8], leading to the recruitment of signal transducing molecules such as protein tyrosine phosphatase 2 (SHP2) and signal transducers and activators of transcription3 (STAT3) [9–11]. It is considered that there are two major cascades downstream of gp130, i.e. JAK/STAT3 and SHP2/ERK [12,13].

In cardiomyocytes, we have reported that LIF induces cardiac hypertrophy [14]. The JAK/STAT3 pathway plays an important role in mediating this cardiac hypertrophy.

On the other hand, we have reported that LIF increases L-type Ca^{2+} current (I_{CaL}) and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$ transient) in cardiomyocytes, and this I_{CaL} augmentation is independent of PKA but dependent on mitogen-activated protein kinase (MEK) [15]. We have also reported that LIF

* Corresponding author. 35 Shinanomachi Shinjuku-ku Tokyo, Japan. Tel.: +81 3 3353 1211; fax: +81 3 3353 2502.
E-mail address: smiyoshi@cpnet.med.keio.ac.jp (S. Miyoshi).

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phosphorylates the serine residue at the position 1829 of the $Cav_{1.2}$ subunit via the actions of extracellular signal-regulated kinase (ERK) and that this phosphorylation increases I_{CaL} in cardiomyocytes [16]. Therefore, the MEK/ERK pathway might be involved in the LIF-mediated increase of I_{CaL}. There may be, however, a possible crosstalk between the JAK/STAT3 pathway and the MEK/ERK pathway downstream of gp130, and therefore the role of the JAK/STAT3 pathway in the LIF-mediated increase of I_{CaL} remains to be elucidated.

In this study, using the knock-in mouse line, we attempted to clarify that it is not the JAK/STAT3 pathway, but rather the SHP2/ERK pathway below gp130 that plays an important role in this I_{CaL} augmentation.

2. Materials and methods

2.1. Materials

The knock-in mouse line [12], in which the SHP2 signal was disrupted by replacing the mouse gp130 gene with the human gp130 mutant cDNA ($gp130^{F759/F759}$ group), and wild-type mice (WT group) were used for this experiment. These mice were 6–10 weeks of age and weighed 21–28 g. In the $gp130^{F759/F759}$ group, only the JAK/STAT3 cascade can be activated, and both the JAK/STAT3 and SHP2/ERK cascades can be activated in the WT group.

2.2. Cell preparation for the patch clamp

After 1000 units of heparin and 50 mg of sodium pentobarbital were administered intraperitoneally, the murine heart was quickly excised and retrogradely perfused with nominally Ca^{2+}-free HEPES–Tyrode's solution, which contained 140 mmol/l NaCl, 4 mmol/l KCl, 0.5 mmol/l $MgCl_2$, 5.5 mmol/l glucose, and 5 mmol/l HEPES (pH adjusted to 7.4 with NaOH) for 3 min, and with the same solution containing 0.5 mg/ml of type II collagenase (Worthington Biochemical, NJ, USA) for 30 min at 37 °C. The ventricles were excised and were gently agitated in high-K^+ storage medium, containing 70 mmol/l glutamic acid-K, 15 mmol/l taurine, 30 mmol/l KCl, 10 mmol/l KH_2PO_4, 0.5 mmol/l EGTA, 0.5 mmol/l $MgCl_2$, 60 mmol/l glucose, and 5 mmol/l HEPES (pH adjusted to 7.4 with KOH) for 10 min to obtain isolated ventricular cells. After small cells, e.g. blood cells or non-myocyte cells, were discarded by use of 20 μm nylon mesh, isolated ventricular cells were stored in high-K^+ storage medium at room temperature for 3–4 h before the patch clamp experiment.

2.3. Whole-cell patch clamp

We performed a whole-cell patch clamp to measure I_{CaL} as described previously [15,17]. HEPES–Tyrode's solution supplemented with 0.5 mmol/l of $CaCl_2$ and 1.3 mmol/l $MnCl_2$ was used for the external solution for the patch clamp experiment. To prevent contamination with other monovalent cation currents, external Na^+ and K^+ in the bath solution were substituted by equimolar choline. The pipette solution contained 115 mmol/l CsCl, 20 mmol/l TEA–Cl, 5 mmol/l MgATP, 0.4 mmol/l TrisGTP, 10 mmol/l BAPTA, and 5 mmol/l HEPES (pH adjusted to 7.2 with CsOH). The resistance of pipettes filled with the internal solution was 1.4 to 1.8 MΩ. Seal resistances <4 GΩ and series resistances >2 MΩ were discarded from the analysis. Currents obtained in this study were normalized to each cell capacitance.

We preincubated the cells in the presence of LIF (1000 U/ml), IL-6 (20 ng/ml), and soluble IL-6 receptor (sIL-6r) (25 ng/ml) or in the absence of it (control) for 20–40 min before the measurement. The effect of PD098059, a specific MEK inhibitor, on I_{CaL} was also observed [15]. The percent inhibition curve was automatically fitted to Hill's equation: $\Delta I/\Delta I_{max} = 1 - C^n/C^n + IC_{50}^n$ by a Chi-square procedure.

See details in the Supplementary data section.

2.4. Cell preparation for the measurement of $[Ca^{2+}]_i$ transient

Instead of HEPES–Tyrode's solution, Ca^{2+}-free $NaHCO_3$-buffered Tyrode's solution, containing 126 mmol/l NaCl, 4.4 mmol/l KCl, 1.0 mmol/l $MgCl_2$, 18 mmol/l $NaHCO_3$, 11 mmol/l glucose, 4 mmol/l HEPES, and 30 mmol/l BDM (pH adjusted to 7.4 at 37 °C with oxygenation; O_2 95%/CO_2 5%), was used for the cell isolation to measure $[Ca^{2+}]_i$ transient. After the washout of blood cells for 3 min, tissue was digested in the same solution with 0.9 mg/ml of type II collagenase and 25 μmol/l of $CaCl_2$ for 15 min at 37 °C. The ventricles were minced and gently agitated in the same solution with 0.9 mg/ml of the collagenase, 20 g/l of albumin Fraction-V (A7906-50G, Sigma, MO, USA), and 200 μmol/l $CaCl_2$. Incubation with the fresh enzyme solution was repeated 3 times at 15-min intervals. The supernatant from each digestion was filtered (100-μm mesh) and centrifuged (500 rpm for 3 min). The cells were then stored in the $NaHCO_3$-buffered Tyrode's solution supplemented with the same amount of minimum essential medium (MEM; 634-04281 WAKO, Tokyo, Japan) and 1 g/l of the albumin at room temperature for 3–4 h before the $[Ca^{2+}]_i$ transient experiment.

2.5. Measurement of $[Ca^{2+}]_i$ concentration

The $[Ca^{2+}]_i$ transient was monitored by use of the fluorescent calcium indicator Fluo-4 AM (Molecular Probes, Eugene, USA), as described previously [15,18]. The $[Ca^{2+}]_i$ transient was recorded from the baseline (0 min) until 30 min after the application of LIF (1000 U/ml) or IL-6 (20 ng/ml) and sIL-6r (25 ng/ml). F/Fo ratio was defined as the following equation:

$$\begin{aligned} F/Fo = {} & (\text{Peak fluorescence intensity} \\ & - \text{minimum fluorescence intensity}) \\ & / (\text{Peak fluorescence intensity at baseline} \\ & - \text{minimum fluorescence intensity at baseline}) \end{aligned}$$

See details in the Supplementary data section.

2.6. Action potential recordings

The action potential from the endocardium of the left ventricle was recorded by standard microelectrodes as described