Endothelin-1 activates Homer 1a expression via mitogen-activated protein kinase in cardiac myocytes

TAKAHIRO KAWAMOTO1, KIYONORI TOGI1, RYOKO YAMAUCHI1, YOSHINORI YOSHIDA1, YASUHIRO NAKASHIMA1, TORU KITA1 and MAKOTO TANAKA1,2

1Department of Cardiovascular Medicine, Graduate School of Medicine, Kyoto University; 2Department of Social Service, Kyoto University Hospital, Kyoto, Japan

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Abstract. Homer proteins are a family of scaffolding proteins which may play an important role in calcium signaling by facilitating the assembly of signaling complexes in neuronal cells. Among the three splice variants of Homer 1, Homer 1a is rapidly up-regulated by neural stimulation and may regulate the disassembly of signaling complexes mediated by Homer proteins. In spite of its potential importance in calcium signaling, the regulation of Homer 1a expression in cardiac myocytes has never been investigated. In this study, we examined the regulation of Homer 1a expression in cardiac myocytes. Homer 1a was significantly up-regulated by several hypertrophic agonists, including endothelin-1 (ET-1), phenylephrine, isoproterenol and angiotensin-II, and ET-1 most strikingly induced Homer 1a expression. The induction of Homer 1a expression by ET-1 peaked at 2 h and inhibitors for mitogen-activated/extracellular signal regulated kinase (MEK) significantly suppressed the induction of Homer 1a. This study first clarified the regulation of Homer 1a expression in cardiac myocytes and demonstrated that ET-1 induced Homer 1a expression through the mitogen-activated protein kinase pathway.

Introduction

Homer proteins are a family of scaffolding proteins, characterized by N-terminal Enabled/Vasp homology (EVH-1) and coiled-coil domains (1). The EVH-1 domain binds a proline-rich motif present in key components of synaptic signal transduction pathways including the metabolic glutamate receptors, mGluR1a and mGlu5a/b, inositol-1,4,5-triphosphate (IP3) receptors and the Shank family of scaffolding proteins (2,3). Homer proteins dimerize with other Homer proteins through the coiled-coil domain and mediate the interaction between cellular proteins that directly or indirectly bind to Homer (4). For example, N-methyl-D-aspartate (NMDA) receptors are associated with Shank via other scaffolding proteins and the ability of Homer proteins to form multimers allows for a physical and functional link between mGluRs, IP3 receptors and NMDA receptors in neuronal cells (1). Thus, Homer proteins are implicated as adaptor proteins that facilitate a physical association between cell surface receptors and intracellular store calcium channels.

Homer 1 comprises three splice variants: Homer 1a, 1b and 1c (4,5). While Homer 1b and 1c are constitutively expressed, Homer 1a is an immediate early gene, induced by synaptic activities including administration of glutamate or brain-derived neurotrophic factor, tetanic stimuli and electroconvulsive seizure (2,5,6). Homer 1a, encoded by a prematurely terminated transcript and lacking the coil-coiled domain (7), may act in a dominant negative fashion by interfering with multimerization and disassembling signaling complexes (1). Indeed, Homer 1a overexpression in neuronal cells attenuated mGluR-evoked intracellular calcium release, suggesting that Homer 1a may regulate coupling between extracellular signals and calcium release from the ER (3). On the other hand, Homer 1a also exerts constitutive activity by disassembling signaling complexes depending on the situation. It was reported that TRPC1, a non-specific cationic channel, could bind to Homer proteins and that the disassembly of a TRPC1-Homer-IP3R complex resulted in channels that were constitutively active (8). Expression of Homer 1a was also shown to induce constitutive activity in mGluR1a and mGluR5 (9).

Homer 1 is expressed in the heart as well as in the brain (4,10). Considering its potential function in neuronal cells, Homer 1a may be also involved in calcium signaling by mediating the interaction between cell surface receptors and ER calcium channels in cardiac myocytes, which plays an important role in cardiac hypertrophy (11,12). However, regulation of Homer 1a expression in the heart is totally unknown. In this study, we investigated the induction and regulation of Homer 1a expression by extracellular signals in the heart using cultured rat neonatal cardiac myocytes. We found that the three splice variants of Homer 1 were all expressed in cardiac myocytes and Homer 1a expression was induced by several hypertrophic agonists. Endothelin-1 (ET-1)
most strikingly up-regulated Homer 1a expression and the
induction by ET-1 was mediated by the mitogen-activated
protein kinase pathway.

Materials and methods

Materials. Endothelin-1, angiotensin II, phenirephrin and
isoproterenol were purchased from Sigma (St. Louis, MO),
and all inhibitors were from WAKO (Tokyo, Japan).

Cell culture. Primary ventricular cardiac myocytes were
prepared from 2- to 3-day-old Sprague-Dawley rats as
described previously (13). Cells were plated at a density of
2x10^4 cells/cm^2 on gelatin-coated dishes and incubated in
Dulbecco's modified Eagle's medium (DMEM) with 10%
fetal calf serum for 24 h. All experiments were performed in
serum-free conditions after 24 h of incubation in serum-free
DMEM.

Northern blot analysis. Total RNA was purified from cultured
cardiac myocytes using TRIzol reagent (Life Technologies,
Gaithersburg, MD). Total RNA (20 μg) was electrophoresed
in 1.0% denaturing (formaldehyde) agarose gels and blotted
onto nylon membrane. After hybridization with a [32P]-labeled
probe, the membrane was washed and exposed to a BAS
5000 Imaging Plate (Fuji, Tokyo, Japan). Densitometry was
performed using NIH image. A full-length cDNA for Homer 1a
was used as a probe.

Cell stimulation and inhibitor assay. After incubation in
serum-free DMEM for 24 h, cardiac myocytes were washed
and maintained in DMEM for 20 min. The cells were
stimulated by the addition of endothelin-1, angiotensin II,
phenirephrin or isoproterenol. After treatment for indicated
times, RNA was extracted for Northern blot analysis. Inhibitors
were added 30 min before stimulation by endothelin-1.

Statistical analysis. Data were analyzed by one-factor ANOVA
followed by Fisher's PLSD as a post-hoc test.

Results

Homer 1 expression in cultured neonatal cardiac myocytes.
We first examined the expression of Homer 1 transcripts in
cardiac myocytes. Northern blotting using full-length cDNA
for Homer 1a as a probe revealed that all three splice variants
of Homer 1 were expressed in cardiac myocytes (Fig. 1A).
However, the expression level of Homer 1a was very low at
basal conditions (Fig. 1A). We next examined whether
hypertrophic agonists could induce Homer 1a expression.
Interestingly, Homer 1a expression was significantly up-
regulated by endothelin-1 (ET-1), phenylephrine, isoproterenol
and angiotensin-II (Ang II) (Fig. 1). Since ET-1 most strikingly
activated Homer 1a expression, we examined the regulation
of Homer 1a expression by ET-1 in cardiac myocytes in sub-
sequent experiments.

ET-1 regulates Homer 1a expression through MEK/ERK
activation. We investigated the time course of Homer 1a
induction by ET-1 in cardiac myocytes. Homer 1a mRNA was

Figure 1. Expression of Homer 1a in cardiac myocytes. Cardiac myocytes
expressed all three splice variants of Homer 1. The immediate early gene
product, Homer 1a, was induced by all hypertrophic agonists examined. ET-1
most strikingly up-regulated Homer 1a expression. (A) A representative blot
showing induction of Homer 1a by several agonists. Cells were incubated
with 50 μM phenylephrine (lane 2, PE), 10 μM isoproterenol (lane 3, IS),
0.1 μM ET-1 (lane 4, ET) or 0.1 μM Ang II (lane 5, AT) for 2 h. Lane 1,
control (without any agonists). Ethidium bromide staining of 28S rRNA as
a reference of RNA loading. (B) Homer 1a mRNA induction by several
agonists. Data are shown as fold induction compared with cells incubated
with ET-1. The mean ± SD of three independent experiments is shown.

Figure 2. The time course of Homer 1a mRNA induction by ET-1. (A) A
representative blot showing the time course of Homer 1a mRNA induction
by ET-1. Cells were incubated with 0.1 μM ET-1 for the indicated times.
Lane 1, 0 h; lane 2, 0.5 h; lane 3, 1 h; lane 4, 2 h; lane 5, 4 h; lane 6, 8 h.
Ethidium bromide staining of 28S rRNA as a reference of RNA loading. (B) Temporal induction patterns of Homer 1a mRNA by ET-1. Data are shown
as fold induction compared with cells incubated with ET-1 for 2 h and
represent the mean ± SD of three independent experiments.
proteins (14,15). Binding of ET-1 to the ET A receptor results in the activation of protein kinase C (PKC) by diacylglycerol and release of Ca\(^{2+}\) from the ER by IP3 (14). We then examined the roles of PKC and IP3 in Homer 1a induction. However, inhibition of PKC (0.1 \(\mu\)M GFX) or the IP3 receptor (2 \(\mu\)M xestospongin C) had no effect on Homer 1a induction, suggesting that the activation of PKC or Ca\(^{2+}\) release from the IP3 receptor was not involved in the induction of Homer 1a by ET-1 (Fig. 3A and B).

In addition to the activation of the Gq class of G proteins, ET-1 rapidly and transiently stimulates the extracellular signal-regulated kinase (ERK) cascade in cardiac myocytes (14). For this cascade, mitogen-activated protein kinase kinase kinase (MAPKKK) is Raf and MAPKK is mitogen-activated/extracellular signal regulated kinase (MEK) (14). We thus investigated the effects of MEK inhibition on Homer 1a induction by ET-1. Although inhibition of p38 (5 \(\mu\)M SB203580) showed no effects, two kinds of MEK inhibitors (5 \(\mu\)M U0126 or 30 \(\mu\)M PD98059) significantly suppressed the induction of Homer 1a, demonstrating that ET-1 induced Homer 1a expression through activation of the MEK-ERK pathway (Fig. 3C and D).

**Discussion**

Calcium signaling in response to extracellular signals plays an important role in cardiac hypertrophy (11,12,16). Because of their potential importance in calcium signaling in neuronal cells, we hypothesized that Homer proteins may be also involved in signal transduction mediating cardiac hypertrophy. However, the expression pattern of Homer 1a has never been examined. In this study, we focused on the expression pattern of the immediate early gene (IEG) product, Homer 1a, because we anticipated that induction of Homer 1a may be involved in feedback regulation of hypertrophic signals. Interestingly, all hypertrophic agonists examined induced expression of Homer 1a, suggesting that Homer 1a induction may be a common phenomenon associated with hypertrophic signal activation.

The mitogen-activated protein kinase (MAPK) pathway regulates cellular proliferation and hypertrophy (14,17). Interestingly, ET-1 activated Homer 1a expression via the MEK-ERK cascade, which also mediates hypertrophic signals (11,18,19), suggesting that Homer 1a may be involved in the modulation of hypertrophic signals. Westhoff et al reported that Homer 1c decreased the ryanodine type 2 receptor (RyR2)-mediated Ca\(^{2+}\) release from microsomes prepared from the sarcoplasmic reticulum (SR) of rat cardiac myocytes but that Homer 1a reversed the effect of Homer 1c, indicating that aggregation of a part of RyR2 population into signaling complexes by Homer proteins may regulate the release of Ca\(^{2+}\) from intracellular stores in cardiac myocytes (20). Therefore, Homer 1a, induced via the MEK-ERK cascade, may regulate disassembly of signaling complexes including SR channels and modulate Ca\(^{2+}\) homeostasis, thus mediating crosstalk between the MAP kinase and Ca\(^{2+}\) signaling pathways during cardiac hypertrophy.

In summary, Homer 1a was induced by several hypertrophic signals and ET-1 most strikingly up-regulated Homer 1a expression in cardiac myocytes. Homer 1a induction by ET-1 was mediated through the MEK-ERK cascade. Homer 1a induction may modulate hypertrophic signals in cardiac myocytes.
cardiac myocytes and Homer proteins may represent a potential therapeutic target in cardiac hypertrophy.

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