Ca\textsuperscript{2+} binding protein-1 inhibits Ca\textsuperscript{2+} currents and exocytosis in bovine chromaffin cells

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Summary

Calcium binding protein-1 (CaBP1) is a calmodulin like protein shown to modulate Ca\textsuperscript{2+} channel activities. Here, we explored the functions of long and short spliced CaBP1 variants (L- and S-CaBP1) in modulating stimulus-secretion coupling in primary cultured bovine chromaffin cells. L- and S-CaBP1 were cloned from rat brain and fused with yellow fluorescent protein at the C-terminal. When expressed in chromaffin cells, wild-type L- and S-CaBP1s could be found in the cytosol, plasma membrane and a perinuclear region; in contrast, the myristoylation-deficient mutants were not found in the membrane. More than 20 and 70\% of Na\textsuperscript{+} and Ca\textsuperscript{2+} currents, respectively, were inhibited by wild-type isoforms but not myristoylation-deficient mutants. The [Ca\textsuperscript{2+}]\textsubscript{i} response evoked by high K\textsuperscript{+} buffer and the exocytosis elicited by membrane depolarizations were inhibited only by wild-type isoforms. Neuronal Ca\textsuperscript{2+} sensor-1 and CaBP5, both are calmodulin-like proteins, did not affect Na\textsuperscript{+}, Ca\textsuperscript{2+} currents, and exocytosis. When expressed in cultured cortical neurons, the [Ca\textsuperscript{2+}]\textsubscript{i} responses elicited by high-K\textsuperscript{+} depolarization were inhibited by CaBP1 isoforms. In HEK293T cells cotransfected with N-type Ca\textsuperscript{2+} channel and L-CaBP1, the current was reduced and activation curve was shifted positively. These results demonstrate the importance of CaBP1s in modulating the stimulus-secretion coupling in excitable cells.

Abbreviations: CaBP – calcium binding protein; CaM – calmodulin; \textit{I}_{Ca} – Ca\textsuperscript{2+} currents; \textit{I}_{Na} – Na\textsuperscript{+} currents; IP\textsubscript{3} – inositol 1,4,5-trisphosphate; NCS-1 – neuronal calcium sensor-1

Introduction

Calcium homeostasis is very important in many physiological events such as neurotransmitter release, muscle contraction, and gene expression [1, 2]. Therefore, the mechanism by which the intracellular Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{i}) is modu-
The change in the amount of freely available cytosolic Ca\(^{2+}\) is vital to the activities of many proteins, which typically have the ability to bind Ca\(^{2+}\) [6, 7]. Calmodulin (CaM) has 4 EF-hand Ca\(^{2+}\)-binding motifs; the binding of Ca\(^{2+}\) induces a conformational change and converts CaM into an active form able to modulate many physiological functions [6, 8]. Recently, a group of calcium binding proteins (CaBP1~5) with structures similar to CaM has been described [9]. These CaBPs may have different subcellular localizations for sensing the changes in local [Ca\(^{2+}\)]\(_i\) homeostasis to differentially modulate cellular activities. CaBP1 has been shown to directly activate the IP\(_3\) receptors [10]; however, it has also been suggested to inhibit the elevation in [Ca\(^{2+}\)]\(_i\) induced by ATP, which opens the IP\(_3\) receptors, in PC12 cells [11].

CaBP1 differs from CaM in that it has an N-terminal myristoylation moiety and an inactive EF-2. It has two alternative spliced variants, L- and S-CaBP1 [12]. L-CaBP1 has been extensively studied for its effects on various types of Ca\(^{2+}\) channels. It binds to the C-terminal CaM binding domain of Ca\(_{v2.1}\) and enhances current inactivation [13]; when binding to the IQ domain of Ca\(_{v1.2}\), it prolongs Ca\(^{2+}\) currents (I\(_{Ca}\)) [14, 15]. These findings suggest that L-CaBP1 differentially modulates the activities of various Ca\(^{2+}\) channels. However, the roles of L- and S-CaBP1 in modulating Ca\(^{2+}\) currents and neurotransmitter release in excitable cells have not been reported.

Here we investigate the physiological roles of L- and S-CaBP1s in modulating Ca\(^{2+}\) channels and neurotransmitter release in excitable cells. L- and S-CaBP1 cloned from freshly isolated rat E14.5 cortex were transiently expressed in bovine chromaffin cells and cortical neurons. To our knowledge this is the first report to show that both L- and S-CaBP1 have similar effects in attenuating stimulus-secretion coupling by diminishing I\(_{Ca}\). Our results reveal the importance of CaBP1 in modulating the Ca\(^{2+}\) signaling and neurotransmitter release in excitable cells.

**Methods**

**Chemicals**

Fura-2 acetomethoxymethyl ester (Fura-2 AM) was purchased from TefLabs (Austin, TX, USA). Ca\(^{2+}\), Mg\(^{2+}\)-free Hank’s Balanced Salt Solution, Neurobasal Medium, B27, DMEM and other chemicals for cell culture were from Invitrogen Inc. (Carlsbad, CA, USA). All other chemicals were commercially available and of reagent grade from Sigma-Aldrich Inc. (St. Louis, MO, USA) unless otherwise indicated.

**Solutions**

The normal bath buffer used for calcium imaging and electrophysiological recording contained (in mM): 145 NaCl, 5 glucose, 10 Na-HEPES, 1 MgCl\(_2\), 5 KCl, and 2.2 CaCl\(_2\), pH 7.3 with NaOH. To record the N-type I\(_{Ca}\) expressed in human embryonic kidney 293 T (HEK293T) cells, NMG buffer (in mM, 140 N-methyl-D-glucamine (NMG), 5 glucose, 10 Na-HEPES, 1 MgCl\(_2\), and 2.2 CaCl\(_2\), pH 7.3 with NaOH) was used as the bath solution. To depolarize the cells, high K\(^+\) buffer (in mM): 150 KCl, 5 glucose, 10 Na-HEPES, 1 MgCl\(_2\), and 2.2 CaCl\(_2\), pH 7.3 with KOH, was used to perfuse the cells locally. The Cs\(^+\)-containing pipette solution for electrophysiological recording contained (in mM): 120 Cs-aspartate, 40 Na-HEPES, 0.1 EGTA, 2 ATP, 0.3 GTP, pH 7.3 with CsOH. 2X BES-buffered saline contained (in mM): 50 N-bis[2-hydroxyethyl]2-aminoethanesulfonic acid (BES), 273 NaCl, and 2.2 NaH\(_2\)PO\(_4\), pH 6.96.

**Isolation and culture of rat E14.5 cortical neuron**

E14.5 embryos were obtained from pregnant Sprague-Dawley rat by Caesarian section complying with the regulations of animal welfare regulation of the National Taiwan University. The forebrain was isolated under dissecting microscope and digested with papain (1 mg/ml in Ca\(^{2+}\), Mg\(^{2+}\)-free HBSS) at 37 °C with gentle shaking for 20 min. The tissue was triturated by 1 ml glass pipette for 20 strokes and centrifuged at 300 \(\times\) g for 1 min. The pellet was resuspended in Ca\(^{2+}\), Mg\(^{2+}\)-free HBSS and adjusted to a density of 10\(^6\) cells/ml. Isolated cells were cultured according to published protocol [16]. To inhibit the growth of glia cells, cytosine arabinoside (1 \(\mu\)g/ml) was added in the culture medium.

**Isolation and culture of bovine chromaffin cells**

Chromaffin cells were prepared by digestion of bovine adrenal gland obtained from local slaugh-