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Research Report

Differential distribution of synGAPα1 and synGAPβ isoforms in rat neurons

Il Soo Moon[a], *Hiroyuki Sakagami*[b], *Jun Nakayama*[c], *Tatsuo Suzuki*[d,*]

[a]*Department of Anatomy, College of Medicine, Dongguk University, Gyeongju 780-714, Republic of Korea*
[b]*Department of Anatomy, Kitasato University School of Medicine, Sagamihara 228-8555, Japan*
[c]*Department of Molecular Pathology, Shinshu University Graduate School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan*
[d]*Department of Neuroplasticity, Shinshu University Graduate School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan*

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ABSTRACT

The synaptic Ras-GTPase activating protein synGAP is a brain-specific protein of approximately 130 kDa and is a negative regulator of Ras. We previously reported 5 C-terminal isoforms of synGAP (α1, α2, β1/2, β3/4 and γ) [Li et al., 2001, J. Biol. Chem. 276: 21417–21424]. In this study, we investigated the expression profiles of the two major isoforms, synGAPα1 and synGAPβ, in the adult rat brain and cultured neurons of the rat hippocampus. Examination of pepsin-pretreated brain sections demonstrated that both isoforms were expressed mainly in the forebrain structures, which suggests their association with postsynaptic density. The distribution of the synGAPα1 and β (β1–4) isoforms in the adult rat brain was clearly different in cerebellum, hippocampus, cerebral cortex, septum and olfactory bulb. In particular, synGAPα1 was specifically localized to the cerebellar glomeruli, dense synaptic sites. From the analysis using cultured neurons, unique expression of synGAPβ was found in a neuron with a sea urchin-like morphology, possibly a star pyramidal neuron, in which the synGAPβ expression was relatively high, in particular, at the distal part of its processes. SynGAPα1 was mostly or specifically localized to excitatory postsynaptic sites, whereas synGAPβ was present at both excitatory and inhibitory postsynaptic sites. Finally, there are more non-synaptic clusters in dendrites in the case of synGAPβ than synGAPα1. Thus, the two synGAP isoforms, α1 and β, distribute differently in neuronal cells and the brain.

1. Introduction

The synaptic Ras-GTPase activating protein synGAP is a brain-specific protein of approximately 130 kDa (Chen et al., 1998; Kim et al., 1998). It is highly concentrated in the postsynaptic density (PSD) fraction prepared from mammalian forebrain. SynGAP is a negative regulator of Ras and its activation is coupled to NMDA receptor activation (Kim et al., 1998; Oh et al., 2004). SynGAP activity is regulated by phosphorylation with Ca^{2+}/calmodulin-dependent protein kinase II (Oh et al., 2004), which is activated by Ca^{2+} influx upon synaptic activation. Phosphorylation of synGAP by Ca^{2+}/calmodulin-dependent protein kinase II increases its RasGAP activity (Oh et al., 2004), which leads to de-activation of Ras and the downstream ERK signaling pathway and activation of P38 MAPK, possibly via an indirect mechanism (Kim et al., 1998;

* *Corresponding author.*
E-mail address: suzukit@shinshu-u.ac.jp (T. Suzuki).
Abbreviations: DIV, days *in vitro*; GAD, glutamic acid decarboxylase; PSD, postsynaptic density; SV2, synaptic vesicle protein 2

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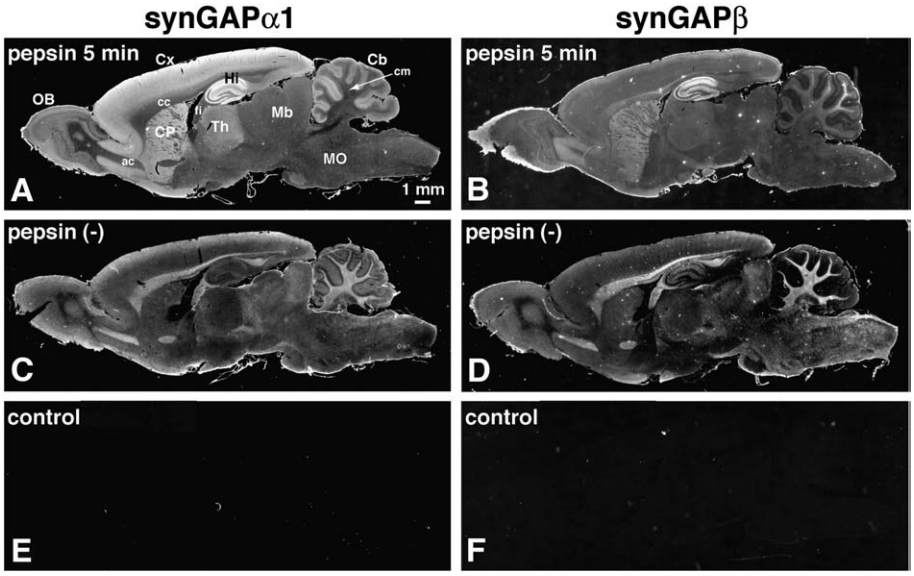

Fig. 1 – Immunohistochemical localization of synGAPα1 and synGAPβ in the adult rat brain and the effects of pepsin pretreatment on the immunostaining. Cryosections were subjected to immunostaining with (A, B) or without (C, D) pepsin pretreatment. Note that pepsin pretreatment enhanced the immunolabeling of both synGAPα1 (A) and synGAPβ (B) in the synaptic fields and attenuated that in the fiber tracts including anterior commissure (ac), corpus callosum (cc), cerebellar medulla (cm) and fimbra of the hippocampus (fi). In the control, no immunolabeling was observed in brain sections pretreated with pepsin when the primary antibody was omitted (E) or anti-synGAPβ antibody was preabsorbed with antigen peptide (F). Cb, cerebellum; Hi, hippocampus; Mb, midbrain; MO, medulla oblongata; OB, olfactory bulb; Th, thalamus.

Rumbaugh et al., 2006). SynGAP is a potent negative regulator of excitatory synaptic transmission and surface expression of the postsynaptic AMPA receptor, through which is regulated the expression of synaptic plasticity such as long-term potentiation, and memory and learning (Kim et al., 2003; Komiyama et al., 2002; Rumbaugh et al., 2006). Studies using mutant mice lacking the synGAP protein suggested a critical role during early postnatal neuronal development by the resulting postnatal lethality (Kim et al., 2003; Porter et al., 2005; Vazquez et al., 2004). SynGAP does not exert any affect on the differentiation of neuronal stem cells into neuronal cells, but does play a role in the regulation of spine and synapse formation (Vazquez et al., 2004) and neurite extension (Tomoda et al., 2004) in the early postnatal period. SynGAP also plays a role in activity-dependent cortical development in the barrel cortex (Barnett et al., 2006) and the survival of neuronal cells (Knuesel et al., 2005).

We previously reported C-terminal isoforms of synGAP at mRNA level: α1, α 2, β1, β2, β3, β4 and γ (Li et al., 2001). Only the α1 isoform has the PDZ domain-binding motif, -TRV, at its C-terminal end, which binds to PSD-95. There are at least seven variants in the 3′ portion of the synGAP mRNA and they encode five C-terminally different protein isoforms: α1, α2, β1/2, β3/4 and γ. All these splice variants are expressed in the brain, although the α2 and γ isoforms appear to be less abundant (Li et al., 2001). In addition, synGAP also has 3 splice variants in the N-terminal portion (a, b and c). Thus, the combination of these isoforms produces a relatively large number of isoforms. In fact, Western blotting of the PSD fraction with anti-synGAP antibodies shows broad immunoreactive bands in the 130-kDa region. Although anatomical data on synGAP are very limited, one paper reported restricted expression of *synGAP* gene in the forebrain and no expression in the cerebellum, thalamus, midbrain, pons, medulla oblongata or brain stem (Porter et al., 2005). Postsynaptic localization (the PSD and dendritic shaft) of synGAP has been shown at the electron microscopic level (Barnett et al., 2006). Tomoda et al. reported the localization of synGAPα2, which lacks the C-terminal PDZ-binding domain, to growth cones, the growing tips of axons (Tomoda et al., 2004). Subcellular distribution of the α1 and β isoforms in the forebrain examined by Western blotting were not substantially different (Li et al., 2001).

In this study, we investigated the expression profiles of the synGAPα1 and synGAPβ isoforms, two major isoforms, in the adult rat brain and the cultured neurons of the rat hippocampus using specific antibodies. We describe differential expression profiles of these two synGAP isoforms in neuronal cells.

2. Results

2.1. *Adult rat brain expression of synGAP*

Previously, we developed antibodies specific to synGAPβ, which recognizes the β1, β2, β3 and β4 isoforms. Using this antibody and a commercially available anti-synGAPα1 antibody, we investigated the expression profiles of these synGAP isoforms in the rat brain and hippocampal neurons in culture. Anti-synGAPα1 and anti-synGAPβ antibodies were produced using C-terminal peptides specific to the two isoforms, respectively. The specificity of these antibodies was verified