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BRAIN RESEARCH

Research Report

A-kinase anchoring protein 150 in the mouse brain is concentrated in areas involved in learning and memory

Anghelus Ostroveanu, Eddy A. Van der Zee, Amalia M. Dolga, Paul G.M. Luiten, Ulrich L.M. Eisel, Ingrid M. Nijholt*

Department of Molecular Neurobiology, Graduate School of Behavioral and Cognitive Neurosciences, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

ARTICLE INFO

Article history:
Accepted 29 January 2007
Available online 2 February 2007

Keywords:
AKAP150
Localization
Immunohistochemistry
cAMP-dependent protein kinase

ABSTRACT

A-kinase anchoring proteins (AKAPs) form large macromolecular signaling complexes that specifically target cAMP-dependent protein kinase (PKA) to unique subcellular compartments and thus, provide high specificity to PKA signaling. For example, the AKAP79/150 family tethers PKA, PKC and PP2B to neuronal membranes and postsynaptic densities and plays an important role in synaptic function. Several studies suggested that AKAP79/150 anchored PKA contributes to mechanisms associated with synaptic plasticity and memory processes, but the precise role of AKAPs in these processes is still unknown. In this study we established the mouse brain distribution of AKAP150 using two well-characterized AKAP150 antibodies. Using Western blotting and immunohistochemistry we showed that AKAP150 is widely distributed throughout the mouse brain. The highest AKAP150 expression levels were observed in striatum, cerebral cortex and several other forebrain regions (e.g. olfactory tubercle), relatively high expression was found in hippocampus and olfactory bulb and low/no expression in cerebellum, hypothalamus, thalamus and brain stem. Although there were some minor differences in mouse AKAP150 brain distribution compared to the distribution in rat brain, our data suggested that rodents have a characteristic AKAP150 brain distribution pattern. In general we observed that AKAP150 is strongly expressed in mouse brain regions involved in learning and memory. These data support its suggested role in synaptic plasticity and memory processes.

1. Introduction

cAMP-dependent protein kinase (PKA) is involved in several intracellular signaling cascades and it regulates multiple cellular functions (Scott, 1991; Skalhegg and Tasken, 2000). A potential mechanism to explain how such a multifunctional and broad substrate kinase mediates precise signaling events, is colocalization of its substrate to specific subcellular compartments. Compartmentalization arises in part from the association of the enzyme with so-called A-kinase anchoring proteins (AKAPs) (Glantz et al., 1993; Lohmann et al., 1984). AKAPs represent a group of more than 70 identified function-

* *Corresponding author*. Fax: +31 50 3632331.
E-mail address: a.ostroveanu@rug.nl (A. Ostroveanu).

Abbreviations: AKAP, A-kinase anchoring protein; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; PP2B/CaN, protein phosphatase 2B/calcineurin; LTD, long-term depression; LTP, long-term potentiation; NMDA, *N*-methyl-D-aspartate; cAMP, cyclic adenosine monophosphate; PKA, cAMP-dependent protein kinase; PSD, postsynaptic density; IR, immunoreactivity; PFA, paraformaldehyde; PB, phosphate buffer; PBS, phosphate-buffered saline

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doi:10.1016/j.brainres.2007.01.117

ally related proteins (Wong and Scott, 2004). Although they share little primary structure similarities, they all have the ability to bind PKA, and therefore to regulate specific cAMP signaling pathways by sequestering PKA to a specific subcellular location. This compartmentalization of individual AKAP–PKA complexes occurs through unique targeting domains that are present on each anchoring protein.

To date, AKAPs have been identified in a wide range of species, tissues and cellular compartments (Angelo and Rubin, 2000; Jackson and Berg, 2002; Sarkar et al., 1984; Wong and Scott, 2004). In the mammalian brain, several AKAPs have been characterized. One of these AKAPs is AKAP79/150. This family of proteins consists of three orthologues: bovine AKAP75, murine AKAP150 and human AKAP79. Initially AKAP75 was identified as a contaminant of PKA regulatory subunit II (RII) purified cytosolic brain preparations (Bregman et al., 1991; Sarkar et al., 1984). In addition, Bregman and colleagues retrieved AKAP150 by screening a rat cDNA library using radiolabeled RIIβ as functional probe (Bregman et al., 1989). Finally AKAP79 was identified as a constituent of postsynaptic densities (PSD) in human cerebral cortex (Carr et al., 1992).

Interestingly, AKAPs function as multi-assembly scaffold molecules interacting with other signaling enzymes. AKAP79/150 has the ability to bind protein phosphatase 2B/calcineurin (PP2B/CaN) (Dell'Acqua et al., 2002) and protein kinase C (PKC) (Faux et al., 1999) besides PKA. By tethering both kinases and phosphatases AKAP79/150 provides a unique platform for integrating opposite signaling events to the same subcellular site.

It has been suggested that in excitatory synapses at the PSD, AKAP79/150 targets its anchored proteins and forms a multi protein complex with AMPA and NMDA receptors (AMPAR and NMDAR), synaptic adhesion molecules, and cytoskeleton proteins. These proteins play an important role in synaptic function (Colledge et al., 2000; Kennedy, 1997; Malenka and Bear, 2004; Yamauchi, 2002; Ziff, 1997).

The first evidence that anchoring of PKA is crucial for the regulation of synaptic function was reported by Rosenmund et al. (1994). In their study, blocking the PKA anchoring to AKAPs prevented the PKA-mediated regulation of AMPA/kainate currents in cultured hippocampal neurons. Moreover, recent findings strongly indicate that anchored PKA is crucial for maintaining AMPA currents during glutamate stimulation (Hoshi et al., 2005). Interestingly, disruption of AKAP–PKA anchoring leads to CaN-dependent, long-term depression (LTD)-like down-regulation of AMPAR currents, implicating an important role for AKAP79/150 in AMPAR regulation (Tavalin et al., 2002). In general, the AKAP79/150 scaffold molecule has emerged as an important element in regulating AMPAR phosphorylation in long-term potentiation (LTP) and LTD at the PSD (Dell'Acqua et al., 2006; Genin et al., 2003; Snyder et al., 2005). Since strengthening or weakening of synaptic transmission is widely considered to be the cellular mechanism that underlies learning and memory, a role of AKAP79/150 in learning and memory can be expected.

To date, only a few immunohistochemical localization studies illustrate the distribution of AKAP79/150 in different brain compartments in various species. In human brain high levels of AKAP79/150 were reported at the PSD of the forebrain (Carr et al., 1992). A more detailed analysis of AKAP150 protein distribution in the rat brain showed that AKAP150 is widely distributed throughout the brain and is expressed in many classes of neurons that constitute the rat CNS (Glantz et al., 1992). A more recent study focused on the distribution of AKAP150 at rat CA1 pyramidal cell asymmetric and symmetric PSD and its colocalization with several markers of excitatory and inhibitory receptors. In this study, the interaction of AKAP150 with components of the excitatory PSD was confirmed, whereas AKAP150 immunoreactivity (IR) was not associated with inhibitory synapses (Lilly et al., 2005).

The distribution of AKAP150 protein in the mouse brain has not been established yet. To elucidate the specific role of AKAP150 in learning and memory processes it may be important to use genetically modified mice in future research. Therefore, besides characterizing AKAP150 expression throughout the whole mouse brain, we specifically focused on AKAP150 expression in areas known to be involved in learning and memory processes. We established the distribution of AKAP150 protein in the mouse brain using immunohistochemistry and Western blot techniques.

2. Results

2.1. *Western blot analysis of AKAP150 expression levels in various brain compartments*

Western blot analysis with two AKAP150 antibodies (a N-terminal or C-terminal antibody) in protein extracts from different mouse brain regions revealed the highest expression level of AKAP150 protein in cortex and striatum (Fig. 1). High expression levels were also detected in the hippocampus and olfactory bulb, while the cerebellum and the hypothalamus revealed low levels of AKAP150 expression. The lowest expression level of AKAP150 was found in the brain stem (Fig. 1). The results of the Western blot expression levels corresponded with the distribution pattern of this protein in the immunohistochemical analysis of the mouse brain (Fig. 2; Tables 1 and 2).

2.2. *General overview of AKAP150 IR in mouse brain*

AKAP150 IR was widely distributed throughout the brain. Highest IR was found in striatum and olfactory tubercle, but in cortex, hippocampus and amygdala AKAP150 expression was also very abundant (Fig. 2). In several brain regions, AKAP150 IR was limited to specific cell layers (e.g. the Purkinje cell layer of the cerebellum) or nuclei (e.g. reticular thalamic nucleus, ethmoid nucleus) (Table 1). Some brain regions did not show any IR for AKAP150 protein (e.g. numerous nuclei in midbrain and hindbrain) (Fig. 2; Table 1).

2.3. *Detailed description of AKAP150 expression in mouse brain*

2.3.1. *Olfactory system*

AKAP150 IR varied from moderate to relatively high in the olfactory bulb and its various layers. Superficial layers (glomer-