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Research Report

Glycoprotein M6a is present in glutamatergic axons in adult rat forebrain and cerebellum

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ABSTRACT

Glycoprotein M6a is a neuronally expressed member of the proteolipid protein (PLP) family of tetraspans. *In vitro* studies suggested a potential role in neurite outgrowth and spine formation and previous investigations have identified M6a as a stress-regulated gene. To investigate whether the distribution of M6a correlates with neuronal structures susceptible to alterations in response to stress, we localized M6a expression in neurons of hippocampal formation, prefrontal cortex and cerebellum using *in situ* hybridization and confocal immunofluorescence microscopy. *In situ* hybridization confirmed that M6a is expressed in dentate gyrus and cerebellar granule neurons and in hippocampal and cortical pyramidal neurons. Confocal microscopy localized M6a immunoreactivity to distinct sites within axonal membranes, but not in dendrites or neuronal somata. Moreover, M6a colocalized with synaptic markers of glutamatergic, but not GABAergic nerve terminals. M6a expression in the adult brain is particularly strong in unmyelinated axonal fibers, i.e. cerebellar parallel and hippocampal mossy fibers. In contrast, myelinated axons exhibit only minimal M6a immunoreactivity localized exclusively to terminal regions. The present neuroanatomical data demonstrate that M6a is an axonal component of glutamatergic neurons and that it is localized to distinct sites of the axonal plasma membrane of pyramidal and granule cells.

1. Introduction

M6a initially attracted attention within the field of stress research because in the adult hippocampal formation of several species, M6a mRNA was found to be reduced in response to chronic stress, whereas antidepressant treatment prevented this effect (Alfonso et al., 2004, 2006). M6a (genetic symbol *Gpm6a*) was cloned as the antigen of the monoclonal M6 antibody (Yan et al., 1993). Although the precise function of M6a remains unclear, there is increasing evidence that it is involved in processes driving plastic changes in neuronal morphology. Using the antibody 2A1, glycoprotein M6a was identified as edge membrane antigen (EMA) which is specifically transported to the leading edge of neuronal growth cones (Sheetz et al., 1990; Baumrind et al., 1992). Neurite formation was found to be severely impaired in cultured cerebellar neurons treated with M6 antibody (Lagenaur et al., 1992). Moreover, overexpression of M6a in cultured primary hippocampal neurons promotes neurite outgrowth and the formation of filopodial protrusions (Alfonso et al., 2005). In contrast, targeted depletion of endogenous

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M6a expression with small inhibitory RNA (siRNA) severely attenuated neurite outgrowth and impaired synapse formation as visualized by a reduction in the number of synaptophysin-positive clusters (Alfonso et al., 2005). During brain development M6a exhibits a temporal pattern of expression coinciding with specific periods of neuronal differentiation and neurite outgrowth (Lund et al., 1986; Yan et al., 1996; Mi et al., 1998).

Sequence analysis has revealed that M6a is a member of the proteolipid protein (PLP) family of hydrophobic tetraspan proteins (Yan et al., 1993; Kitagawa et al., 1993). In mammals, this protein family consists of three members: PLP, M6a, and M6b. PLP (genetic symbol *Plp1*) is expressed by oligodendrocytes and represents the major protein component of myelin in the central nervous system (CNS) (Milner et al., 1985). Proteolipid M6b (genetic symbol *Gpm6b*) was detected in both neuronal and glial populations (Yan et al., 1996; Werner et al., 2001), whereas M6a expression in the mammalian CNS is restricted to neurons (Lagenaur et al., 1992). All three proteolipids are abundant in the CNS but were also found in non-neuronal cell types, and their orthologs are also present in non-mammalian species (Kitagawa et al., 1993; Yoshida et al., 1999; Schweitzer et al., 2006).

Remodeling of pyramidal neuron dendrites in response to stress has been observed within the hippocampal CA3 subfield

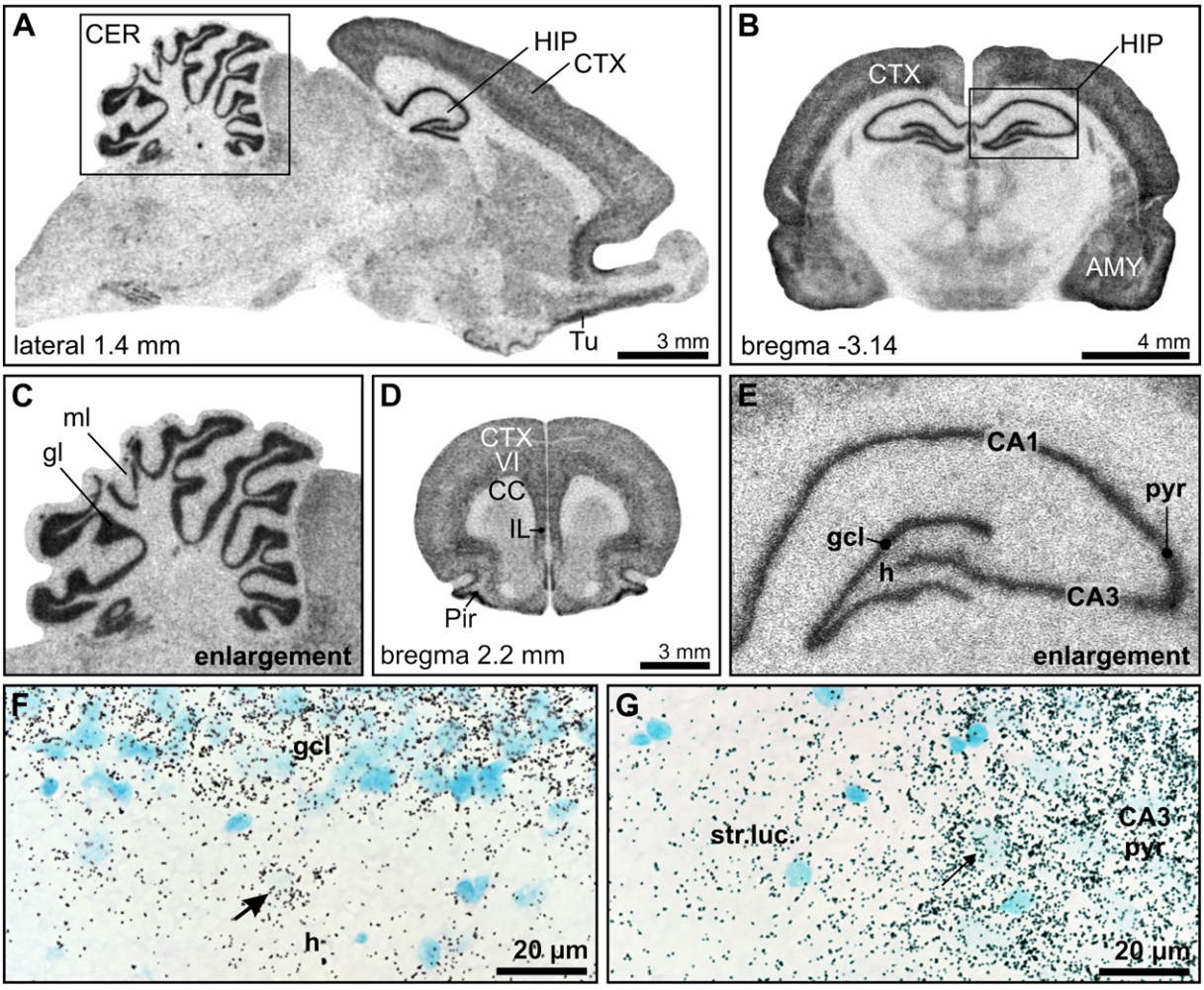

Fig. 1 – M6a mRNA expression in the rat brain. (A) Strong hybridization signals were detected by *in situ* hybridization with a ^{33}P-dUTP-labeled riboprobe in cerebellum (CER), hippocampus (HIP), different cortical regions (CTX) and olfactory tubercle (Tu) (sagittal section). (B) Note the strong hybridization signals in the hippocampal formation (HIP), throughout cortical regions (CTX) and in the amygdala formation (AMY) (coronal section). (C) Cerebellum (enlargement of box shown in A): Note the strong hybridization signal in the granule cell layer (gl). The molecular layer (ml) is unlabeled. (D) In the prefrontal cortex, M6a expression is slightly enriched in cortical layer VI (VI), in the medial prefrontal cortex including the infralimbic cortex (IL) and in the piriform cortex (Pir) (coronal section). Corpus callosum, CC. (E) Hippocampal formation (enlargement of box shown in B): Note the strong hybridization signal over pyramidal neuron layer (pyr) in CA1–CA3 and in the dentate gyrus with the granule cell layer (gcl). (F) Emulsion autoradiography showing a dense pattern of silver grains representing M6a mRNA in granule cells (gcl) in the dentate gyrus. Cell nuclei were counterstained with toluidine blue. Arrow denotes labeled neuron in the hilus (h), possibly a mossy cell. (G) Emulsion autoradiography showing a dense pattern of silver grains representing M6a mRNA in CA3 pyramidal neurons (pyr). Cell nuclei were counterstained with toluidine blue. Arrow indicates a strongly labeled pyramidal neuron with silver grains surrounding the (lightly stained) nucleus. Str.luc, stratum lucidum.