IDENTIFICATION OF NOVEL cDNAs EXPRESSED IN MURINE CEREBELLUM

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ABSTRACT

This investigation was designed to identify novel genes expressed in the cerebellar granule cells (GC) that would aid the exploration of the molecular characteristics of GC. One dimensional (1D) and 2D-SDS PAGE (polyacrylamide gel electrophoresis) comparisons of cerebellar proteins from wild-type (+/+) and granuloprival weaver (*wv/wv*) mutant mice failed to identify cerebellar proteins expressed specifically in GC. An antiserum was raised against proteins of isolated cerebellar GC. In western blot analyses, the antiserum cross-reacted with several proteins one of which (26 kDa) is missing from granuloprival adult weaver (*wv/wv*) mutant cerebella. Light- and electron-microscopic immunocytochemistry of +/+ cerebellum showed immunopositivity in the cytoplasmic compartment of GC and in neuronal processes of the GC layer neuropil. In the molecular layer immunostaining is probably of the granule cell axons. The antiserum was used to screen a cDNA expression library derived from postnatal day one (P1) *wv*/+ cerebella. Among 750,000 clones, 22 were immunopositive. The 5' end of these latter were sequenced. Search of GenBank entries for 100-200 nucleotides of the "end"-sequences showed that 14 clones represent novel cDNAs. Southern blot analyses of mouse genomic DNA confirmed the existence of the novel genes in the murine genome. Nine of the clones showed hybridization *in situ* in the GC layer of +/+ cerebellum. Some of them also hybridized to hippocampal and cortical neurons. Clone GCAP-8 (insert size 1.1 kb) was sequenced. It is a partial cDNA that represents a single-copy gene with multiple RNA transcripts in mouse brain. The GCAP-8 gene was mapped to the distal region of mouse Chromosome 5 by analyses of two multilocus crosses. Its cellular localization was studied by *in situ* hybridization during normal ontogeny and in adult +/+ and cerebellar mutant mice including *wv/wv*, "Purkinje cell (PC) degeneration" (*pcd/pcd*), and reeler (*rl/rl*). In mouse brain GCAP-8 (present in embryonic CNS at least as early as gestational day 14) is expressed in cerebellar GC, PC, and deep nuclei, hippocampus, substantia nigra, striatum and olfactory bulb. In the mutants, GCAP-8 expression correlates with their respective anatomical deficits. In human cerebellum GCAP-8 is expressed in the GC layer. These novel cDNAs may serve as molecular markers for study of GC intrinsic neuronal properties, their role in development, and function within the cerebellum.

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