Available online at www.sciencedirect.com
SCIENCE DIRECT
Neuroscience Research 53 (2005) 376–383
Neuroscience Research
www.elsevier.com/locate/neures

Spatial diversity in gene expression for VDCCγ subunit family in developing and adult mouse brains

Masahiro Fukaya [a,*], Maya Yamazaki [b], Kenji Sakimura [b], Masahiko Watanabe [a]

[a] Department of Anatomy, Hokkaido University Graduate School of Medicine, Sapporo 060-8638, Japan
[b] Department of Cellular Neurobiology, Brain Research Institute, Niigata University, Niigata 951-8122, Japan

Received 17 June 2005; accepted 22 August 2005
Available online 19 September 2005

Abstract

The γ subunit of voltage-dependent Ca^{2+} channels (VDCCs) is characterized by molecular diversity and regulation of AMPA-type glutamate receptors as well as VDCCs. In the present study, we examined expressions for the VDCCγ1–8 subunit mRNAs in developing and adult mouse brains by in situ hybridization. In adult brains, the γ2 and γ7 subunit mRNAs were widely expressed in various grey matter regions with the highest level in cerebellar Purkinje cells and granule cells. The γ3 and γ8 subunit mRNAs predominated in the telencephalon, with the latter being at striking levels in the hippocampus. The γ4 subunit mRNA was enriched in the olfactory bulb, striatum, thalamus and hypothalamus. The γ5 subunit mRNA was abundant in the olfactory bulb, hippocampal CA2, thalamus, inferior colliculus and Bergmann glia. Transcripts of these subunits were detected in embryonic brains: some showed well-preserved spatial patterns (γ2, γ5, γ7 and γ8), while others underwent developmental up- (γ3) or down-regulation (γ4). In contrast, the γ1 and γ6 subunit mRNAs were negative or very low throughout brain development. Therefore, the present study has revealed spatial diversity in gene expression for individual VDCCγ subunits, presumably reflecting functional diversity of this protein family and their differential involvement in neural function.

Keywords: VDCCγ subunit; Stargazin; TARPs; Development; mRNA; In situ hybridization; Brain

1. Introduction

The γ subunit of voltage-dependent Ca^{2+} channels (VDCCs) has been originally identified as an auxiliary subunit of 1,4-dihydropyridine (DHP)-sensitive or L-type VDCCs in skeletal muscles (Bosse et al., 1990; Jay et al., 1990; Powers et al., 1993). The second subunit, γ2 subunit or stargazin, was identified as a gene responsible for the spontaneous mutant mouse, *stargazer*, which is characterized by absence epilepsy and ataxia (Letts et al., 1998). Subsequent studies have revealed six additional subunits and, hence, there are eight members to date in the VDCCγ family (Black and Lennon, 1999; Burgess et al., 1999, 2001; Klugbauer et al., 2000; Chu et al., 2001; Moss et al., 2002). Each γ subunit contains four putative transmembrane domains with intracellularly located N- and C-termini (Chu et al., 2001; Black, 2003). The γ1, γ2, γ3, γ4, γ5 and γ7 subunits have been shown to affect the function of L-type, T-type and P/Q-type VDCCs, when expressed in various combinations (Wei et al., 1991; Eberst et al., 1997; Letts et al., 1998; Klugbauer et al., 2000; Freise et al., 2000; Rousset et al., 2001; Green et al., 2001; Moss et al., 2002; Held et al., 2002). On the other hand, the γ2 subunit and its structurally-related members, γ3, γ4 and γ8 subunits, are crucial for cell surface expression, synaptic targeting, recycling, channel activity and gating of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, and hence named as transmembrane AMPA receptor regulatory proteins (TARPs) (Chen et al., 2000; Tomita et al., 2003, 2004, 2005; Yamazaki et al., 2004; Priel et al., 2005). The γ subunit family is expressed in various tissues, including the brain, skeletal muscle, heart, lung and testis (Chu et al., 2001). In the brain, all γ subunits, except the γ1 subunit, are expressed (Chu et al., 2001). Of these, four TARPs have been reported to display high and distinct expressions in the developing and adult brains (Klugbauer et al., 2000; Chen et al., 2000; Tomita et al., 2003). However,

* Corresponding author. Tel.: +81 11 706 5030; fax: +81 11 706 5031.
E-mail address: mfukaya@med.hokukdai.ac.jp (M. Fukaya).

0168-0102/$ – see front matter
doi:10.1016/j.neures.2005.08.009

comparative and systematic analysis on the expression of the VDCCγ family has not yet been performed. In the present study, we examined expressions for the VDCCγ1-8 subunit mRNAs in developing and adult mouse brains by in situ hybridization with [^{33}P]dATP-labeled antisense oligonucleotide probes, and have revealed their distinct regional and cellular expression in the brain.

2. Materials and methods

2.1. Probes

To detect mRNAs for each VDCCγ subunit, specific antisense oligonucleotide probes were synthesized as follows: 5′-gtgctctggctcagcgtccatgcaggattccccaggggttctgagg-3′ for the γ1 subunit (GenBank accession No. AJ006306), 5′-gatgcgggtgatggcggaggcctggaggtagtcggtggcgcgggc-3′ for the γ2 subunit (accession No. AF077739), 5′-cggcaggcgcgcaaatgtagacttcttcaggagctctgaatggga-3′ for the γ3 subunit (accession No. AJ272044), 5′-ggcgtaaggagaggaggaagaggcctaaggaactcccgcttggt-3′ for the γ4 subunit (accession No. AJ272045), 5′-catctggtcatagtctgggcacttgagcaaagctgggtagttgct-3′ for the γ5 subunit (accession No. AF361347), 5′-ttgggccaccccacttggggcacagtgacctccagggccaggaag-3′ for the γ6 subunit (accession No. AF361348), 5′-gcgatagtgaaagtactgctcagagctgctgggcctgttcat-3′ for the γ7 subunit (accession No. AF361349), and 5′-acaccacaaaccctctcttcattccagcgtttcaatgactccag-3′ for the γ8 subunit (accession No. AF361350). Oligonucleotide probes were labeled with [^{33}P]dATP using terminal deoxyribonucleotidyl transferase (Invitrogen, Carlsbad, CA).

2.2. In situ hybridization

Under deep pentobarbital anesthesia, the brains were freshly obtained from C57BL/6J mice at embryonic days 13 (E13), E18, postnatal days 1 (P1), P7, P14, P21 and adult (4 months). The day after overnight mating was designated as E0, and the day of birth as P1. Fresh frozen sections (20 μm thickness) were cut with a cryostat (CM1900, Leica, Nussloch, Germany) and mounted on glass slides precoated with 3-aminopropyltriethoxysilane. Probe labeling and hybridization were performed as described (Fukaya et al., 2005) with minor modifications. Sections were treated at room temperature with the following incubation steps: fixation with 4% paraformaldehyde–0.1 M sodium phosphate buffer (pH 7.2) for 10 min, 2 mg/ml glycine–phosphate-buffered saline (pH 7.2) for 10 min, acetylation with 0.25% acetic anhydride in 0.1 M triethanolamine–HCl (pH 8.0) for 10 min and prehybridization for 1 h in a buffer containing 50% formamide, 50 mM Tris–HCl (pH 7.5), 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin, 0.6 M NaCl, 0.25% SDS, 200 μg/ml tRNA, 1 mM EDTA and 10% dextran sulfate. Hybridization was performed at 42 °C for 12 h in the prehybridization buffer supplemented with 10,000 cpm/μl of [^{33}P]dATP-labeled oligonucleotides. Slides were washed twice at 55 °C for 40 min in 0.1× SSC containing 0.1% sarcosyl. Sections were exposed either to BioMax (Kodak, Rochester, NY) or to Nuclear Track emulsion (NTB-2, Kodak)

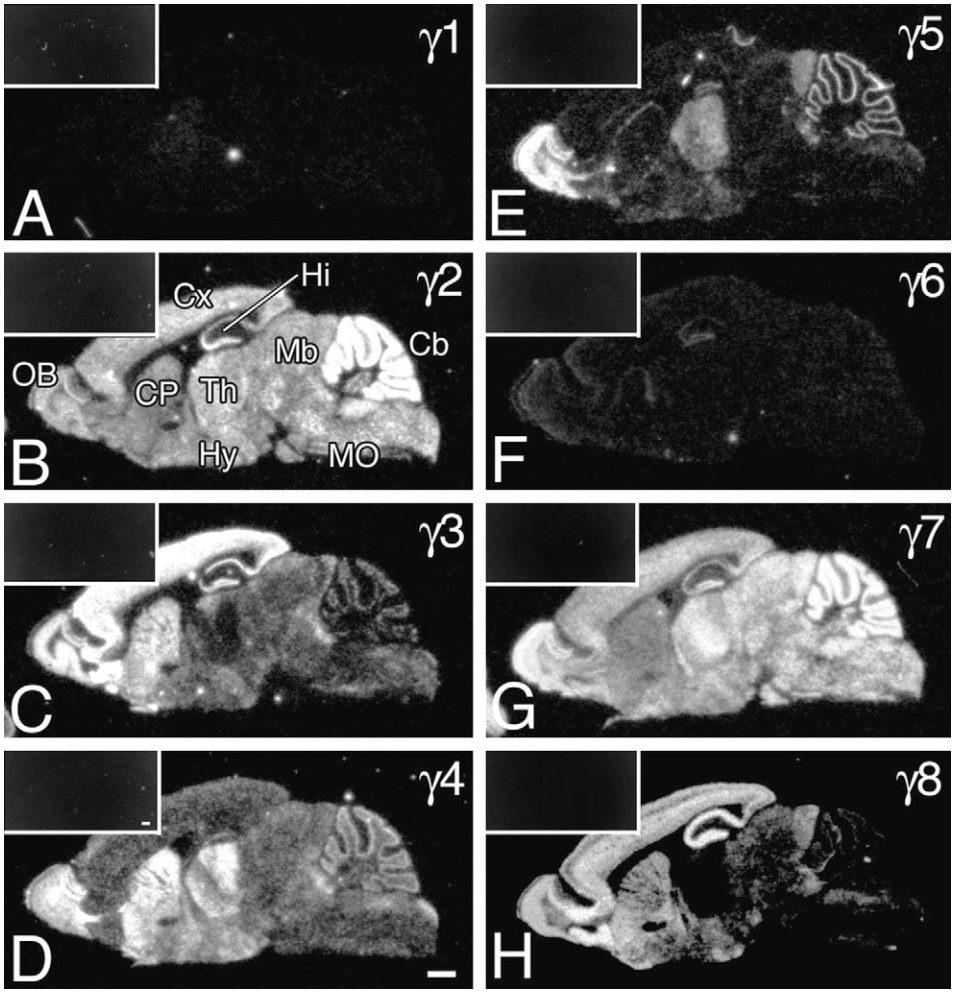

Fig. 1. Distribution of VDCCγ1 (A), γ2 (B), γ3 (C), γ4 (D), γ5 (E), γ6 (F), γ7 (G) and γ8 (H) subunit mRNAs in the adult mouse brain. Images were made from parasagittal brain sections exposed to an X-ray film. Insets show negative hybridizing signals by adding unlabeled probes. Cb, cerebellum; CP, caudate-putamen; Cx, cerebral cortex; Hi, hippocampus; Hy, hypothalamus; Mb, midbrain; MO, medulla oblongata; OB, olfactory bulb; Th, thalamus. Scale bars, 1 mm.