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Immunohistochemistry of Voltage-Gated Calcium Channel α_{1B} Subunit in Mouse Cerebellum

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Abstract

Secretion of neurotransmitters is initiated by voltage-gated calcium influx through presynaptic, voltage-gated N-type calcium channels. However, little is known about their cellular distribution in the mouse cerebellum. In the cerebellum, α_{1B} immunoreactivity is found mainly on the cell bodies of all Purkinje cells. In addition, the immunoreactivity was detected on a subset of Purkinje cell dendrites, clustered to form a parasagittal array of bands. In the anterior lobe vermis, immunoreactive Purkinje cell dendrites form narrow stripes separated by broad bands of unstained dendrites. Moving caudally through the vermis, these stripes become thicker as a larger fraction of the Purkinje cell dendrites become immunoreactive. This localization study of the α_{1B} pore-forming subunits in mouse cerebellum may guide future investigations of the role of calcium channels in neurological pathways.

Key words : N-type calcium channels, Purkinje cell, Cerebellum

Introduction

Calcium ions play a major role in many different physiological functions including synaptic transmitter release and membrane excitability [2], neurite outgrowth and plasticity [5], gene expression patterns [7]. The main route of extracellular Ca^{2+} entry into neurons is through voltage-gated calcium channels (VGCCs) that are multi subunit complexes composed of a pore-forming/voltage-sensing α_1 subunit and several regulatory subunits, including α_2, β, δ, and γ [9]. On the basis of pharmacological and physiological properties, at least six distinct types of VGCCs have been identified, designated as L, N, P, Q, R, and T [15]. Among these classes of calcium channels, the N-type channel has been shown to play a significant role in regulation of neurotransmitter release [8, 14]. For example, dopamine release from nerve terminals in the striatum is regulated by N-type calcium channels [1]. The gene encoding the N-type α_{1B} subunit has been identified, and several different isoforms of the N-type α_{1B} subunit have been cloned from rat and rabbit brain tissue, and mouse and human cell lines [6, 10, 12]. Recently, it has been suggested that in addition to allowing Ca^{2+} influx to trigger exocytosis, N-type calcium channels regulate neurotransmitter release by specifically binding to presynaptic proteins such as syntaxin 1A, SNAP25, and synaptotagmin, that are tightly coupled with the release process [13, 16]. This interaction provides an additional mechanism by which neurons fine-tune neurotransmitter release. The VGCCs are thought to perform different functions in different parts of the neuron. To understand the functions of Ca^{2+} channels in specific parts of the neuron and the differences between the subtypes, it is essential to have an overall view of their subcellular distribution.

In the present study, the spatial distributions of channel proteins for N-type VGCCs have been revealed in the adult mouse cerebellum by using immunohistochemistry.

Materials and Methods

Adult (90-day-old) normal BALB/C mice were obtained from the Korea Research Institute of Bioscience and Biotechnology. All experimental procedures were carried out

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in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals. All animals were deeply anaesthetized with sodium pentobarbital (60 mg/kg body weight) and transcardially perfused with 0.9% NaCl in 0.1M phosphate buffer (PB, pH 7.4) followed by 100ml of 4%-paraformaldehyde in 0.1M PB (pH 7.4). The brains were then placed immediately in the same fixative at 4℃ for 24 hours. The post-fixed brains were transferred to 0.1 M PB, and after the brains were cryoprotected in 10%, 20% and 30% sucrose in 0.1M PB, they were cryostat sectioned in the frontal plane (40m). Rabbit anti-rat α_{1B} subunit of VGCC antibodies (Alomone Labs, Jerusalem, Israel, product No. ACC-002) were used diluted 1:25 in blocking solution (0.1M PB, pH 7.4, plus 10% normal goat serum, 0.3% Triton X-100). After several rinses in 0.1M phosphate-buffered saline (PBS, pH 7.4), the sections were quenched for 10 minutes in 1% H_2O_2, and rinsed in 0.1M Tris phosphate-buffered saline solution (TPBS; 8.5 mM $Na_2HPO_4 \cdot 7H_2O$, 3 mM KH_2PO_4, 125 mM NaCl, 30 mM Tris-HCl, 0.03 mM NaN_3, pH 7.7). Sections were incubated overnight at room temperature in primary antibodies. They were then washed three times for five minutes in 0.1 M TPBS, and incubated in 1:100 peroxidase-conjugated anti- rabbit IgG (Dakopatts Inc., Mississauga, Canada) for two hours at room temperature. After three additional rinses in TPBS, antibody-binding sites were revealed by a 15-minute incubation in 0.2% diaminobenzidine in TPBS. Sections were then dehydrated through graded alcohols and mounted in DPX.

Results

The present study demonstrated that the α_{1B}-immunoreactivity occurred in a distinct pattern in the mature mouse cerebellum. In the cerebellum, α_{1B}-immunoreactivity is found mainly on Purkinje cells. Reaction product was deposited on the cell bodies of Purkinje cells (Fig. 1A, B). These characteristic distributions were noted throughout the whole cerebellum including anterior vermis, posterior vermis and hemispheres. However, α_{1B}-immunoreactivity in Purkinje cell dendrites reveals a different pattern. Immunoreactivity is associated predominantly with Purkinje cell dendrites, and is relatively dense along the entire length. The immunoreactive Purkinje cell dendrites are clustered to form a parasagittal array of bands that is generally reproducible between individuals and symmetrical about the midline (Fig. 2). In the anterior lobe vermis, immunoreactive Purkinje cell dendrites form narrow stripes separated by broad bands of unstained dendrites (Fig. 2). Moving caudally through the vermis, these stripes become thicker as a larger fraction of the Purkinje cell dendrites become immunoreactive (Fig. 3). Similarly, alternating stripes of α_{1B}-immunoreactivity are revealed in the hemispheres (Fig. 4). At higher magnification, a punctuate-like pattern of staining was apparent along the cell bodies and dendrites of Purkinje cells. These punctuate structures may be either spines or synaptic terminals.

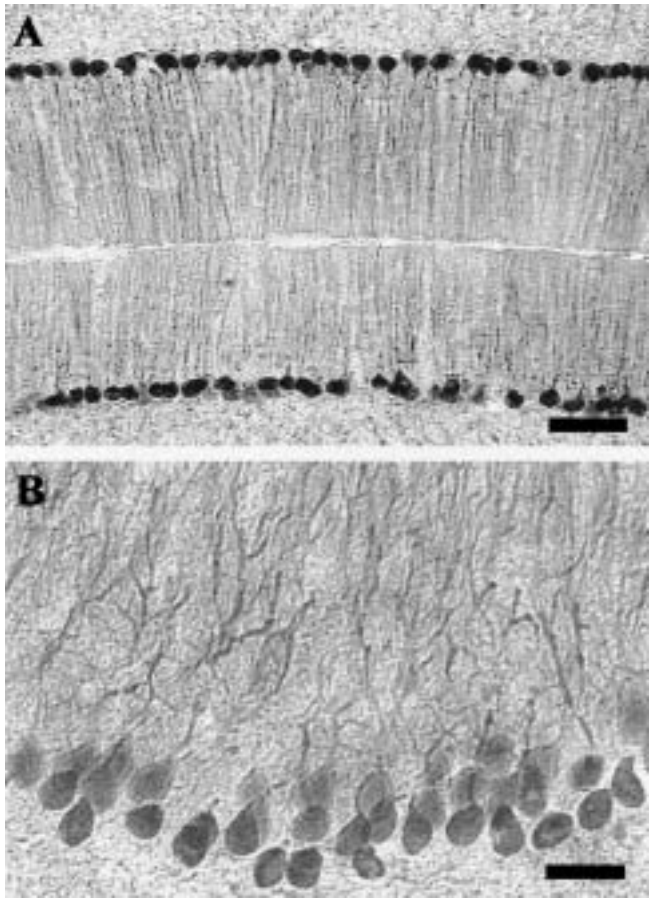

Fig. 1. Anti-α_{1B} subunit of VGCC immunoperoxidase-stained, 40-μm frontal section (A) and sagittal section (B) through the vermis of the mouse cerebellum. Strong immunoreactive Purkinje cell bodies and their dendrites were observed. Scale bar = 80μm (A) and 40μm (B)

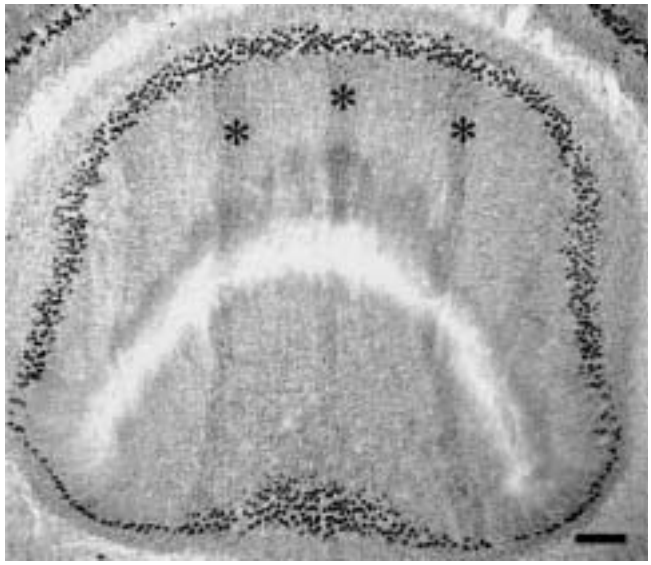

Fig. 2. Anti-α_{1B} subunit of VGCC immunoperoxidase-stained, 40-μm frontal sections through the vermal lobule III

of the mouse cerebellum. Note that the Anti-α_{1B} subunit of VGCC immunoreactive parasagittal three bands (*). Scale bar = 200μm

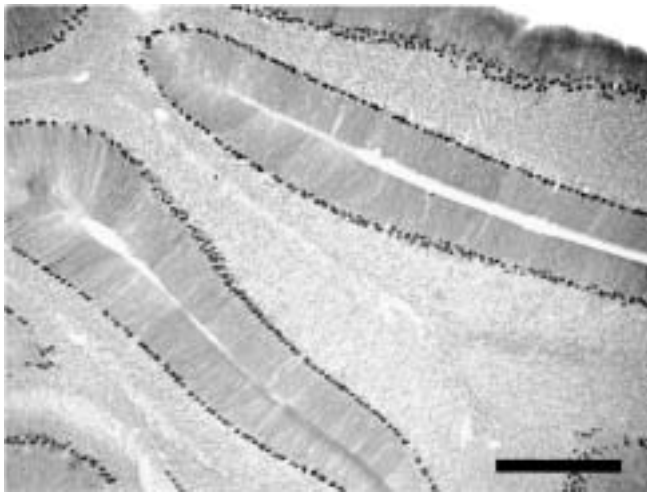

Fig. 3. Photomicrographs of cerebellar hemisphere immunoperoxidase-stained by using anti-α_{1B} subunit of VGCC immunocytochemistry (40μm frontal sections). Note that the band-like structures of molecular layer. Scale bar = 250μm

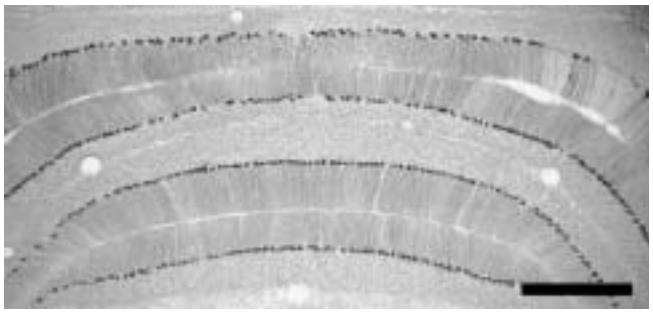

Fig. 4. Anti-α_{1B} subunit of VGCC immunoperoxidase-stained, 40-μm frontal sections through the vermal lobule X, IX and VIII of the mouse cerebellum. Note that the Anti-α_{1B} subunit of VGCC immunoreactive thick band structures. Scale bar = 250μm

Discussion

The cerebellum is one of the few sites in the central nervous system where the pattern of intrinsic connections is known in considerable detail. This knowledge has been the catalyst for many of the models and theories of cerebellar function. We describe here the preparation of antibodies for α_{1B} and their application in comprehensive localization of the normal mouse cerebellum. The detailed analysis of á subunit expression in serial sections of Purkinje cells provides important new data on the intracellular distribution of VGCC.

The present study shows that the α_{1B} subunit is found in the soma and dendrites of Purkinje cells. This localization of α_{1B} subunits was different from that described in protein or mRNA studies in the rat [17, 18]. However, Westenbrok et al, [4] demonstrated that the cell bodies of Purkinje cells were labeled at a low level and they also showed the α_{1B}-immunoreactivity was localized in areas with dense synaptic formation which was in agreement with the results that α_{1B} subunit was localized predominantly in dendritic shafts and presynaptic terminals [4]. Thus, although there are some arguments, our results show a good correlation with the distribution observed in the rat cerebellum. In addition, in the present study, α_{1B}-immunoreactive Purkinje cell dendrites are clustered to form a parasagittal array of stripes separated by bands of unstained dendrites. There are numerous previous reports of molecules expressed in stripes in the cerebellum. However, this report is first time for α_{1B} subunit and its related with the Purkinje cell functions. The most-studied of these is zebrin II [11, 20], which cloning studies suggest is the respiratory isoenzyme aldolase c [3]. The pattern of zebrin II expression in the adult mouse is described in detail in reports of Eisenman and Hawkes [11].

The functional significance of the α_{1B} channel type is not clear. N-type currents are important in mediating neuropeptide release, since ω-conotoxin GVIA can block 50-70% of electrically evoked release [19]. The detailed features of this study will extend our current knowledge of α_{1B} subunit distribution in terms of the number of neuroanatomical areas examined, by investigating α_{1B} subunit protein expression, for the first time.

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