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Immunohistochemical Study on the Distribution of the Voltage-gated Ion Channels in Gerbil Cerebellum

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There is growing evidence that alterations in Ca^{2+} homeostasis may play a role in processes of brain aging and neurodegeneration. However, few have focused on voltage-gated Ca^{2+} channel (VGCC) subunits, much less on expression of other voltage-gated ion channels, i.e. voltage-gated K^{+} (Kv) and Na^{+} (Na_v) channels.

In the present study, we have investigated the spatial patterning of VGCCs, Kv1 and Na_v channels by immunohistochemistry. This study have shown clearly that the VGCCs, Kv1 and Na_v channels have differential distribution in the cerebellum of gerbil, which is used as an ischemia and epilepsy animal model.

Immunoreactivities for Cav2.1, Cav1.2 and Cav1.3 were observed in the cell bodies and dendritic branches of Purkinje cells. In particular, Cav1.3 immunoreactivity was most prominent in the cell bodies and dendritic arborizations. A distinct band of punctate immunoreactivity for the Cav2.1, Cav2.2, Cav1.2 and Cav1.3 were observed in cerebellar nuclei. Strong immunoreactivities for Kv1.3, Kv1.4, Kv1.5 and Kv1.6 were observed in the Purkinje cell bodies, whereas Kv1.2 immunoreactivity was found in the basket cell axon plexus and terminal regions around the Purkinje cells. In the cerebellar nuclei, Kv1.2, Kv1.4 and Kv1.6 proteins were clearly detected in the soma of cerebellar output neurons. The most intense staining for $Na_v1.1$ was observed in the granular layer, whereas strong immunoreactivity for $Na_v1.2$ were seen in the Purkinje cell bodies, and extended into their dendrites.

The overall results have demonstrated the expression patterns of VGCCs, Kv1 and Na_v channels in gerbil cerebellum. Further studies are needed to define changes in other Ca^{2+} channel types to determine whether any channel changes represent selective loss of specific receptors or of cell loss, and to determine whether changes in Kv and Nav channels are linked to Ca^{2+} channel changes.

Key words : Voltage-gated calcium channel (VGCC), Voltage-gated potassium (Kv) channel, Voltage-gated sodium (Na_v) channel, Gerbil, Cerebellum, Immunohistochemistry

Introduction

Calcium plays physiological roles as a cellular mediator and pathological roles during Ca^{2+} overload (Cheung et al. 1986). Many neuronal processes are regulated by Ca^{2+} influx through voltage-gated Ca^{2+} channels (VGCCs). On the basis of their pharmacological or electrophysiological properties, at least six distinct types of VGCCs have been identified and are designated L, N, P, Q, R, and T (Randall 1998). Neuronal VGCCs are composed of at least three gene products: the pore-forming α_1 subunit and the struc-

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tural/regulatory α_2-δ and β subunits (Isom et al. 1994). Multiple isoforms of the principal α_1 subunit of VGCCs, designated class A-E and S, have been cloned from the rat brain, and they correspond to each subtype of VGCCs although there controversy still remains (Randall 1998). Previous studies have examined gene/mRNA expression specifically in the aged or Alzheimer's brain. However, few have focused on VGCC subunits, much less on expression of other voltage-gated ion channels, i.e. voltage-activated potassium (Kv) and sodium (Na_v) channels which are known to be major players in neuronal excitability.

The differential and neuronal cell type-specific expression of specialized Kv channel subtypes in the nervous system probably reflects the wide range of functions which Kv channels may exert in neuronal physiology as key determinants of membrane excitability. The Kv channels consist of α and β subunits (Rettig et al. 1994) and Kv channel α subunits belong to a superfamily on the basis of structural relatedness (Coetzee et al. 1999). At least 23 different alpha subunits of Kv channel family (Kv1.1-1.8, Kv2.1-2.2, Kv3.1-3.4, Kv4.1-4.3, Kv5.1, Kv6.1, Kv8.1, and Kv9.1-9.3) have been cloned (Coetzee et al. 1999). Most likely, different combinations of Kv channel α and β subunit isoforms into heteromultimeric Kv channels may substantially contribute to the generation of Kv channel diversity in the nervous system (Rettig et al. 1994). In the present study, we have found that staining patterns of six Kv1 channel subunits overlap in some areas of the rat and gerbil cerebellum, but each has a unique pattern of expression.

Na_v channels in the mammalian central nervous system are multisubunit protein complexes composed of one large pore-forming α-subunit polypeptide, and two other glycoprotein β subunits, β1 and β2 (Marban et al. 1998). Molecular cloning studies have identified a single family of nine related functional α-subunits (Na_v1.1-Na_v1.9) that appear to have arisen by gene duplication (Catterall 2000). Recent papers have focused on the expression patterns of the Na_v1.1, Na_v1.2, Na_v1.3, Na_v1.5 and Na_v1.6 α-subunits as well as the β1 and β2 subunits in human and rat brain (Whitaker et al. 2000, Burbidge et al. 2002, Wu et al. 2002, Lindia and Abbadie 2003). In human cerebellum, all subtypes are expressed in the granular layer, whereas specific expression of Na_v1.1, Na_v1.6, β1 and β2 mRNAs has been observed in Purkinje cells (Whitaker et al. 2000). Such data confirm that human brain sodium channel mRNAs have a distinct regional distribution, within distinct neuronal subpopulations.

Therefore, this approach towards an understanding of distinct VGCCs and other two kinds of voltage-gated ion channels demonstrate the expression patterns of these channel subunits in gerbil cerebellum. To pursue these goals, we have investigated the spatial patterning by immunohistochemistry using anti-peptide antibodies directed against a unique sequence in each subunit of VGCCs, Kv and Na_v channels.

Materials and Methods

Five adult (8-10 week old) Mongolian gerbils (*Meriones unguicularis*) were examined. To avoid the animals from suffering, these animals were treated in accordance with the 'Principles of Laboratory Animal Care' (NIH publication No. 86-23, revised in 1985). The animals were perfused transcardially with cold phosphate buffered saline (PBS, 0.1 M, pH 7.4), and then with ice-cold 4% paraformaldehyde for 10 min at a flow rate of 50-60 ml/min. Brains were removed immediately and sliced into blocks 4-6 mm thick. These were immersed in a cold fixative for 6-12 hours and then washed in a series of cold sucrose solutions of increasing concentration. Frozen sections were cut at 40 μm in the coronal plane, and immunohistochemistry was performed in accordance with the free-floating method described earlier (Chung et al. 2001a, b). Rabbit anti-rat $Ca_v2.1/\alpha_{1A}$, $Ca_v2.2/\alpha_{1B}$, $Ca_v1.2/\alpha_{1C}$

and $Ca_v1.3/\alpha_{1D}$ antibodies (product No. ACC-001, 002, 003, and 005, Alomone Labs, Jerusalem, Israel) were used as primary antibodies at a dilution of 1 : 400, 1 : 25, 1 : 300, and 1 : 400, respectively. Polyclonal anti-Kv1.1, Kv1.2, Kv1.3, Kv1.4, Kv1.5 and Kv1.6 (product No. APC-009, 010, 002, 007, 004 and 003, Alomone Labs) antibodies were also used as primary antibodies at a dilution of 1 : 200, 1 : 200, 1 : 70, 1 : 200, 1 : 70 and 1 : 70, respectively. In the staining of Na_v channels, rabbit anti-$Na_v1.1$ and $Na_v1.2$ antibodies (product No. ASC-001 and 002, Alomone Labs) were used at a dilution of 1 : 200 for both of them.

A sample of sections was reacted without primary antiserum, and a different sample was exposed to a primary antiserum that had been preincubated for 24 hours with control antigen peptides. No sections from these samples exhibited any of the immunoreactivity described in this report. Visual assessment and densitometric measurement using a NIH image program (Scion Image) determined the staining intensity.

Results

1. Expression of $Ca_v2.1$, $Ca_v2.2$, $Ca_v1.2$ and $Ca_v1.3$

Within the gerbil cerebellar cortex (Fig. 1), intense staining of $Ca_v2.1$ was observed in the soma of Purkinje cells, with a weaker signal in cells of both the molecular and granular layers. In the experiment using anti-$Ca_v2.2$ antibodies, the cytoplasm of Purkinje cell bodies were hardly stained and there was little evidence of concentrated labeling in their dendritic or axonal projections. The most intense staining for the $Ca_v1.2$ was found in the cell bodies and dendrites of Purkinje cells. The cell bodies of Purkinje cells were

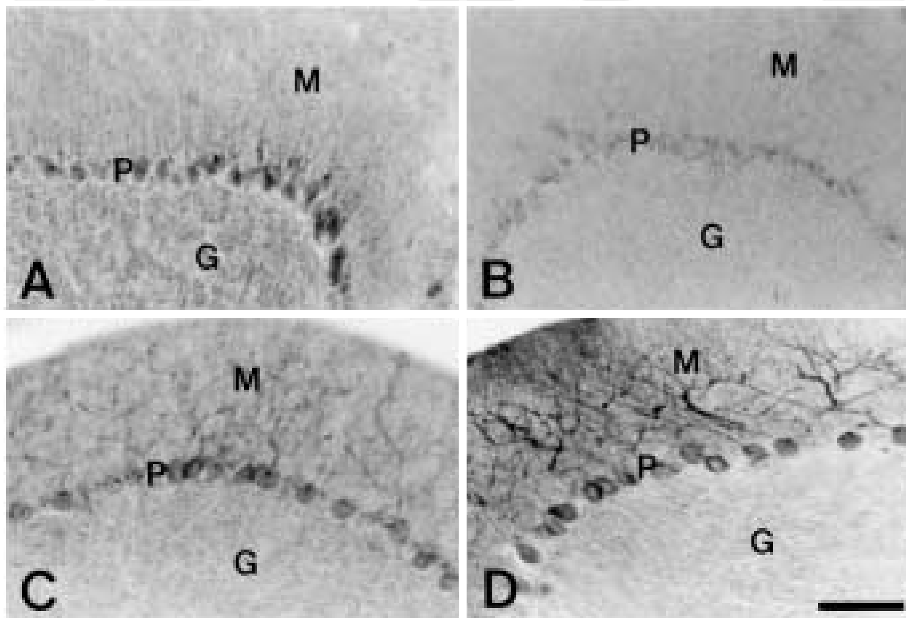

Fig. 1. Cellular localizations of $Ca_v2.1$ (A), $Ca_v2.2$ (B), $Ca_v1.2$ (C), and $Ca_v1.3$ (D) in the cerebellar cortex, Immunoreactivity for $Ca_v2.1$ in the Purkinje cells was seen in the cell bodies, and extended into the apical dendrites. The most intense staining for $Ca_v1.2$ and $Ca_v1.3$ was found in the cell bodies and dendrites of Purkinje cells. M: molecular layer, P: Purkinje cell layer, G: granular layer. Scale bar = 50 μm

heavily stained for $Ca_v1.3$ and many dendritic branches were strongly stained.

In the cerebellar nuclei (Fig. 2), the large cell bodies of cerebellar output neurons in the nucleus medialis, interpositus and lateralis displayed moderate immunoreactivity for $Ca_v2.1$. $Ca_v2.1$ immunoreactivity was also observed in the surrounding neuropil, the region where the Purkinje cell axons terminate. $Ca_v2.2$-immunoreactive cell bodies were hardly found in the surrounding neuropil. The large cell bodies of cerebellar neurons in the cerebellar nuclei showed moderate immunoreactivity for $Ca_v1.2$. $Ca_v1.3$-positive cell bodies of cerebellar output neurons were rarely found in the nucleus medialis, interpositus and lateralis. We graded immunoreactivities according to the % of maximal mean density level, and then showed quantitative data (Table 1).

2. Expression of Kv1.1-1.6 channels

Prominent staining of Kv1 channel proteins was observed in neuronal cell bodies together with uniform immunoreactivity throughout many regions of the neuropil. Immunohistochemical analysis revealed that

Table 1. Immunoreactivities of VGCCs in gebil cerebellum

Cerebellar region	VGCC			
	$Ca_v2.1$	$Ca_v2.2$	$Ca_v1.2$	$Ca_v1.3$
Cerebeller cortex				
Molecular layer	+	+	++	+++
Purkinje cell layer	+++	++	+++	+++
Granular layer	++	+	++	+
Cerebellar nuclei				
Cerebellar output neurons	+++	+	+++	+
Surrounding neuropil	+	+	+	++

[a]Immunoreactivity was classified into three categories according to the % of maximal mean density level (+, weak; ++, moderate; +++, heavy).

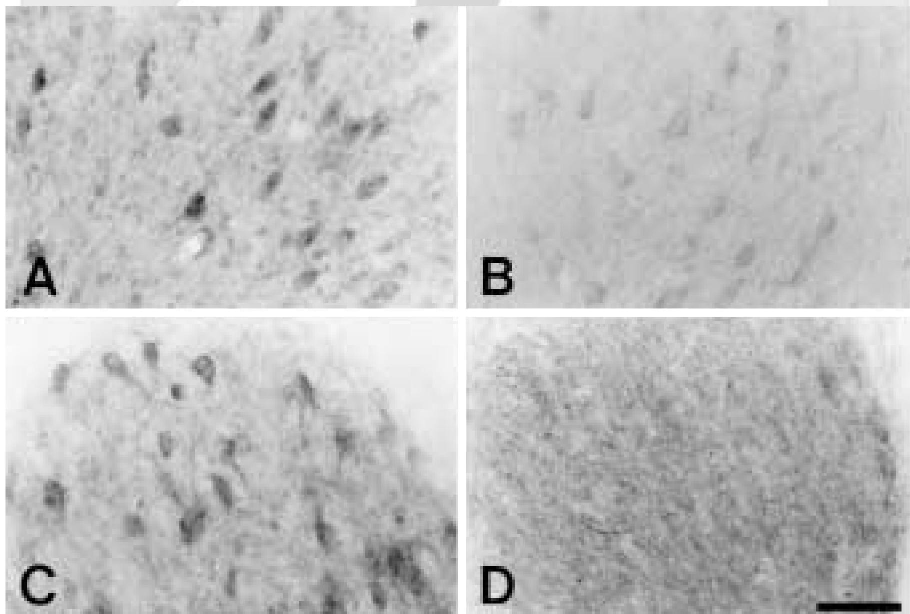

Fig. 2. Cellular localizations of $Ca_v2.1$ (A), $Ca_v2.2$ (B), $Ca_v1.2$ (C), and $Ca_v1.3$ (D) in the cerebellar nuclei, The large cell bodies of cerebellar output neurons displayed moderate immunoreactivity for $Ca_v2.1$ and $Ca_v1.2$ in the nucleus medialis, interpositus and lateralis. In particular, $Ca_v1.3$ immunoreactivity was strong in the surrounding neuropil. Scale bar = 50 μm

despite reproducible differences in signal intensity, immunoreactivities for Kv1.1, Kv1.2, Kv1.3 and Kv1.6 were found in the cell bodies of the basket and stellate cells as well as in the neuropil (Fig. 3).

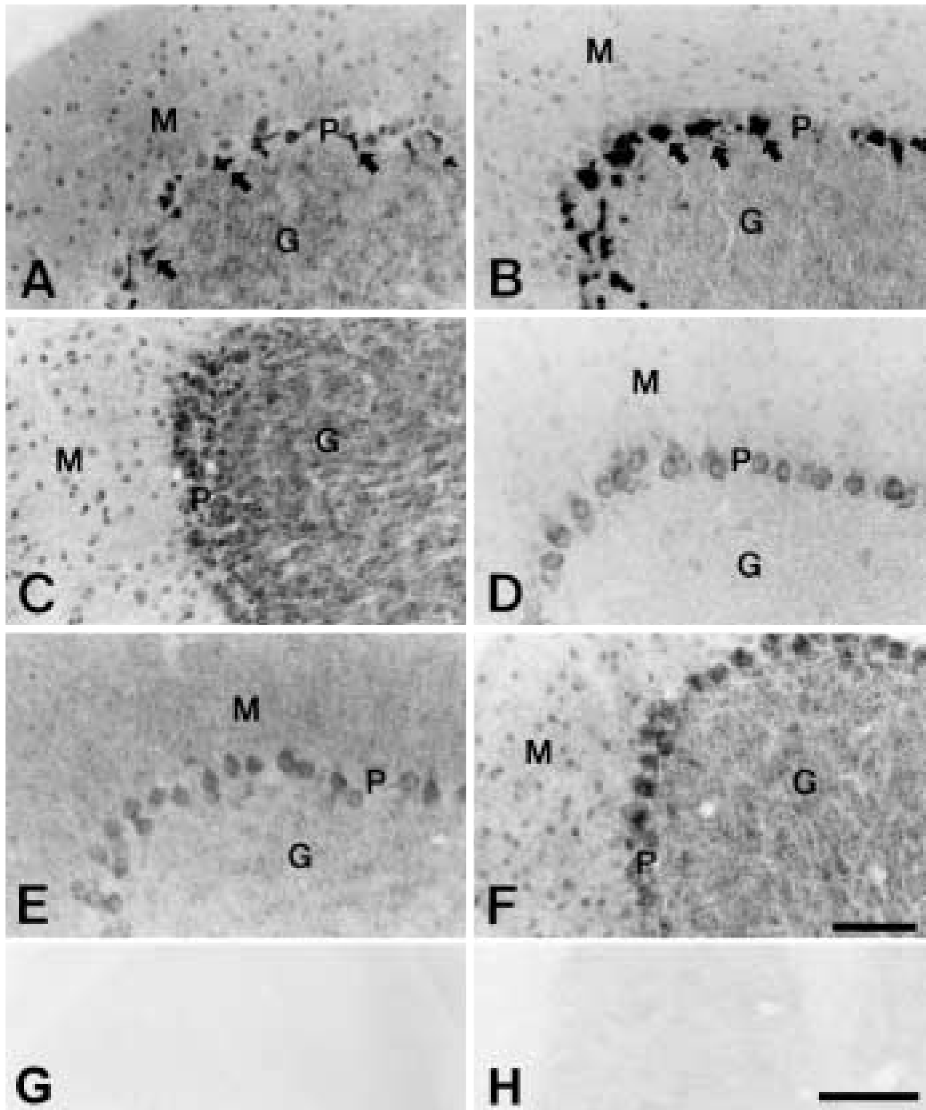

Fig. 3. Cellular localizations of Kv1.1 (A), Kv1.2 (B), Kv1.3 (C), Kv1.4 (D), Kv1.5 (E), and Kv1.6 (F) subunits in the gerbil cerebellar cortex, Strong immunoreactivities for Kv1.3, Kv1.4, Kv1.5 and Kv1.6 were observed in the Purkinje cell bodies, whereas a weak signal of Kv1.2 was observed in the cell bodies. Immunoreactivity for Kv1.2 was prominent in the basket cell axon plexus and terminal regions around the Purkinje cells (B, arrows). Relatively low immunoreactivity for Kv1.1 was also concentrated in this area (A, arrows). A sample of sections was reacted without primary antiserum (G), and a different sample was exposed to a primary antiserum that had been preincubated for 24 hours with control antigen peptides (H). No section from either group exhibited any of the immunoreactivity described in this report (G, H). M: molecular layer, P: Purkinje cell layer, G: granular layer. Scale bar = 50 μm (A–F), 100 μm (G, H)

In the Purkinje cells, the present studies showed that Kv1 channels were clearly expressed at apparently different levels and extensions into the dendritic processes (Fig. 3). Strong immunoreactivities for Kv1.3, Kv1.4, Kv1.5 and Kv1.6 were observed in the Purkinje cell bodies, whereas a weak signal of Kv1.2 was observed in the cell bodies. Like the localization in the rat cerebellum, immunoreactivity for Kv1.2 was found in the basket cell axon plexus and terminal regions around the Purkinje cells. Although the staining intensity was relatively lower than that of Kv1.2, immunoreactivity for Kv1.1 was also concentrated in this area. In the granular layer, strong staining for Kv1.1, Kv1.3 and Kv1.6 was found in the granule cell bodies. In the cerebellar nuclei, a distinct band of immunoreactivities for Kv1 channels was observed in the region where Purkinje cell axons form inhibitory GABAergic synapses with the cerebellar output

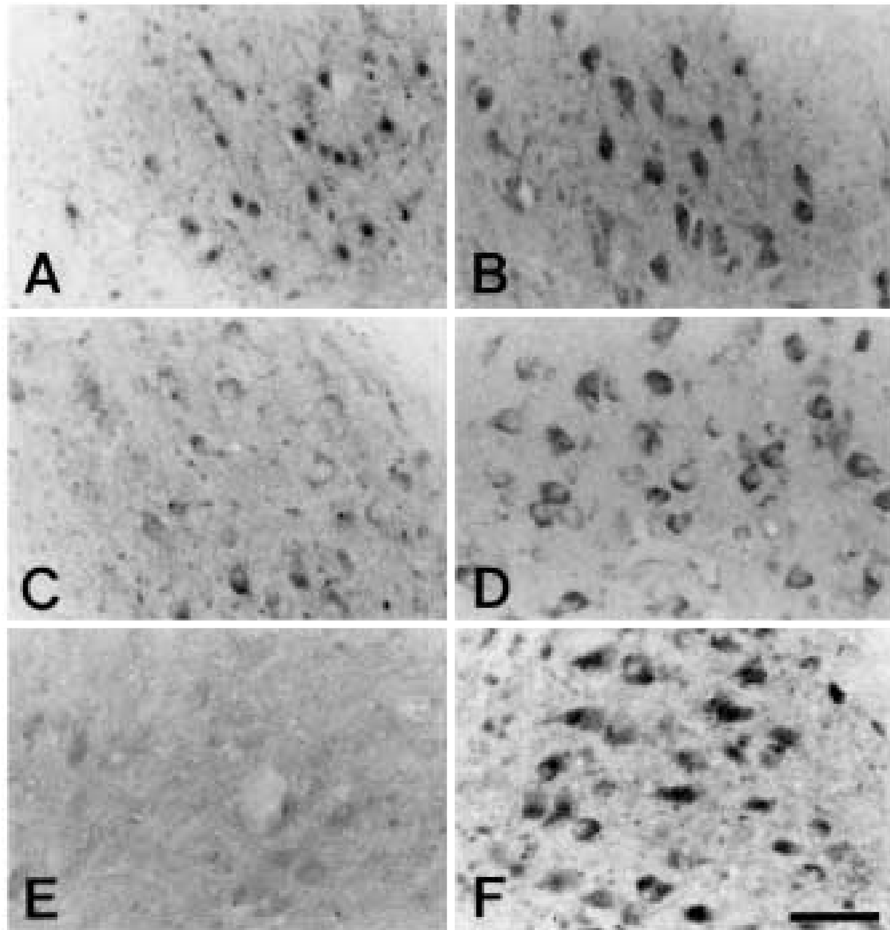

Fig. 4. Cellular localizations of Kv1.1 (A), Kv1.2 (B), Kv1.3 (C), Kv1.4 (D), Kv1.5 (E), and Kv1.6 (F) subunits in the gerbil cerebellar nuclei, Kv1.2, Kv1.4 and Kv1.6 proteins were clearly detected in the soma of cerebellar output neurons, whereas Kv1.1 and Kv1.3 were weakly stained in the cell bodies. The predominant staining for Kv1 channels was also found in the surrounding neuropil. Scale bar = 50 μm

neurons (Fig. 4). Kv1.2, Kv1.4 and Kv1.6 proteins were clearly detected in the soma of the pyramidal neurons, whereas Kv1.1 and Kv1.3 were weakly stained in the cell bodies. Kv1.5 immunoreactive cell bodies were hardly detected. The predominant staining for Kv1 channels was also found in the surrounding neuropil.

3. Expression of $Na_v1.1$ and $Na_v1.2$ channels

In the present study, prominent staining of $Na_v1.1$ and $Na_v1.2$ channels were observed in neuronal cell bodies, together with uniform immunoreactivity throughout the region of the neuropil. However, $Na_v1.1$ and $Na_v1.2$ channels were clearly expressed at apparently different levels and extensions into the dendritic processes (Figs. 5, 6). In the cerebellar nuclei, their staining pattern was similar to that in the rat cerebellar nuclei.

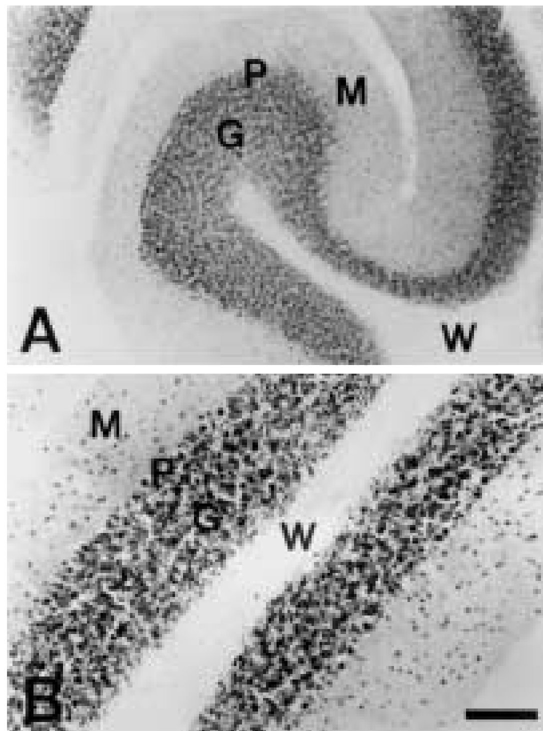

Fig. 5. Cellular localizations of $Na_v1.1$ in the cerebellar cortex, At a low magnification, the most intense staining for $Na_v1.1$ was observed in the granular layer (A). Uniform labeling was observed throughout the molecular layer, which included the round cell bodies of basket and stellate cells (A, B). The cytoplasm of Purkinje cell bodies were hardly stained, and reaction products were almost absent from the white matter. M: molecular layer, P: Purkinje cell layer, G: granular layer, W: white matter Scale bar = 250 μm (A), 100 μm (B)

Within the cerebellar cortex, uniform labeling for $Na_v1.1$ was observed throughout the molecular layer, which included the round cell bodies of basket and stellate cells (Fig. 5). The cytoplasm of Purkinje cell bodies was hardly stained and there was little evidence of concentrated labeling in their dendritic or axonal projections. Strong uniform staining was also observed in the granular layer, but reaction products were almost absent from the white matter. In the experiment using anti-$Na_v1.2$ antibodies, the most intense staining was observed in the soma of Purkinje cells, with a strong signal in the molecular layer (Fig. 6). It was noted that strong immunoreactivity in the Purkinje cells were seen in the cell bodies, and extended into their dendrites. Intense staining was also observed throughout the molecular layer. On the other hand, granule cells were hardly stained.

Discussion

Expression of VGCC proteins

In the present study, we investigated the distribution of $Ca_v2.1$, $Ca_v2.2$, $Ca_v1.2$ and $Ca_v1.3$ in gerbil cerebellum. In the Purkinje cells of gerbil cerebellum, the staining pattern was quite different from those of rat Purkinje cells. Immunoreactivity for $Ca_v2.1$, $Ca_v1.2$ and $Ca_v1.3$ were observed in the cell bodies and the dendritic branches. In particular, $Ca_v1.3$-IR was most prominent in the cell bodies and dendritic arborizations. The P-type Ca^{2+} channel has been suggested as the predominant channel type in the

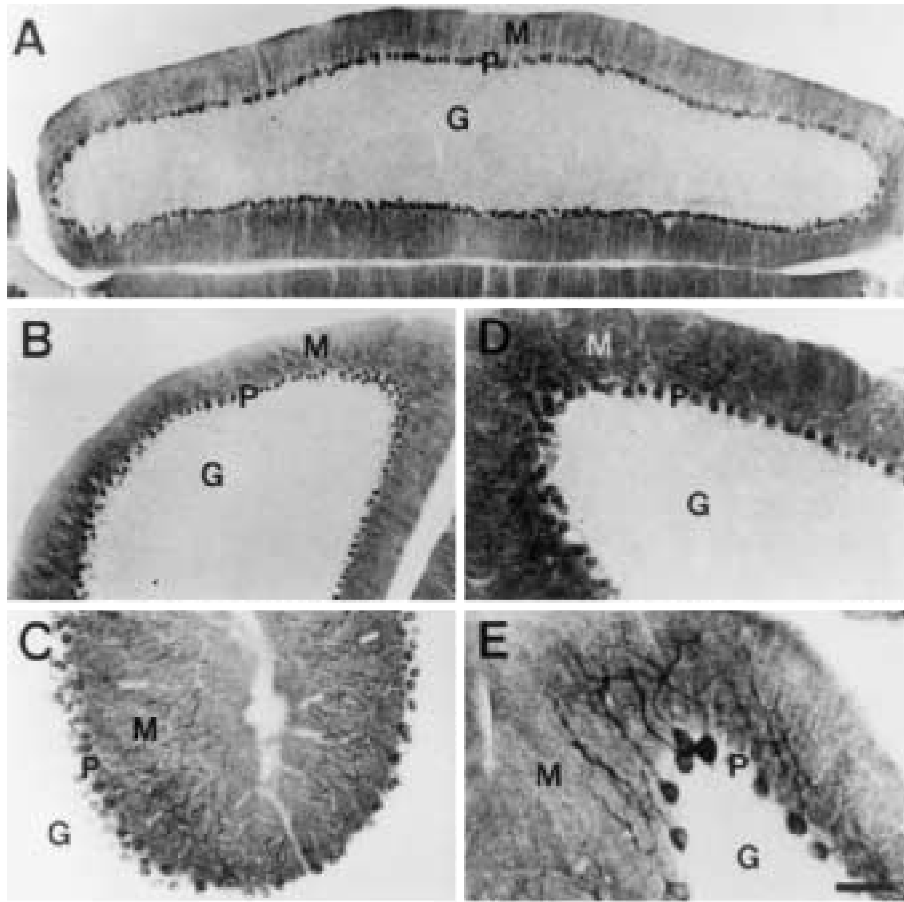

Fig. 6. Cellular localizations of $Na_v1.2$ in the hemisphere (A–C) and intermediate lobe (D, E) of the cerebellum, At a low magnification, the most intense staining for $Na_v1.2$ was observed in the soma of Purkinje cells, with a strong signal in the molecular layer (A). It was noted that strong immunoreactivity in the Purkinje cells were seen in the cell bodies, and extended into their dendrites. On the other hand, granule cells were hardly stained. M: molecular layer, P: Purkinje cell layer, G: granular layer Scale bar = 250 μm (A, B), 100 μm (C, D), 50 μm (E)

Purkinje neurons (Mintz et al. 1992a). The importance of P-type ($Ca_v2.1$) Ca^{2+} channels in cerebellum is evident in the 'autosomal-dominant pure cerebellar ataxia' disorders in humans that are marked by CAG-expansions in the $Ca_v2.1$ gene (Ishikawa et al. 1997), and in the neuropathological consequences of the mouse tottering (tg) and leaner (tg (la)) mutations of $Ca_v2.1$ (Fletcher et al. 1996).

In the cerebellar nuclei, a distinct band of punctate immunoreactivity for the $Ca_v2.1$, $Ca_v2.2$, $Ca_v1.2$ and $Ca_v1.3$ were observed in the region where Purkinje cell axons form inhibitory GABAergic synapses with the cerebellar output neurons (Fig. 2). $Ca_v1.3$-IR was detected in the surrounding neuropil, not in the cell bodies in the gerbil. Using an anti-P type channel antibody, immunoelectronmicroscopic signals were observed in rat Deiter's nucleus in fields containing Purkinje cell preterminal axons and synaptic boutons

(Hillman et al. 1991). Other broadly applicable data has been noted in patch clamp studies of thin cerebellar slices where, for example, the selective P/Q channel antagonist ω-agatoxin IVA (Mintz et al. 1992b) was seen to markedly suppress both potassium (Momiyama and Takahashi 1994) and electrically induced GABA release in rat cerebellar nuclear cells: ω-conotoxin GVIA was also active in this model (Takahashi and Momiyama 1993). Taken together, such data may suggest that the P/Q type containing $Ca_v2.1$ and N type containing $Ca_v2.2$ are located in this region, which is in keeping with the present study.

The overall results of the above localization study have shown different staining patterns of VGCCs from those in gerbil cerebellum. To understand the role of calcium channels in neurological pathways, it will be necessary to investigate VGCC subunit association at the subcellular level by immunoelectron microscopy and biochemical analyses.

Expression of Kv1 proteins

The localization of different Kv channel subunits to dendritic and to axonal domains may be recognized in immunocytochemical experiments in many neuronal cell types at the light microscope level. Therefore, we have employed antibodies directed against Kv channel subunits for mapping Kv channels in the cerebellum. In the Purkinje cells, Kv1 channels were clearly expressed at apparently different levels and extensions into the dendritic processes (Fig. 3). Strong immunoreactivities for Kv1.3, Kv1.4, Kv1.5 and Kv1.6 were observed in the Purkinje cell bodies, whereas a weak signal of Kv1.2 was observed in the cell bodies. Like the localization in the rat cerebellum, immunoreactivity for Kv1.2 was found in the basket cell axon plexus and terminal regions around the Purkinje cells. Combined with previous reports, three major conclusions may be extracted from our results: (i) different neuronal cell types express different combinations of Kv1 channel subunits; (ii) in some cases, Kv1 channel subunits are targeted to contrasting subcellular compartments; (iii) distinct combinations of Kv1 channel subunits are co-localized in different neuronal cell types.

Recently, accumulating evidence has indicated that many neurons undergo apoptosis after global or focal ischemia. Studies have shown that increased K^+ efflux might be a primary step leading to apoptosis, and Kv1.2 was observed to respond to ischemic insult in neurons under both *in vivo* and *in vitro* conditions (Conforti et al. 2000, Qiu et al. 2003). In recent epileptic research, it is assumed that a disturbance of Kv channel function is involved in epileptogenesis by leading to decreased neuronal stability (Rho et al. 1999). Although gerbils used in this study were not divided into SS or SR, the present study on the differential localization patterns of Kv1 channel subunits in the gerbil cerebellum may provide helpful guidelines for correlating current types with particular channels and useful data for the future investigations on the pathological conditions such as ischemia and epilepsy.

Expression of Na_v channels

Given this high degree of similarity, it is somewhat surprising that these two polypeptides have virtually nonoverlapping subcellular distributions in the central nervous system. Although $Na_v1.1$ and $Na_v1.2$ are simultaneously expressed in many of the same adult central neurons (Felts et al. 1997), $Na_v1.1$ is found in the somatodendritic membrane, whereas $Na_v1.2$ is predominantly found on axons and at or near axon terminals. This implies that $Na_v1.1$ may play a key role in mediating dendritic excitability, an important component of synaptic signal processing (Johnston et al. 1996), whereas $Na_v1.2$ are involved in action potential initiation at the initial segment and propagation along axons. Westenbroek et al. (1989) demonstrated that the somata of Purkinje cells were immu-

noreactive to antibodies specific for $Na_v1.1$ throughout the rat cerebellum. In contrast, $Na_v1.2$ was not observed in the Purkinje cell bodies, although a low level of immunoreactivity would be obscured by the intense staining of the molecular layer. Thus, they have concluded that in the cerebellum, $Na_v1.1$ is preferentially localized in the cell bodies of Purkinje cells, whereas $Na_v1.2$ is preferentially localized in areas rich in unmyelinated fibers. In the present study, $Na_v1.1$ and $Na_v1.2$ were clearly expressed at apparently different levels and extensions into the dendritic processes in gerbil cerebellum (Figs. 5, 6).

The overall results of the above localization study have shown clearly that $Na_v1.1$ and $Na_v1.2$ have differential distribution in gerbil cerebellum. To understand the role of Na_v channels in neurological pathways, it will be necessary to determine the functional properties of $Na_v1.1$ and $Na_v1.2$ channels in the cell bodies of central neurons by direct physiological recording. It will be also interesting to investigate Na_v channels association at the subcellular level by immunoelectron microscopy and biochemical analyses. The present study may provide useful data for such future investigations.

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저빌 소뇌에서 전압의존성 이온채널 분포에 관한 면역조직화학적 연구

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간추림 : 최근 칼슘(Ca^{2+}) 항상성의 변화가 뇌의 노화와 신경퇴행성 질환에서 어떤 역할을 할 것이라는 보고들이 증가되고 있다. 하지만, 전압의존성 Ca^{2+} 채널(VGCC)에 대한 연구는 별로 없을 뿐 아니라, 신경 흥분성에서 중요한 역할을 하는 다른 종류의 전압의존성 채널인 Kv 채널과 Na_v 채널에 대한 연구 역시 거의 없는 실정이다.

따라서, 본 연구에서는 허혈과 간질의 동물모델로 사용되는 저빌의 소뇌에서 면역조직화학을 이용하여 VGCC, Kv1 채널, Na_v 채널의 분포를 관찰하였다. 실험 결과, VGCC, Kv1 채널, Na_v 채널은 저빌의 소뇌에서 서로 다른 분포양상을 나타내었다.

Cav2.1, Cav1.2 및 Cav1.3에 대한 염색성은 조롱박 세포의 세포체와 돌기에서 관찰되었고, 그 중에서 Cav1.3의 염색성이 가장 뚜렷하게 나타났다. 소뇌핵에서는 조롱박 세포의 축삭과 소뇌핵의 세포가 억제성 연접을 이루는 부위에서 Cav2.1, Cav2.2, Cav1.2 및 Cav1.3에 대한 염색성이 특이적인 띠모양으로 관찰되었다. Kv1 채널들은 조롱박 세포에서 분명하게 다른 정도로 발현되었으며 세포돌기에서의 발현도 차이가 있었다. 조롱박 세포의 세포체에서 Kv1.3, Kv1.4, Kv1.5 및 Kv1.6에 대한 염색성은 강했으나, Kv1.2에 대한 염색성은 조롱박 세포 주변의 바구니 세포 축삭얼기와 끝부분에서 특히 강했다. 소뇌핵에서는 소뇌핵 신경세포의 세포체에서 Kv1.2, Kv1.4 및 Kv1.6의 염색성이 뚜렷했다. Na_v1.1의 가장 강한 염색성은 과립층에서 관찰되었고 조롱박 세포체에서는 거의 발견되지 않았다. 반면, Na_v1.2은 조롱박 세포체와 돌기에서 강하게 발현되었다.

앞으로는 이러한 Ca^{2+} 채널의 변화가 특정 수용체나 세포의 소실을 나타내는 것인지, 또한 Kv 채널과 Na^{+} 채널의 변화가 Ca^{2+} 채널의 변화와 어떻게 연관이 되는지에 대한 더 많은 연구가 계속 진행되어야 할 것이다.

찾아보기 낱말 : 전압의존성 Ca^{2+} 채널, Kv 채널, Na_v 채널, 저빌, 소뇌, 면역조직화학

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