J. Vet. Sci. (2003), 4(2), 113-116

JOURNAL OF
Veterinary
Science

Expression of PSD95 in the Rat Sciatic Nerve

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Received April 13, 2003 / Accepted July 30, 2003

Abstract

This study was designed to elucidate the existence of PSD95 in the rat sciatic nerve. Immunohistochemical stains of cryosection and teased fiber of sciatic nerves were performed with goat polyclonal antibody against PSD95. Western blot analysis was also accomplished with the same antibody. We got an interesting result that the rat sciatic nerve obviously showed PSD95 immunoreactivity especially in the nodal and paranodal regions, and we also identified a distinct band of PSD95 by western blot. These results suggest PSD95 exists in the sciatic nerve as well as it does in the central nervous system. We suppose PSD95 may have some important roles in ion channel clustering, junctional plasticity and signal transduction in the peripheral nerves as well.

Key words: PSD95, sciatic nerve, immunocytochemistry, western blot

Introduction

Postsynaptic density 95 (PSD95) was first identified as an abundant cytoskeleton-associated protein found in the post-synaptic density fraction of rat synaptosomes [2]. Recent studies have suggested that it plays an important role in the assembly and organization of excitatory postsynaptic architecture [3, 10, 13].

PSD95 has been identified to the dendrites of hippocampal neurons [2], to the presynaptic plexus of cerebellar basket cells [8] and to the basket cell terminal pinceau of cerebellar cortex and postsynaptic dendrites in both intact and lysed forebrain synaptosomes [5]. This molecule is characterized by three N-terminal PDZ domains, an SH3 domains, and a C-termianl guanylate kinase (GK)-like domain, thus making that members of the membrane associated guanylate kinase (MAGUK) superfamily [1, 10, 13]. Each of the PDZ, SH3, and GK domains exhibit functions as a site for protein-protein interaction.

Septate like junctions, between the axolemma and the membrane of Schwann cells at the paranodal region, are very similar structure of the junction between Purkinje cell soma and basket cell terminal pinceau. Althouth the localization of PSD95 has not yet been reported in the peripheral nerves, it was supposed the PSD95 might be present in the septate junctional area between the axolemma and the membrane of Schwann cells. We have accomplished this study to identify whether the PSD95 is localized in the sciatic nerve or not.

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Materials and Methods

Animals

Ten adult Sprague-Dawley rats, weighing from 250 to 300 gm and aged from 10 to 16 weeks, were used. All animals were kindly provided by the National Veterinary Research & Quarantine Services (Anyang, Korea). Feed (Samyang, Korea) and water were provided ad libitum. Animals were housed individually in plastic-bottomed cages under constant temperature (20±2℃) and moisture (50±7%) on a twelve: twelve-hour light : dark cycle.

Immunocytochemistry

Animals were anesthetized by inhalation of ethyl ether, and then slowly perfused transcardially with 0.1 M PBS (pH 7.4) followed by 4% paraformaldehyde (PFA) in 0.1 M PBS.

Sciatic nerves of both limbs and cerebellar cortex were carefully dissected out and fixed in 4% PFA for 24 hours. After washing with 0.1M PBS for 1 hour, tissues were infiltrated by phosphate buffered sucrose (10% for 24 h, 20% for 24 h and finally 30% for 72 h) for cryoprotection, and then quickly embedded with OCT compound (Tissue-Tek, Canada), and got 10㎛ sections by using of cryostat (Leica, Germany).

Sciatic nerves for teasing were incubated for 10 min. in PBS containig 0.1% Triton X-100. Individual nerve fibers were seperated by using acupuncture needles under the stereomicroscope.

Each slide was washed 3 times with 0.1 M PBS for 10

min., and put into PBS containing 1% BSA, 0.2% Triton X-100 and 0.5% H_2O_2 for 30 min to block endogenous peroxidase activity. Slides were moved into 0.05 M glycine in PBS containing 1% BSA (PBSA) for 10 min. After washing with PBSA, slides were incubated in 1.5% blocking normal rabbit serum (Vector, U.S.A.) in PBSA for 1 hour.

Slides were incubated overnight at 4℃ in 1 : 50 goat anti-PSD95/SAP90 (Santacruz, U.S.A.) in PBSA containing 1.5% blocking serum. After washing 3 times with PBSA for 10 min, slides were incubated for 1 hour in biotinylated rabbit anti-goat IgG (Vector, U.S.A.). After washing 3 times with PBS for 10 min, they were incubated for 1 hour in avidin-biotin horseradish peroxidase complex (Vector, U.S.A.). Then slides were washed 3 times with PBS for 10 min. respectively. Sections were treated with 0.02% 3,3'-diaminobenzadine tetrahydrochloride (DAB, Sigma, U.S.A) in 0.05% H_2O_2 for 10 min and washed off with distilled water. After counterstained with hematoxylin, they were observed with microscope.

Western blotting

Collected sciatic nerves and small part of cerebellar cortex for control were washed with ice cold 25 mM Tris, 1 M EDTA solution and grinded by the glass homogenizer. The lysates were spinned down at 10,000 g for 10 min. at 4℃ and supernatant was taken. Protein concentration of each sample was determined by the microassay (Bio-Rad) and stored at -70℃. Samples were mixed with equal volume of 2×sample-loading buffer[50mM Tris-HCl (pH 6.8), 2% SDS, 5% 2-mercaptoethanol, 20% glycerol, 0.05% bromophenyl blue], then boiled at 100℃ for 5 min., and cooled down to room temperature. In each sample, 20 ㎍ of protein was subjected to 10% SDS-polyacrylamide gel electrophoresis. Proteins were then transferred electrophoretically to a nitrocellulose membrane. Membrane was stained with Ponceau S (Sigma, U.S.A.), and washed with distilled water. Membranes were blocked for 1 hour with 5% skimmed milk in TBS-T buffer [250 mM Tris-HCl, 150 mM NaCl, 0.5% Tween 20, 5% skimmed milk], and incubated for 2 hours in 1:1,000 goat anti-PSD95/SAP90 diluted with blocking buffer. After washing, membranes were incubated with 1:5,000 biotinylated conjugated rabbit anti-mouse IgG for 1 hour. After washing 3 times with TBS-T for 15 min, they were incubated for 30 min. in avidin-biotin horseradish peroxidase complex. Then slides were washed 3 times with TBS-T for 10 min. Membrane was transferred to the 0.02% 3,3'-DAB with 0.05% H_2O_2 for 10 min., then washed off with distilled water.

Retults

Immunocytochemistry

In the normal rat cerebellum, the strong immunoreaction of PSD95 was obtained mainly around the Purkinje cells (Fig. 1a). When observed in higher magnification, around the initial part of Purkinje cell axonal membrane was immunostained very strongly with PSD95 (Fig. 1b). It is supposed to be the junctional portion where the descending branches of the basket cell axon envelop the Purkinje cell soma. In the normal rat sciatic nerve fibers immunostained with goat anti-PSD95, distinct immunorection in the teased fibers was observed (Fig. 2b). Sciatic nerves expressed a moderate immunoreaction in the nodal and paranodal regions. Especially, paranodal axolemma was the most strong immunoreactive area in the sciatic nerve (Fig. 2a). As seen in Fig. 2b, teased sciatic nerve fibers expressed fine immunoreaction of PSD95, and it was very easy to identify compared with that of sections (Fig. 2a). The immunoreaction was clearly detected on the axolemma especially at the paranode.

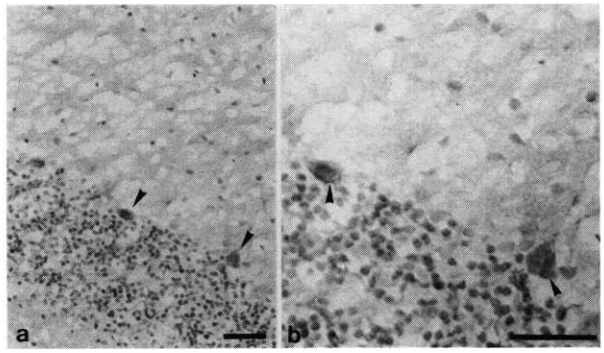

Fig. 1. a, Normal rat cerebellum immunostained with anti-PSD95 and counterstained with hematoxylin. Strong immunoreaction is found around the Purkinje cell (arrow heads). b, Higher magnification of a. PSD95 immunoreaction product (brown color) is very strongly stained around the Purkinje cell membrane which is presumable region of terminal pinceau of basket cells. bar = 50 ㎛.

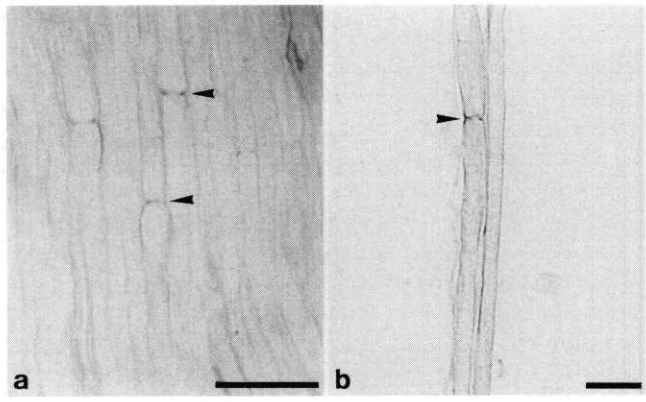

Fig. 2. a, Normal rat sciatic nerve immunostained with anti-PSD95 and counterstained with hematoxylin. Nodal and paranodal regions show strong immunoaction of PSD95 (arrow heads). bar = 50 ㎛. b, Teased fibers of normal sciatic nerve express moderate PSD95 immunoreaction especially at the nodal and paranodal regions (arrow heads). bar = 50 ㎛.

Western blot analysis

From the western blot analysis, the lysate of normal adult rat sciatic nerve definitely expressed distinct band just below the level of 98kD (Fig. 3), and this band was also identified more strongly in the sample of cerebellar cortex. These distinct bands exhibit sciatic nerves obviously express PSD95.

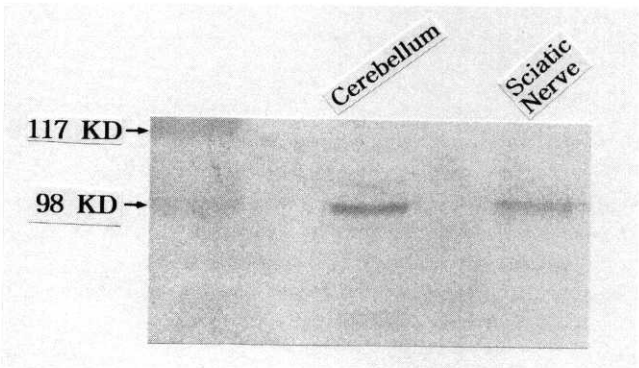

Fig. 3. Western blot analysis of PSD95 in the cerebellar cortex and the sciatic nerve. Sciatic nerve expresses a distinct band just below 98kD level weakly than that of cerebellar cortex.

Discussion

The postsynaptic density (PSD), a specialization of the cytoskeleton at the synaptic junction, lies adjacent to the cytoplasmic face of the postsynaptic membrane. It is recently apparent that the PSD provides a structural matrix, which clusters ion channels in the postsynaptic membrane [4, 6, 7] and anchors signaling molecules such as kinases and phosphatases at the synapse [9]. The NMDA receptor, which required for several forms of synaptic plasticity [14], is clustered at the postsynaptic membrane by the components of PSD [13]. Thus, the PSD contributes to the critical features of synaptic integration and regulation[15].

The first new core protein that was identified by cDNA cloning of protein from the PSD fraction was termed post synaptic density 95 (PSD95) by Cho *et al* [2], and it has been also called by synaptic associated protein 90 (SAP90) by Kistner *et al* [8].

To determine the more reliable subcellular localization of PSD95 at forebrain synapses, Hunt *et al* [5] used postembedding immunoelectron microscopy, and the results supported their original hypothesis that PSD95 is a component of the PSD. Compared with the dlg and ZO-1, PSD95 is distributed asymmetrically at forebrain synaptic junctions, and is associated principally with the postsynaptic side. Thus, any functional role of PSD95 would be exerted disproportionately on the postsynaptic side of the junction, most likely in association with NMDA type glutamate receptors [10].

The high expression of PSD95 in the presynaptic plexus of cerebellar basket cells was reported by Kister *et al* [8] and Hunt *et al* [5], and its absence in the postsynaptic Purkinje neurons means it's not the representative distribution of PSD95 in most synapses. The explanation for the aberrant high expression of PSD95 in the terminal plexus of basket cell may lie in its unusual structure, which is unique in the mammalian brain [12]. Converging axons of several basket cells form a "basket" around the soma of each Purkinje cell and then descend and branch into a dense plexus termed a "pinceau" that surrounds the base of the axon between the soma and the beginning of the myelin sheath. PSD95 was also identified in the postsynaptic dendrites of forebrain synaptosomes [5]. From these reports, it has been verified the PSD95 has some roles for signalling between the synaptic junctions and it has likely to play some roles in the septate-like junctions as well.

Each large pinceau makes only a few chemical synapses onto the Purkinje cell axon at a position about one third of the way from the soma of Purkinje cell to the myelin sheath. Otherwise the axon is shielded from the pinceau by a neuroglial sheath [5].

Although PSD95 may have various binding and signaling functions in different part of neurons, this study demonstrates that PSD95 is clearly detected at the paranodal and nodal axolemma in sciatic nerve. By the teasing fiber technique, PSD95 was also detected on the nodal and paranodal regions, and they are presumative regions of septate-like junctions at the paranodal loops.

This study is first data of localization of PSD95 at the sciatic nerve and it's still an early stage for the understanding of function of this molecule in the PNS. We expect the result of this study which represents the obvious expression of PSD95 protein in the sciatic nerve might be a very useful base for the future study of PSD95 and its association of peripheral nerves.

Referneces

1. **Anderson, J. M.** Cell signaling: MAGUK magic. Curr. Biol. 1996, **6**, 382-384.
2. **Cho, K. O., Hunt, C. A and Kennedy, M. B.** The rat brain postsynaptic density fraction contains a homolog of the Drosophila disc-large tumor suppressor protein. Neuron. 1992, **9**, 929-942.
3. **Craven , S. E., El-Husseini, A. E. and Bredit, D. S.** Synaptic targeting of the postsynaptic density protein PSD-95 mediated by lipid and protein motifs. Neuron, 1999, **22**, 497-509.
4. **Ehlers, M. D., Mammen, A. L., Lau, L. F. and Huganir, R.L.** Synaptic targeting of glutamate receptors. Curr. Opin. Cell Biol. 1996, **8**, 484-489.
5. **Hunt, C. A., Schenker, L. J. and Kennedy, M. B.** PSD95 is associated with the postsynaptic density and not with the presynaptic membrane at forebrain synapse. J. Neurosci. 1996, **16(4)**, 1380-1388.

6. **Kennedy, M. B.** The postsynaptic density at glutamatergic synapses. Trends Neurosci. 1997, **20(6)**, 264-268.
7. **Kenedy, M. B.** The postsynaptic density. Curr. Opin. Neurobiol. 1993, **3**, 732-737.
8. **Kistner, U., Wenzel, B. M., Veh, R. W., Cases-Langhoff, C., Garner, A. M., Papeltauer, U., Voss, B., Gundelfinger, E. D. and Garner, C. C.** SAP90, a rat presynaptic protein related to the product of the drosophila tumor suppressor gene dlg-A. J. Biol. Chem. 1993, **268**, 4580-4583.
9. **Klauck, T. M. and Scott, J. D.** The postsynaptic density: A subcellular anchor for signal transduction enzymes. Cell Signal. 1995, **7(8)**, 747-757.
10. **Kornau, H. C., Seeburg, P. H., and Kennedy, M. B.** Interaction of ion channels and receptors with PDZ domain proteins. Curr. Opin. Neurobiol. 1997, **7**, 368-373.
11. **Kornau, H. C., Schenker, L. T., Kennedy, M. B. and Seeburg, P. H.** Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. Science. 1995, **269**, 1737-1740.
12. **Palay, S. L. and Chan-Palay, V.** The basket cell. *In* Cerebellar cortex, pp. 180-216, Springer-Verlag, New York, 1974.
13. **Sheng, M.** PDZ and receptor/channel clustering: rounding up the latest suspects. Neuron. 1996, **17**, 575-578.
14. **Wilson, M. A. and Tonegawa, S.** Synaptic plasticity, place cells and spatial memory: Study with second generation knockouts. Trends Neurosci. 1997, **20**, 102-106.
15. **Ziff, E. B.** Enlightening the postsynaptic density. Neuron. 1997, **19**, 1163-1174.