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Drebrin expression is increased in spinal motoneurons of rats after axotomy

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Abstract

Drebrin has been known to act on actin filaments at dendritic spines to cause morphological change, and might be related to the plasticity of synaptic transmission. In the present study, changes of drebrin were examined immunohistochemically in the spinal motoneurons of rats following unilateral sciatic nerve transection. Drebrin-immunoreactivity (-ir) in the motoneurons was significantly increased on the lesioned side after 3 days. Confocal laser-scanning microscopic images of the motoneurons revealed conspicuous increase in drebrin in the submembranous region of the cells. After 10 weeks, drebrin-ir on the lesioned side decreased to a level not significantly different from that on the unlesioned side. The results suggested that drebrin played important roles in synaptic restoration in axotomized motoneurons.

Keywords: Drebrin; Motoneuron; Axotomy; Immunoreactivity; Synapse; Regeneration

Motoneurons become unresponsive to synaptic input after axotomy [19]. The loss of synaptic contacts on to axotomized neurons has been suggested to underlie this lost responsiveness [4,18]. Previous studies demonstrated that both glutamate and acetylcholine receptors are down-regulated in motoneurons after axotomy [11–13], and that the synaptic restoration occurs after muscle reinnervation is achieved again [3,5,18]. However little is known about the cellular mechanisms of synaptic stripping and restoration after axotomy.

Drebrin is an actin-binding protein expressing mainly in neurons (for reviews, see Refs. [14,17]). Drebrin was first described as a developmentally regulated brain protein whose expression is maximal during embryogenesis and decreases thereafter [15]. In the embryo, drebrin is accumulated in the somata of migrating neurons and in neurite processes of postmigratory neurons. In the adult brain, an embryonic type of drebrin is replaced by an adult type by alternative RNA splicing mechanisms [14], and the adult type of drebrin (drebrin A) was abundant in the cerebral cortex and hippocampus, but present only at low levels in the cerebellar cortex, pons medulla, and spinal cord [6]. Our recent studies indicate that drebrin is responsible for the dynamic remodeling of actin filaments in dendritic spines and alters the spine shape [7,8]. These findings suggest that drebrin plays a role in the structure-based plasticity of the synapse [6]. The present study investigated the change in drebrin expression in motoneuron after sciatic nerve transection to examine the roles of drebrin in axotomized motoneurons.

Twenty-eight female Wistar rats weighing 200–250 g were anesthetized with halothane. The right sciatic nerve was exposed at mid-thigh level and sectioned with scissors. Following recovery from surgery and survival for 1, 3, 7 days and 2, 4, 6 and 10 weeks ($n = 4$ for each survival date), the animals were anesthetized with an overdose of pentobarbital (75 mg/kg) and perfused transcardially with 4% paraformaldehyde in phosphate buffer. The lumbar spinal cords were then removed, postfixed by immersion in the same fixative for 30–60 min, rinsed in 10% sucrose, frozen, and sectioned at 14 μm on a cryostat. Sections were processed for immunohistochemistry with a monoclonal antibody against drebrin M2F6 [16], followed with secondary fluorescence-labeled anti-mouse antibody (Cappel, Durham, NC). The antibody M2F6 reacts with both the embryonic (drebrin E) and adult (drebrin A) isoforms of drebrin. Specimens were

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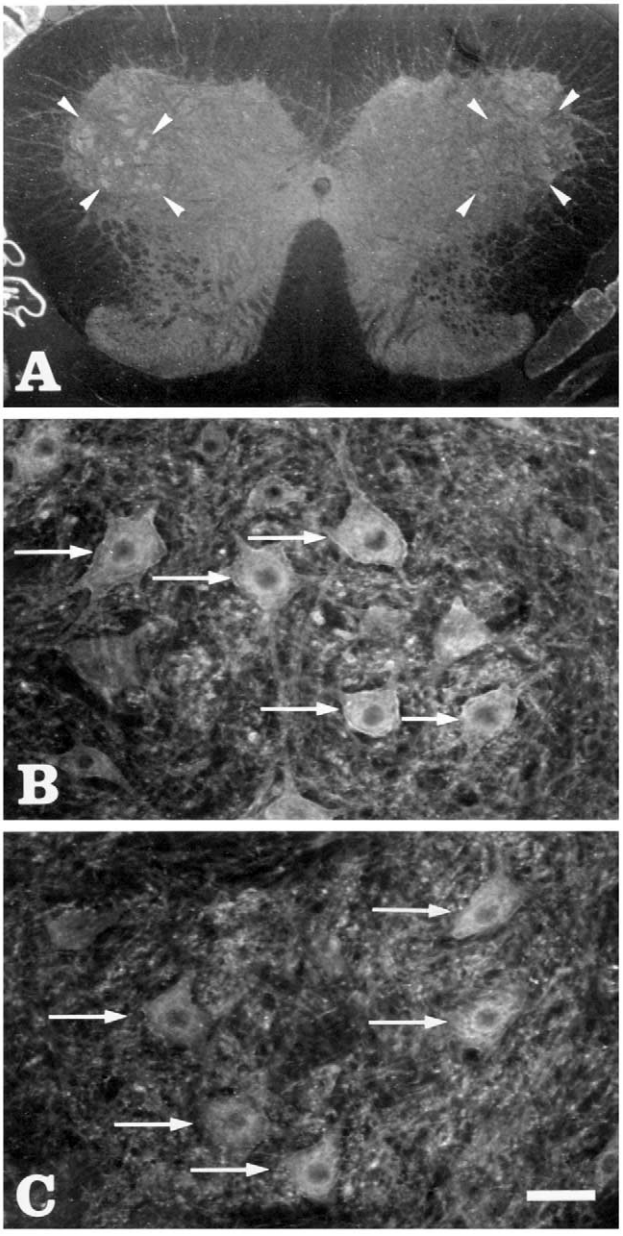

Fig. 1. Immunofluorescence micrographs demonstrating distribution of drebrin-ir in rat lumbar segments following unilateral sciatic nerve transection. (A) Low magnification micrograph showing the distribution of drebrin-ir on the lesioned (left side of the micrograph) and unlesioned side of the spinal cord at the L5/6 segment transition, 7 days after sciatic nerve transection. Arrowheads outline the regions occupied by dorsolateral motoneuron pools. Drebrin-ir was increased in the lesioned motoneurons. (B) Lesioned and (C) unlesioned side of a section immunostained for drebrin 7 days after sciatic nerve transection. Increase of drebrin was observed in neuropil and motoneurons giving axons to the sciatic nerve on the lesioned side (arrows in B), whereas motoneurons were weakly immunopositive on the unlesioned side (arrows in C). Scale bar, 400 μm (A), 50 μm (B,C).

observed under a confocal laser-scanning microscope (MRC1024; Bio-Rad, Richmond, CA). Drebrin-immunoreactivity (-ir) of motoneurons was measured in the sciatic pool of the lumbar sections 1, 3, 7 days and 10 weeks after the nerve transection. The density of drebrin-ir was measured with a computer-assisted imaging analysis system (NIH Image software) coupled with a CCD camera (8 bits/pixel) by an investigator without knowledge of which side was lesioned. Each density value ranging from 0 to 256, where 0 represents a completely black region and 256 a completely white region. Significance of differences in the density was determined with Mann–Whitney *U*-tests. A probability value of $P < 0.05$ was considered significant.

An antibody against drebrin (M2F6) stained gray matter of the spinal cord in normal control rats (data not shown). The densely populated neuropils were stained for drebrin. The white matter was not immunostained, but there was immunoreactivity along the neuronal processes adjacent to the anterior horns. Drebrin-ir increased in the motoneurons on the lesioned side from 3 days to several weeks after sciatic nerve transection (Fig. 1A). Strong drebrin-ir was observed to surround the neuronal somata and the proximal dendrites (Fig. 1B). Drebrin-ir in the cytoplasm of the neuron was also increased. On the unlesioned side, motoneurons were weakly immunopositive (Fig. 1C). The diffuse and coarsely granular drebrin-ir was demonstrated in the cytoplasm and the neuropils. The quantitative evaluation of the drebrin-ir demonstrated that the density values on the lesioned and unlesioned side were 63.1 ± 2.8 ($n = 46$) vs. 58.6 ± 2.4 ($n = 54$) after 1 day, 69.8 ± 2.4 ($n = 58$) vs. 55.6 ± 1.9 ($n = 47$) after 3 days, 77.4 ± 2.7 ($n = 60$) vs. 60.6 ± 1.9 ($n = 74$) after 7 days, 53.6 ± 1.4 ($n = 44$) vs. 54.1 ± 1.6 ($n = 52$) after 10 weeks, respectively (Fig. 2). All values are expressed as mean ± standard error of mean (SEM). Only a slight, not significant, tendency toward increase was seen on the lesioned side after 1 day. There was a significant increase in the drebrin-ir in the motoneurons on the lesioned side compared with that of those on the

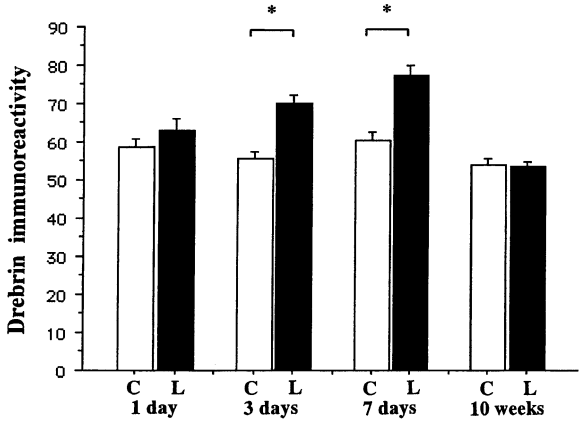

Fig. 2. Histogram showing the density values of drebrin-ir in motoneurons 1, 3, 7 days and 10 weeks after sciatic nerve transection. The results are expressed as mean density values on the unlesioned side ('control' side, C: open bars) and the lesioned side (L: filled bars). Asterisks indicate significant differences between the unlesioned and lesioned sides (Mann–Whitney *U*-tests; $*P < 0.001$, error bars = SEM).

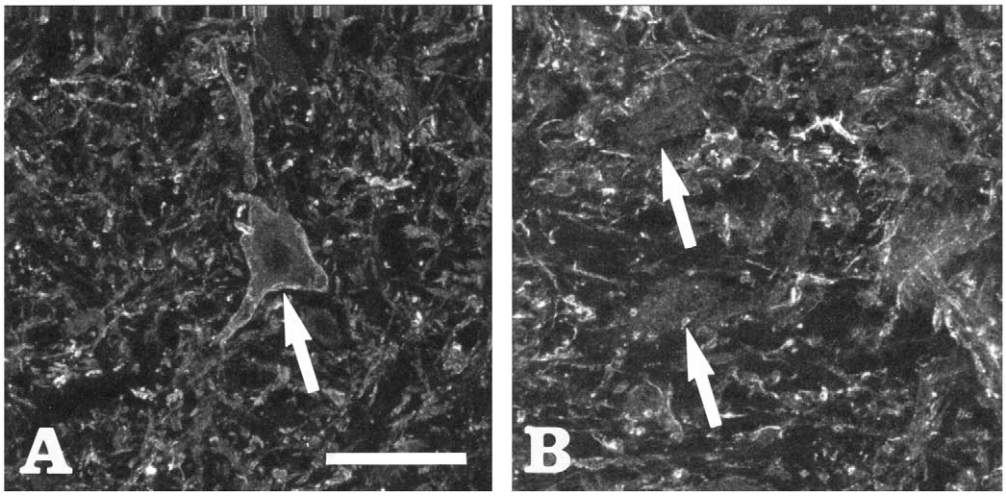

Fig. 3. Confocal laser-scanning microscopic images of (A) axotomized and (B) control motoneurons stained with an antibody against drebrin. (A) The conspicuous increase in drebrin was observed in the submembranous region of dendrites and cell body of motoneurons (arrow) 7 days after axonal transection. (B) Drebrin staining was observed as fine scattered dots in cell bodies of the control motoneurons (arrows). Each cell was identified by a differential-interference-contrast microscope. Scale bar, 50 μm.

unlesioned side after 3 and 7 days ($P < 0.001$; Mann–Whitney *U*-test). Drebrin-ir on the lesioned side decreased to a level not significantly different from that on the unlesioned side after 10 weeks.

Confocal laser-scanning microscopic images of the motoneurons revealed a conspicuous increase of drebrin in the submembranous region 7 days after axonal transection (Fig. 3A). Drebrin staining was observed as fine scattered dots in dendrites and cell body of the control motoneurons (Fig. 3B).

In the present study, we showed that drebrin expression in the motoneurons increased from 3 to several weeks after sciatic nerve transection, and returned to constitutive level by 10 weeks. The loss of synaptic terminals from the cell bodies of adult motoneurons within 1 week after axotomy is well documented [4]. Recent studies have demonstrated that down-regulation of glutamate receptors in motoneurons 3 days after axotomy are obvious using in situ hybridization and immunohistochemistry [11,12]. The electromicroscopic quantitative study revealed that the synaptic cover (length of synaptic terminal apposition per 100 μm motoneuronal cell body plasma membrane length) of motoneurons was reduced at 8–16 days after axotomy and returned to the control level 32 days following nerve crush [9]. Thus, the time course of increase in drebrin expression was largely coincident with that of synaptic loss and restoration in the lesioned motoneurons. Confocal laser-scanning micrographic findings in this study were in line with our previous study in the cerebral cortex, which reported the localization of drebrin at the synapses [6]. Drebrin is known to bind to actin filaments in the dendritic spines, causing morphological changes, which might be directly related to the plasticity of synaptic transmission [6,7]. These facts suggest that drebrin plays important roles in synaptic restoration in axotomized motoneurons.

Post-synaptic density (PSD) contains receptors, signal transducing proteins and cytoskeletal proteins [1]. Drebrin, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and α-actinin-2 are highly dependent on F-actin for their localization, whereas NMDA-type glutamate receptors and post-synaptic density-95 (PSD-95) are independent. Signals of mRNA for NMDA-type glutamate receptors and PSD-95 in the motoneurons decreases following axotomy [3,11]. By contrast, drebrin expression increased rapidly in response to axotomy, suggesting that the alteration of the microfilament arrays by the actin-associated components of PSD is one of the most crucial processes for synaptic regeneration.

Previous studies indicate that synaptic restoration depends on muscle reinnervation [5,18]. Injury to the axons of facial motoneurons stimulates increases in the synthesis of actin, tubulin and GAP-43 and decreases in the synthesis of neurofilament proteins [2]. These changes in cytoskeletal protein synthesis following axotomy are regulated by a variety of factors including loss of target-derived trophic factors. A recent report demonstrated that calcitonin gene-related peptide in motoneurons is upregulated after axotomy, and its expression is regulated by trophic factors such as fibroblast growth factors [10]. It is possible that muscle-derived trophic factors regulate drebrin expression in the axotomized motoneurons.

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