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NMDA receptor triggered molecular cascade underlies compression-induced rapid dendritic spine plasticity in cortical neurons

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ABSTRACT

Compression causes the reduction of dendritic spines of underlying adult cortical pyramidal neurons but the mechanisms remain at large. Using a rat epidural cerebral compression model, dendritic spines on the more superficial-lying layer III pyramidal neurons were found quickly reduced in 12 h, while those on the deep-located layer V pyramidal neurons were reduced slightly later, starting 1 day following compression. No change in the synaptic vesicle markers synaptophysin and vesicular glutamate transporter 1 suggest no change in afferents. Postsynaptically, N-methyl-D-aspartate (NMDA) receptor trafficking to synaptic membrane was detected in 10 min and lasting to 1 day after compression. Translocation of calcineurin to synapses and enhancement of its enzymatic activity were detected within 10 min as well. These suggest that compression rapidly activated NMDA receptors to increase postsynaptic calcium, which then activated the phosphatase calcineurin. In line with this, dephosphorylation and activation of the actin severing protein cofilin, and the consequent depolymerization of actin were all identified in the compressed cortex within matching time frames. Antagonizing NMDA receptors with MK801 before compression prevented this cascade of events, including NR1 mobilization, calcineurin activation and actin depolymerization, in the affected cortex. Morphologically, MK801 pretreatment prevented the loss of dendritic spines on the compressed cortical pyramidal neurons as well. In short, we demonstrated, for the first time, mechanisms underlying the rapid compression-induced cortical neuronal dendritic spine plasticity. In addition, the mechanical force of compression appears to activate NMDA receptors to initiate a rapid postsynaptic molecular cascade to trim dendritic spines on the compressed cortical pyramidal neurons within half a day.

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Introduction

Plasticity of cortical circuitry was once thought to occur primarily during early postnatal development. Subsequent discovery that large-scale functional reorganization of somatosensory map can be achieved following peripheral nerve injury (Garraghty and Kaas, 1991) demonstrates plasticity in adult cortex as well. In the adults, functional remapping might occur simultaneously in subcortical nuclei that relay the message to and from the affected cortex as well. These demonstrate the profound influence of cortical circuitry changes. In this regard, trauma or disease that compromise brain functions is likely to have caused cortical circuitry changes. For instance, the mechanical pressure generated by intracranial swelling or hematoma can have dramatic and permanent influences on the body and can be fatal if unattended. Meningioma and diseases could cause chronic compression of the brain (Spacek, 1987; Watanabe et al., 2001) In the case of cerebral compression, patient's vital functional abnormalities, Glasgow Coma Scale, hematoma volume, presence of comorbidity and degree of midline shifting are indications of surgical intervention (Bullock et al., 2006), however, whether and when to intervene surgically remain ambiguous. Early surgery presents benefits but remains controversial (Lee et al., 1998) and there is no consensus on the time frame of what constitutes early surgery. In this regard, understanding the acuteness and the extent of the effects of compression on cerebral cortex at the cellular and molecular levels is eagerly awaited. Using rats as a model, we demonstrated that epidural compression of the somatosensory cortex distorted the dendritic arbors of the underlying cortical pyramidal neurons instantaneously and neurons underwent dramatic dendritic reorganization in 3 days (Chen et al., 2003, 2010a). In addition, dendritic spines on these neurons were also reduced within 3 days (Chen et al., 2003), which was irreversible with delayed decompression (Chen et al., 2004). Since dendritic spines are the sites of excitatory neurotransmission (Gray, 1959) on pyramidal neurons, the main output neurons, of the cerebral cortex, their irreversible loss suggests long-term alteration of neuronal circuits and cognitive dysfunction (Segal, 2005) in patients experienced cerebral compression.

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Appropriate presynaptic activity is crucial to maintain dendritic spines and reduction or excess presynaptic activity can lead to dendritic spine retraction (Rutledge et al., 1972). Loss of presynaptic elements due to deafferentation leads to the elimination of dendritic spines (Globus and Scheibel, 1966). Augmentation of presynaptic glutamate release activates glutamate receptors and increases calcium influx and could lead consequently to the collapse of dendritic spines (Halpain et al., 1998). This shows that alteration of spines structurally could be initiated by changes in neuronal calcium concentration driven by presynaptic and postsynaptic neuronal activity (Segal et al., 2000). Pruning of dendritic spines could be an active process involving the activation of N-methyl-D-aspartate (NMDA) receptors and consequent alteration of postsynaptic activity (Bock and Braun, 1999; Halpain et al., 1998). In addition, NMDA receptors (NMDAR) have been shown to be critical to the regulation of synaptic activity and determining the morphology of dendritic spines (Fox et al., 1996; Huang and Pallas, 2001). Synaptic NMDARs are localized to postsynaptic densities (PSDs) and involved in the induction of long-term potentiation and long-term depression (Hunt and Castillo, 2012). NMDAR are glutamate receptors comprising of two essential NR1 subunits and two modulatory NR2 subunits (Paoletti and Neyton, 2007). Besides being ligand-gated, NMDAR are mechano-sensitive and can be stimulated by changes of membrane tension (Casado and Ascher, 1998). Activation of NMDAR located on dendritic spines is expected to alter dendritic spine calcium concentration, which can activate calcineurin (CaN), also known as protein phosphatase 2B (Pallen and Wang, 1985), to regulate the phosphorylation status of molecules in dendritic spines. Among these, activated CaN can dephosphorylate and consequently activate the actin severing protein cofilin (Kurz et al., 2008) to ultimately cause the depolymerization of actin filaments (F-actin) (Kurz et al., 2008), the principal cytoskeleton of dendritic spine, into soluble monomeric globular actin (G-actin). The disruption of cytoskeleton leads eventually to the disruption and retraction of dendritic spines. Thus, regulating the trafficking and activation of NMDAR in the event of central neuronal injury could be a way to modulate central neuronal morphology and consequently their functions. In this regard, whether and how compression modulates NMDAR on cerebral neurons is the issue we explored in the present study.

In this study, we used a rat epidural bead implantation model to mimic the compression of cerebral cortex in diseases (Chen et al., 2003). We studied the mechanisms underlying the rapid reduction of dendritic spines of cortical neurons under compression. Both the synaptic vesicle marker synaptophysin and the glutamate packaging protein vesicular glutamate transporter 1 (VGLUT1) were examined to find out whether compression alters presynaptic inputs and/or activities. Synaptoneurosomes containing both pre and post-synaptic components were prepared from the compressed cortex and studied for compression-induced postsynaptic changes in the dendritic spines including NR1, CaN, G-actin and F-actin expressions. In addition, we also assessed the activity of the CaN and the phosphorylation status of the cofilin in the compressed tissue over time. These allowed us to find out whether this cascade of molecular changes occurred at a time course compatible to that of the reduction of dendritic spines on cortical pyramidal neurons following compression. Results from the present study will not only clarify the pathophysiological changes but also reveal the molecular processes and time windows for treatment/intervention consideration in the cerebral cortex under compression.

Materials and methods

Young adult male Wistar rats (Charles River strain, Animal Center of the Medical College of National Taiwan University), 8 weeks old were used in this study. Animal experiments were approved by the Animal Care and Use Committee of the Tzu Chi University and the rats were housed and cared for accordingly. All efforts were made to minimize animal suffering during and following surgery.

Experimental designs and groups

Three lines of experiments were conducted. First, to investigate the time course of compression-induced reduction of dendritic spines using intracellular dye injection technique, normal, sham-operated and epidural-bead-implanted rats surviving from 3 h to 3 days post-surgery were studied. Details of animal groups and survivals were listed in Table 1. At the end of the survival, rats were processed for intracellular dye injection as described below.

The second part investigated whether blocking NMDAR withholds the compression-induced reduction of dendritic spines. Animals were treated with 2 doses of the noncompetitive NMDAR antagonist MK801 (dizocipline 1 mg/kg; Sigma-Aldrich, St. Louis, MO) intraperitoneally, one at 20 min before bead implantation and another 2 days after compression. This dosage of MK801 was reported to result in no significant neuronal deficits when applied to rat cerebral cortex under compression (Yang et al., 2006). Animals were processed for intracellular dye injection 3 days following compression. Sham-operated and/or vehicle-treated rats served as the controls. Animal numbers in this part of the study are shown in the right 4 columns of the upper half of Table 1. Since data from sham-operated rats treated with or without vehicle were indistinguishable, they were pooled in the analyses. Similarly, data from rats subjected to 3 days of compression with or without vehicle treatment were also pooled for analyses.

The third part of the experiments used biochemical methods to investigate the cellular mechanisms underlying the compression-induced retraction of dendritic spines. Rats were divided into groups and the expressions of a number of proteins in total tissue lysate and in subcellular fractions including synaptoneurosomes, cell-surface membrane and PSD-associated fractions of the synaptoneurosomes, and crude synaptosomal plasma membrane (SPM) fraction were studied. Groups and survivals of the rats were listed in Table 1. In this part of the experiments, the effects of MK801 pretreatment, a single dose before compression (dosage similar to that described above), on the expressions of several dendritic spine retraction-related proteins were also studied. Please see the lower far right part of Table 1 for animal groups and numbers.

Data from the sham-operated 3-hours-survival rats with and without saline treatment were indistinguishable and hence pooled for subsequent analyses. Similarly, sham-operated and 3-days-survival rats with or without saline treatment were also pooled respectively for analyses.

Epidural bead implantation over the somatosensory cortex

We used the rat epidural bead implantation model to focally compress the cerebral cortex (Chen et al., 2003). A hemispherical polyethylene bead 1.5 mm in thickness and with a flat surface area of 5.0 mm in diameter was used to compress the cortex. For implantation, rats were anesthetized with intraperitoneal injection of 30 mg/kg tiletamine plus zolazepam (Zoletil 50®; Virbac, Carros, France) and 8 mg/kg xylazine (Rompun; Bayer, Leuverkeusen, Germany) and then mounted on a stereotactic device. The scalp was incised and an elliptical opening on the skull over the right somatosensory cortex was carefully made with a drill. The plastic bead with the flat surface up was slid into the epidural space to its final destination with the center of the bead located approximately 2.0 mm caudal to the bregma and 2.5 mm lateral to the midline. Sham-operated rats were subjected to craniotomy without bead implantation.

Intracellular dye injection and visualization of the studied neuron

To prepare the brain for intracellular dye injection, rats were deeply anesthetized and subjected to intracardiac perfusion with 2% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4. The brain was immediately sectioned with a vibratome into 350-μm-thick coronal slices. Slices were pre-treated with 10^{-7} M 4′, 6-diamidino-2-phenyl-indole

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