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Neuroprotective effects of mexiletine on motor evoked potentials in demyelinated rat spinal cords

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ABSTRACT

This study was conducted to whether the administration of mexiletine, a Na^+ channel blocker, impacts the recovery from demyelination. Under anesthesia, 0.1% ethidium bromide was injected into the dorsal funiculus (T3), followed by a mexiletine or saline treatment. Motor evoked potential (MEP) recordings and luxol fast blue stainings were performed at one, seven, 14, and 21 days post-operatively. Conduction was delayed during demyelination, but the mexiletine-injected group demonstrated shortened latencies and reductions in the demyelination area when compared to the control. These results suggest that systemic mexiletine plays a positive role in protecting neural tissues from demyelination.

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1. Introduction

During myelin damage, the corresponding axon is degenerated according to the severity of the disease. An important consequence of demyelination is the conduction block, which results in a variety of symptoms. Focal demyelinating lesions can be induced via an intraspinal injection of ethidium bromide (EB) in experimental models (Hegarty and Portenoy, 1994; Guazzo, 2005). Spontaneous remyelination can occur in demyelinated central nerve fibers, including multiple sclerosis (MS) plaques.

The acute functional deficits that occur following damage to the ascending afferent fibers in many species are poorly defined and not well-understood (Davidoff et al., 1989; Glendinning and Vierck, 1993). Studies of locomotion in decerebrate cat preparations have demonstrated that stimulation of either the mesencephalic locomotor or pontomedullary locomotor regions produces locomotive movements via pathways projecting to the pontomedullary medial reticular formation and the ventrolateral (VLF) or dorsolateral funiculus (DLF) (Shik and Iagodnitsyn, 1978; Noga, 1999). A previous study demonstrated that important pathways for locomotion may also exist in the dorsal white matter. Motor evoked potentials (MEPs) have been widely used both experimentally and clinically to study the functions of the motor system (Lee et al., 2005, 2007).

Some current studies have indicated that the sodium channel blocker protects axons as a result of its neuroprotective effects that lead to functional recovery following spinal cord injury (SCI) (Bryan et al., 2004; Ates et al., 2007). In this study, we aimed to determine whether the intraperitoneal administration of mexiletine, an antiarrhythmic and use-dependent Na^+ channel blocker, impacts the demyelination model in electrophysiological studies.

2. Materials and methods

2.1. Animals

Ninety-six adult male Sprague-Dawley rats (300–320 g) were used. The animals were kept at a constant temperature on a 12 h light/dark cycle with free access to food and water. All animal experiments were approved by the Institutional Animal Care and Use Committee of Yonsei University Health System.

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2.2. Experimental demyelination with ethidium bromide

Rats were anesthetized with intraperitoneal pentobarbital (50 mg/kg), and a dorsal laminectomy was conducted in the third thoracic vertebrae. Demyelination was induced with a 0.9 mm deep injection of 1 μL 0.1% EB solution into the dorsal funiculus through a fine-tipped glass pipette connected to a Hamilton syringe. More than 30 rats from the EB-injected group received 0.1 M mexiletine (1.0 mL/mg) via a daily intraperitoneal injection from immediately after EB injury to the day before the electrophysiological testing. In the control (SA + SA), a 1 μL saline injection was used in place of EB in the spinal cord and mexiletine in the intraperitoneal cavity.

2.3. Motor evoked potential recording

The rats were ventilated following the induction of urethane anesthesia at each experimental time point. A craniotomy of the right frontoparietal lobe was performed with a drill, and a laminectomy was conducted at T6. Rats were placed in a stereotaxic frame (Narishige Scientific Instrument Laboratory; Tokyo, Japan) and artificially respirated using a small animal respirator (Model 683, Rodent Ventilator, Harvard; Holliston, MA). The MEP was recorded from the dorsal spinal cord at T6 using a bipolar disc electrode (NE-120, David Kopf; Woodland Hills, CA), which was specifically designed to record evoked potentials from the cerebral cortex. The MEP was recorded (sweep time 10 ms, band pass filter 30–3000 Hz, average 100–300×) following stimulation of the right sensorimotor cortex (2 mm posterior and 2 mm lateral to bregma) using another disc electrode (stimulating intensity 0.1–10 mA, 0.1 ms duration, 1–4 Hz repetition rate). A ground electrode was inserted into the tail. The effect of electrical stimulation with intensity of 6 mA on MEPs was analyzed in the wave forms by latencies. The latencies of MEPs were classified as Initial (initial rising phase of MEP), N1 (first negative peak), and P1 (first positive peak).

2.4. Histological examination

Following the MEP recording, each animal was deeply anesthetized, and the heart was perfused with 350 mL of 4% glutaraldehyde in a phosphate buffer (pH 7.4). The lesioned spinal cord was immediately removed and placed in 4% glutaraldehyde for 2 h, followed by 30% sucrose in PBS overnight. A serial section of the spinal cord was obtained using a cryostat (10 μm thick; HM500V, Microm; Walldorf, Germany). In order to identify the lesion site as well as demyelination or remyelination, selected sections were stained with Luxol Fast blue (LFB) which stained myelinated fibers. The spinal cord tissue was then incubated at 60 °C for two h in 0.1% LFB dissolved in 95% ethanol and 10% acetic acid. Stained sections were differentiated in 0.05% lithium carbonate and 70% ethanol. Sections were counterstained with cresyl violet, dehydrated, and then mounted. The myelinated areas of the dorsal column in 5 sections with a 0.25 mm interval from each tissue were automatically measured using the MetaMorph Imaging System (Universal Image Co., Downingtown, PA, USA). The percent of the lesion area was calculated: (% lesion area) = (total dorsal column area − area stained by LFB)/total dorsal column area) × 100.

2.5. Statistical analysis

The mean values for latency were expressed as the means ± SEM. Mean values of the MEP peaks following electrical stimulation of various time lapses were tested with one-way ANOVA. Dunnett's post-hoc comparison was used to compare the experimental groups with the EB group. Histological data were analyzed by an independent *t*-test at each time point. *p*-values of less than 0.05 were considered significant.

3. Results

3.1. Motor evoked potentials during acute demyelination one day following EB injection

Each group of 10 injured rats was post-operatively recorded after one, seven, 14, and 21 days. We observed two types of ascending evoked potential waveforms: negative (N1) and positive (P1) peaks. The MEP latency reflected the time between the onset of the stimulus artifact and the initial rising phase of the first positive or negative peak. This latency period was compared with the initial, N1, and P1 peaks of the different groups for each recording time.

A representative waveform became evident for the different groups (Fig. 1A). We observed that the amplitude increased depending on the stimulus intensity. Serial recordings from the control (SA + SA) demonstrated high action potentials with low intensity stimulation, but no response was elicited from either EB-injected group with the same low stimulus intensity. During acute demyelination, the latency period for both EB-injected experi-

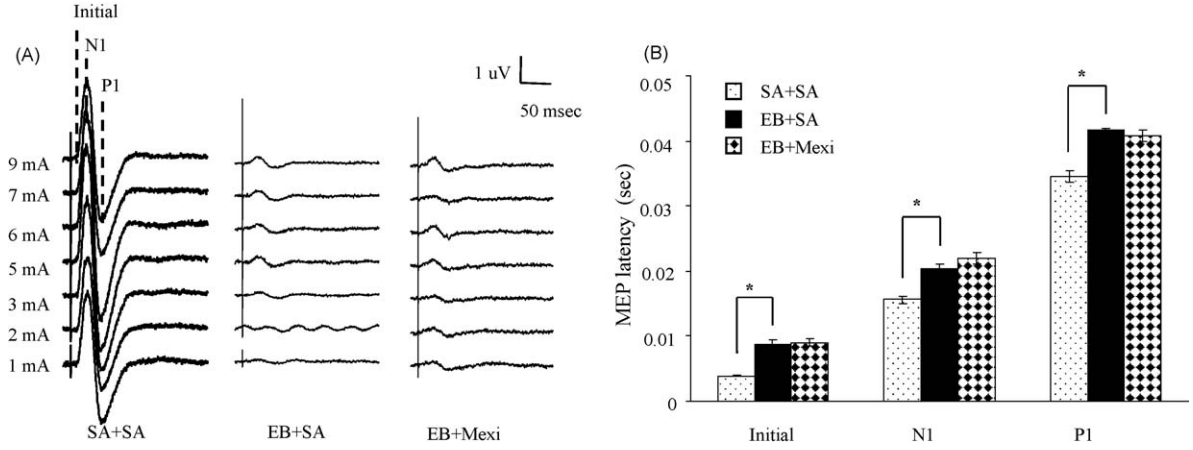

Fig. 1. Representative MEP waveforms and latencies one day after EB injury. The contralateral motor cortex was stimulated, and the MEP of the dorsal spinal cord at T6 was recorded using a bipolar disc electrode. Latency and amplitude were significantly reduced at one postoperative day in the EB- and EB + mexiletine-injected groups, but saline-injected animals exhibited minimal deficits (A). The EB-injected injury group demonstrated significant increases in latency compared to SA + SA (B, $^*p < 0.05$). Initial; initial rising phase of MEP, N1; first negative peak, P1; first positive peak, SA; saline, EB; ethidium bromide, Mexi; mexiletine.

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