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Research article

Acetyl-L-carnitine enhances myelination of regenerated fibers of the lateral olfactory tract

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HIGHLIGHTS

- The effects of acetyl-L-carnitine on myelination were investigated in lateral olfactory tract injury.
- The lateral olfactory tract was transected in neonatal rats.
- Acetyl-L-carnitine accelerates myelination of the regenerated fibers after lateral olfactory tract injury.

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ABSTRACT

It is well known that acetyl-L-carnitine (ALC) has various neuroprotective effects against neurodegenerative diseases. In addition, it has been reported that ALC facilitates myelination of regenerated axons after peripheral nerve injuries. We previously reported that spontaneous regeneration of the lateral olfactory tract (LOT), the main fiber tract of the central olfactory system, consistently occurred in newborn rats and a majority of these regenerated fibers were unmyelinated in neonatally LOT-transected young adult rats. To investigate the effects of ALC treatment on myelination in LOT, neonatal rats were treated with ALC after LOT transection. Immunohistochemistry for myelin basic protein showed more positive areas in ALC-treated rats than in control rats. Moreover, the number of myelinated axons of regenerated fibers was assessed using electron microscopy and was found to be statistically higher in ALC-treated rats compared to control rats. The study revealed that ALC accelerates myelination of regenerated fibers in neonatally LOT-injured young adult rats.

1. Introduction

L-Carnitine or acetyl-L-carnitine (ALC), the acetyl derivative of L-carnitine, has various neuroprotective effects against neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, ischemia, and traumatic brain injury [1–5]. Moreover, it has been reported that L-carnitine or ALC facilitates myelination of regenerated axons after peripheral nerve injuries [6–8]. In the central nervous system, L-carnitine or ALC is also reported to enhance myelin formation, but this finding is limited to a few studies [9,10].

The lateral olfactory tract (LOT) is the main fiber tract of the central olfactory system and exclusively consists of myelinated axon fibers. We have recently demonstrated that the neonatally transected LOT can be replaced by newly-formed axon fibers connecting the olfactory bulb and the olfactory cortex (the olfactory tubercle and piriform cortex) via the transected site at a later stage [11–14]. Because the regenerated LOT is primarily composed of unmyelinated axon fibers, LOT injury is a good animal model to examine the effects of ALC on myelin formation in the developing brain. Therefore, we used neonatal rats in our injury model of the central olfactory tract to investigate whether ALC accelerates myelination and increases the number of myelinated axon fibers in the regenerated LOT of young adult rats.

Abbreviations: ALC, acetyl-L-carnitine; FB, fast blue; LOT, lateral olfactory tract; MBP, myelin basic protein; P, postnatal day; SD, standard deviation.

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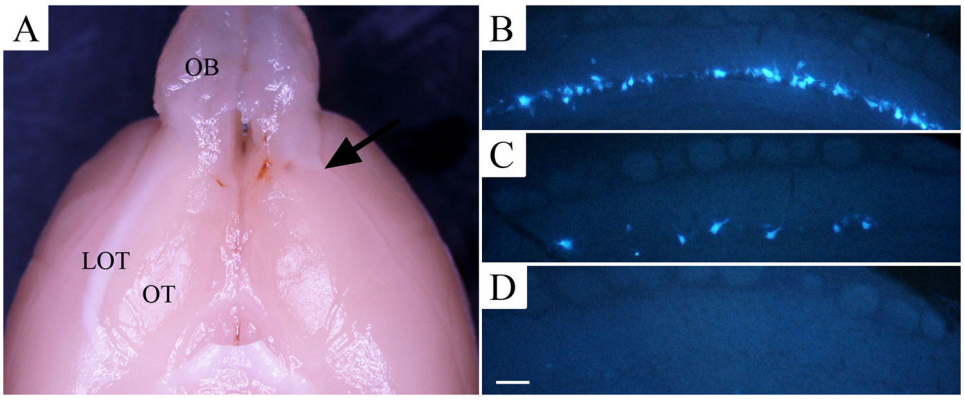

Fig. 1. The image shows the ventral view of a completely LOT-transected case (A). An arrow points to the transected site and the left transected LOT cannot be seen as a white myelinated band due to lack of myelination. LOT, lateral olfactory tract; OB, olfactory bulb; OT, olfactory tubercle. The images (B–D) show the bulbar mitral cell layers of incompletely LOT-transected cases (B and C) and a completely LOT-transected case (D). FB-positive mitral cells can be seen in the left olfactory bulb of incompletely LOT-transected cases (B and C), but they are absent in the completely LOT-transected case (D). Scale bar, B–D, 100 μm.

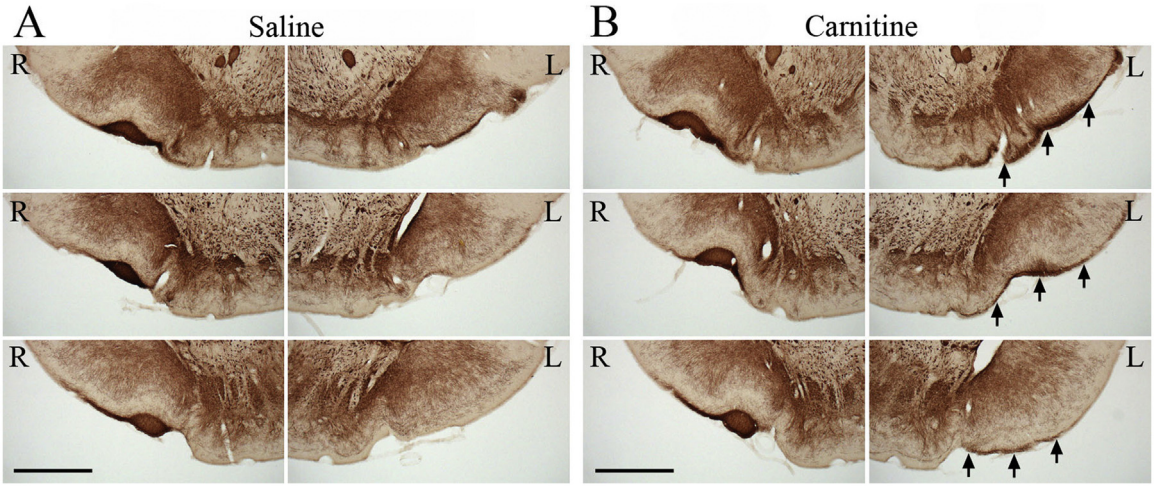

Fig. 2. The images show the sections processed for immunohistochemistry using an anti-MBP antibody from the control (A) and ALC-treated (B) rats. Upper, middle, and lower images are from the level of the rostral one-third, middle, and caudal one-third of the olfactory tubercle, respectively. Note the increase in MBP-positive myelinated fibers (arrows) on the left LOT-transected side in the ALC-treated rat. Scale bars, 1 mm.

2. Materials and methods

2.1. Animals

Newborn Wistar rat pups (Japan SLC Inc., Hamamatsu, Japan) of both sexes were used for this study. Postnatal day (P) 0 refers to the first 24 h after birth. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and protocols were approved by our Institutional Animal Care and Use Committee. All efforts were made to minimize the number of animals used and their suffering. Surgical manipulations were performed in hypothermic conditions using a freezer (−20 °C, 15–20 min).

2.2. LOT transection and retrograde tracer injection

LOT transection was performed in P2 pups ($n = 70$) unilaterally on the left side, as described previously [11–13]. Briefly, the LOT was transected at the posterior half of the olfactory stria by inserting the tip of a knife (Ophthalmic Scleral MVR Knife, 25 gauge; Alcon, Tokyo, Japan) from the ventrolateral aspect of the head. Immediately after LOT transection, a retrograde fluorescent tracer, Fast Blue (FB) (Polysciences Inc., Warrington, PA, USA), was injected into the left olfactory cortex to confirm the completeness of LOT transection. FB (1%, 0.1 μL) was injected into the posterior part of the olfactory cortex situated far caudal to the site of LOT transection. After surgery, the pups were housed with their dam in a single cage (26.0 × 42.0 × 18.0 cm) under standard laboratory conditions with a 12-h light/dark cycle and room temperature of 22 °C. Food and water were supplied *ad libitum*. In total, 14 pups that underwent LOT transection on P2 showed a continual decrease in body weight and died by P5.

2.3. ALC injection and tissue preparation

The LOT-transected rat pups were randomly divided into two groups, based on sex and weight, ALC-treated ($n = 28$) and control ($n = 28$) groups. Each rat in the ALC-treated group was injected intraperitoneally once daily from P5 to P20 with 100 mg/kg body weight of ALC (Sigma-Aldrich, Saint Louis, MO, USA). The rats in the control group were injected with an equivalent volume of saline. On P30, rats were euthanized with sodium pentobarbital (100 mg/kg, intraperitoneally) and perfused through the heart with 50 mL of 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were removed, postfixed for 2 days in the same fixative, soaked in 30% sucrose for 2 days, and divided into 2 regions (olfactory bulbs and other brain regions). Using a freezing microtome, 50 μm sagittal sections of the olfactory bulb were cut at 150 μm intervals (3 sets).