Nitric Oxide Synthase Inhibitor Facilitates Functional Recovery after Sciatic Nerve Injury[*]

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It has been shown that nitric oxide(NO) is a mediator of nerve injury or the physiological response to injury. The present study was conducted to determine whether NO synthase(NOS) inhibitor enhances functional recovery after nerve injury or not. Under pentobarbital anesthesia, male Sprague-Dawley rats were subjected to one of four different types of sciatic nerve injury (crush, cut, ligation, and sham injury models). The rats received i.p. injections of N^{ω}-nitro-L-arginine methyl ester(L-NAME), a NOS inhibitor, for ten days from the day of surgery. Twenty five days after injury, the rats were anaesthetized with urethane and the compound muscle action potentials(CMAPs) were recorded following sciatic nerve stimulation. L-NAME significantly shortened the latencies of the CMAPs in crush injury model compared to the vehicle treated rats. In the case of cut or ligation injury model, L-NAME tended to shorten the latencies but the differences between L-NAME and vehicle treated rats were not statistically significant. These results suggest that L-NAME facilitates functional recovery after sciatic nerve injury.

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Nitric oxide(NO) may act as a neurotransmitter (Lefebvre, 1995), and NO synthase(NOS) isozymes have been shown in the central nervous system (Paakkari & Lindsberg, 1995), the dorsal root ganglia(DRG). It has been shown that NO is also a mediator of nerve injury(Fiallos-Estrada, Kummer, Mayer, Bravo, Zimmermann, & Herdegen, 1993; Yu, 1994) or the physiological response to injury and may be responsible for abnormal neural activity and pain behavior in animals (Wiesenfeld-Hallin, Hao, Xu, & Hokfelt, 1993).

In the peripheral nervous system, NO and NOS have been studied on the effects of axonal injury. For example, after ligation of nerve roots, NOS activity was bilaterally decreased in the spinal cord and ipsilaterally increased in the DRG (Choi, Raja, Moore, & Tobin, 1996). Peripheral nerve sectioning resulted in an increase in NOS messenger ribonucleic acid(mRNA) in the corresponding DRG(Verge, Xu, Xu, Wiesenfeld-Hallin, & Hokfelt, 1992; Wiesenfeld-Hallin et al., 1993).

Alternatively, NO may be involved in regeneration of injured peripheral nerve(Zochodne, Misra, Cheng, & Sun, 1997). According to Gonzalez-Hernandez and Rustioni(1999a,b), NOS are overexpressed after rat sciatic nerve ligature and NOS activity lasts long in growth cones, suggesting that NO contributes to regeneration. However, conflicting results have also been reported. In a study, a NOS inhibitor enhanced regeneration of the proximal stump of transected sciatic nerve, suggesting that NO may have a negative impact on the regeneration(Zochodne, Levy, Zwiers, Sun, Rubin, Cheng, & Lauritzen, 1999). Therefore, the effects of NO on the regeneration following peripheral nerve injury is still unknown. The present study was conducted to determine whether NOS inhibitor enhances functional recovery after injury or not.

MATERIALS AND METHODS

Subjects and Surgery

A total of seventy five male Sprague-Dawley rats(Daehan Biolink, Korea) with body weights between 250-350g were used. Anaesthesia was induced by intraperitoneal injection of sodium pentobarbital(Entobar[®]). The left sciatic nerve was exposed where it emerged from beneath the middle gluteal muscle. Each rat received one of four different types of injury. For example, in the case of crush(crush group), the nerve was crushed at this marked point using a pair of forceps with 0.5-mm-wide tips. In the case of cut group, the left sciatic nerve was exposed and completely transected with fine surgical scissors. In ligation group, the nerve was tightly ligated with 4.0 silk thread. In sham group, the surgery was identical except for leaving the nerve intact.

The wound was closed in layers, and the rat allowed to recover from anaesthesia. During

recovery the rats were observed continuously until the righting reflex was restored and then returned to their home cage, where they were group housed.

The rats of each group were randomly assigned to receive a 10 day course of N^{ω}-nitro-L-arginine methyl ester(L-NAME, 10mg/kg in a volume of 0.1ml); 0.1ml; given once on the day of surgery then twice daily for 10 days intraperitoneally) or an identical course of its vehicle.

Electrophysiological Recording

Multifiber motor conduction studies of sciatic-tibial fibers were carried out under urethane anaesthesia(1.25g/kg) by stimulating the sciatic nerve at the proximal part to injury and recording the compound muscle action potentials with needle electrodes placed in the gastrocnemius muscle of the hind paw. The intensity of the stimulating current ranged from 0.1 to 1.0 mA. As 0.4 mA square pulses tended to produced consistent CMAPs, data from CMAPs evoked by 0.4 mA current were analyzed. For each measurement, the responses to 20 stimuli were collected and averaged using a CED 1401 interface and a 586 personal computer.

Statistical Analysis

Statistical analysis was performed on the raw data from CMAPs using the SigmaStat software package. Differences between groups(L-NAME vs. vehicle) in each injury type were tested with Student's t-test. Differences for which $p < 0.05$ were taken to be statistically significant.

RESULTS

Results of the electrophysiological studies are summarized in the figures. At 25 days, the latencies of the compound action potentials (CAPs) in the L-NAME treated rats tended to be substantially shortened than those of the vehicle treated rats, indicating functional recovery from nerve injury. Figure 1 shows representative wave forms of compound muscle action potentials evoked by stimulation of the sciatic nerve in each group with different types of nerve injury. The amplitudes of the CMAPs tended to be higher in the L-NAME treated rats than those of the vehicle treated rats. However, as the trend of changes in the amplitudes were not consistent, the data of amplitudes were not analysed statistically. In contrast, the latencies of CMAPs were consistent and analysed statistically.

In figure 2, the mean latencies of the CMAPs evoked by sciatic stimulation with 0.4 mA square pulses were compared. Generally, L-NAME appeared to shorten the latencies of the CMAPs. In crush injury model, the latencies of the CMAPs of L-NAME injected rats(n=11) were shortened significantly compared to those of the
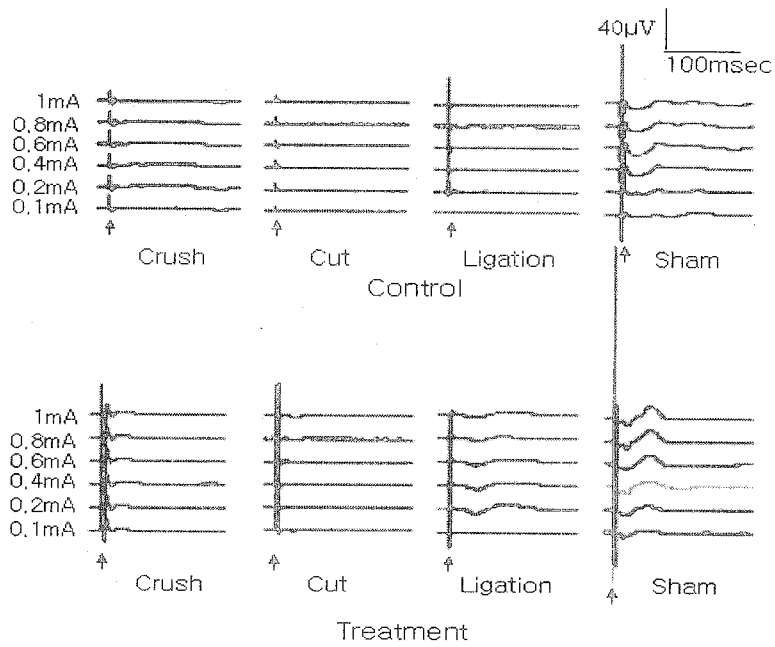
Figure 1. Representative wave forms of compound muscle action potentials from each group on day 25 p.o. Arrow indicates stimulus artifact.
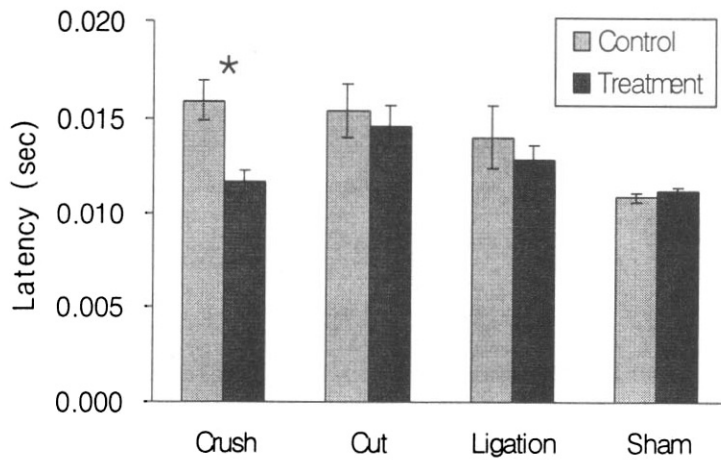

Figure 2. Comparisons of mean latencies of the compound muscle action potentials following sciatic nerve stimulation on p.o. 25 day. (*p<0.05).

vehicle treated rats (n=9) (t(18)=3.34, p<0.01).

In the case of cut injury model, the latencies of the CMAPs of L-NAME injected rats(n=9) tended to be shortened compared to those of the vehicle treated rats(n=10) but the differences were not significant(t(17)=0.41, p>0.05). In ligation injury model, L-NAME tended to shorten the latencies but the differences between L-NAME(10) and vehicle treated rats(8) were not statistically significant (t(16)=0.58, p>0.05). The latencies of sham operated rats were almost similar in both L-NAME(n=9) and vehible treated rats(n=9) (t(16)=0.95, p>0.05).

DISCUSSION

The findings in this work indicate that inhibition of NOS with L-NAME is associated with substantial functional recovery compared to the vehicle. The better outcome in the L-NAME group was indicated by more complete electrophysiological recovery in crush injury model.

In a similar study, the latency of the spinal cord potentials was prolonged by spinal cord compression and NOS inhibition significantly reduced this increase in latency(Suzuki, Tatsuoka, Chiba, Sekikawa, Nemoto, Moriya, Sakuraba, & Nakaya, 2001). These results suggest that normalization of the latency period might reflect the better recovery of the neurological function in rats with NOS inhibition.

In the present study, systemic administration of L-NAME significantly enhanced the functional recovery of sensory axons in the sciatic nerve of the rat after a crush injury, whereas there were no effects in the other injury model. This phenomenon is not easy to explain. Two hypothesis may be helpful to explain as in the role of L-NAME.

Firstly, because a crush injury causes only very local damage to the non-neuronal supporting cells and does not disrupt the endoneurial tubes, L-NAME may act on neuronal cell directly. In the absence of a gap, any enhancement of functional recovery by L-NAME is likely to be attributable to increased axonal extension, rather than due to an indirect effect mediated by enhancing the outgrowth of supporting cells. Secondly, however, L-NAME may also have indirect effects on neuronal cells, via an action on the non-neuronal supporting cells in peripheral nerve(Unsicker et al., 1993).

There is evidence that endothelial NOS (eNOS) is mainly produced by endothelial cells and may be produced by Schwann cells(Levy & Zochodne, 1998; Ma, Morita, & Murota, 1994).

Therefore, L-NAME has some beneficial effects on neuronal cells. The mechanism that L-NAME exerts its neuroprotective effects on injured neurons may be explained.

There is evidence that NO might reversibly inhibit growth cones which can guide axonal

regeneration(Hess, Patterson, Smith, & Skene, 1993). NO reacts with the superoxide anion to produce the oxidant peroxynitrite, with actions that could include lipid oxidation and nitration of proteins such as neurofilaments or Trk-receptors for neurotrophines(Dawson & Dawson, 1995; Radi, Beckman, Bush, & Freeman, 1991). Most of the neurotoxic actions of NO have been studied at the level of the cell body, where NO may act to cause DNA strand breaks, and activate ADP-ribose synthetase in turn, depleting cellular energy stores(Dawson & Dawson, 1995). Following axotomy, there is transcriptional upregulation of NOS in DRG neurons and there is also increased expression in motor neurons after root avulsion (Verge et al., 1992; Wu & Li, 1993). In the latter model, NOS expression preceded motor neuron death(Wu, Liuzzi, Schinco, Depto, Li, Mong, Dawson, & Snyder, 1994).

In separate work from proximal nerve stumps and chronic constriction nerve injuries, Zochodne et al.(Levy & Zochodne, 1996; Zochodne & Nguyen, 1997) have found evidence for the presence of NO just proximal to the zone of acute axonal degeneration from nerve transection or ligation. Non-specific inhibition of NOS, using L-NAME reduced hyperaemia at this site at 48 hours and 14 days following the creation of the injury in both models. Immunohistochemistry identified the increase of NOS staining following injury. The work has suggested that there is local generation of NO at the distal tip of proximal nerve stumps of transected nerves.

In accordance with these studies, L-NAME as a NOS inhibitor has been shown to enhance regeneration of the proximal stump of transected sciatic nerve(Zochodne, et al., 1999). There is also evidence that the demyelinization can be attenuated by NOS inhibition(Suzuki et al., 2001). Therefore, L-NAME may exerts neuroprotective effects resulting in functional recovery by enhancing regeneration or inhibiting demyelination.

However, to understand the precise role of NO or NOS inhibitors in functional recovery is not easy. For example, it is unclear exactly when L-NAME was of benefit after injury, that is time window of beneficial effects. Further study is required to understand detailed mechanisms of NO or NOS inhibitors in response to nerve injury.

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좌골신경 손상 후 Nitric oxide synthase inhibitor의 주입에 의한 기능회복 촉진 효과

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Nitric oxide(NO)는 손상에 대한 생리적 반응 또는 신경 손상을 매개하는 물질로 알려져 왔다. 본 연구는 NO synthase(NOS) inhibitor가 신경손상 후 기능회복을 증진시키는가 아닌가를 규명하기 위해 수행되었다. 수컷 Sprague-Dawley종 흰쥐를 pentobarbital로 마취하고 압착(crush), 절단(cut), 결찰(ligation), 모의(sham) 손상 모델 등 네 가지 유형으로 좌골신경을 손상시켰다. 실험동물은 수술한 날부터 시작하여 10일 동안 매일 NOS inhibitor인 N^{ω}-nitro-L-arginine methyl ester (L-NAME)을 복강내에 주사하였다. 신경손상 후 25일째 되는 날 쥐를 urethane으로 다시 마취하고, 좌골신경의 자극에 따른 복합근활동전위(compound muscle action potentials; CMAPs)를 기록하였다. 그 결과 L-NAME은 crush 손상 모델에서 용매를 주입한 통제집단에 비해 CMAP의 잠재기가 유의미하게 단축되었다. 절단이나 결찰 손상모델에서, L-NAME은 잠재기를 단축시키는 경향이 있었지만 L-NAME과 용매를 주입한 동물들간의 차는 유의미하지 않았다. 이러한 결과는 L-NAME이 좌골신경손상후 기능회복을 촉진시킨다는 것을 시사한다.