Ultrastructural and Neurophysiological Changes Observed Following Injection of Morphine, Meperidine and Pentazocine in the Sciatic Nerves of Rabbits

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= 국문 초록 =

가토의 좌골신경에 Morphine, Meperidine, Pentazocine을 주사한 후 미세형태학적 및 신경생리학적 변화

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Morphine, meperidine 및 pentazocine을 가토의 좌골신경에 주입한 후 마약제의 신경차단 유무와 약제 주입 후 4시간, 24시간 및 1주에 좌골 신경을 절취하여 신경조직학적 변화를 관찰하였다.

좌골신경에 약제를 주입한 후 신경자극에 의한 반응과 뒷다리 운동을 관찰한 결과, morphine군은 신경차단 효과가 없었고 meperidine군과 pentazocine군은 약제주입 5분 후부터 근육이완이 시작되어 10분 후부터 근육수축이 나타나지 않았으며 뒷다리에 마비증상은 약제주입 60분 후부터 부분적으로 회복되기 시작하여 90분 후에는 정상으로 회복되는 양상의 신경차단 효과가 있었다.

광학 현미경적 소견으로는 모두 4시간부터 1주까지의 표본에 특기할만한 변화가 없었으며, 전자 현미경적 소견에서 morphine군은 1주 후 소견에서 유수신경섬유와 무수신경섬유에 경미한 수포양을 보였다. Meperidine군은 4시간 후 소견으로 유수신경섬유의 축삭돌기에 경미한 수포양이 있었고 무수신경의 마이엘린화되는 소견이 있었으며, 24시간 후 유수신경섬유에 경미한 수포양이 있었고 무수신경섬유가 정상으로 되었으며 1주 후 특기할 만한 변화가 없었다.

Pentazocine군은 약제주입 4시간 후 유수신경섬유에 경미한 수포양을 보였으며 24시간 후 유수신경섬유와 무수신경섬유에 중등도의 수포양이 나타났으며 1주 후 경미한 수포양을 나타내었다. 주입된 약제 중 morphine이 가장 수포양이 적었으며 pentazocine이 더 심한 변화를 나타내었고, 전단계 쥐 실험에서 나타났던 meperidine주입 1주 후의 심한 신경조직 손상은 본 실험에서 나타나지 않았다.

Key Words: Narcotics, Peripheral nerve, Neurophysiological, Histological change

INTRODUCTION

Since the findings of the first opiate receptors[1], several subtypes have been identified and the subtypes' functions differed by the receptors[2]. It has been reported that a small amount of morphine injected into the subarachnoid space prolongs analgesia[3], and epidural morphine has also been known to have a similar effect[4].

Administration of intraspinal(intrathecal or epidural) narcotics has been widely used and has been well acknowledged for pain manage-

ment. The action mechanism of intraspinal narcotics has been well defined; the furthermore, meperidine and pentazocine have been reported to be used as good anesthetic agents for spinal anesthesia[5~9]. The effectiveness of meperidine and pentazocine as local anesthetics in patients scheduled for various surgeries was also reported[5~9]. The effectiveness of meperidine and pentazocine as local anesthetics in patients scheduled for various surgeries was also reported[5~9]. These reports were followed by experimental evaluation of the toxic effects of the above mentioned narcotics on the sciatic nerves of rats[10] as well as dogs[11]. There was a difference, however, between the experimental results in the nerve tissue of the rats and the dogs. Severe fatty degeneration of the cytoplasm and severe vacuolar changes of the axon were revealed one week after injection of meperidine in the rat sciatic nerve but not in the dog sciatic nerves.

To evaluate the differences in our preliminary experiments, the objective of this study was to examine the early histologicl changes of the nervous system and the acute effects of the neurophysiological action within one week after injection of morphine, meperidine, and pentazocine into the sciatic nerves of rabbits.

METHODS

This study used twenty seven adult rabbits, weighing an average of 800 to 1,000 gm and were divided into three groups. The protocol was approved by the hospital ethics committee. All the rabbits were anesthetized intramuscularly with ketamine 10 mg/kg. Their hindquaters were shaved and 5 cm incisions were made over the anterior head of the biceps femoris and just to the greater tuberosity of the femur. After carefully separating the head of the biceps from the gluteus maximus and the vastus lateralis, the sciatic nerves were exposed.

1) Neurophysiological test

The exposed sciatic nerves were stimulated using 8 mA, 4 times per second by a nerve stimulator(WR medical electrode) to observe myoneural responses. The nerves were then raised by a curved forceps under a microscopic field without causing trauma, and isolated sciatic nerves were injected a total amount of 0.2 ml of 0.1% morphine in Group 1, 0.5% meperidine in Group 2, and 0.3% pentazocine in Group 3 using a 25-gauge needle and a tuberculin syringe. The injection site was identified by applying a 6-0 black silk suture into the adjacent muscle. The sciatic nerves were stimulated for 20 minutes at 5 minute interval, and gait changes were carefully observed in the recovery room in order to see the myoneural activity. After neurophysiological evaluation, muscles were sutured with a 2-0 chromic and skins were sutured with a 3-0 silk.

2) Nerve biopsy and histological preparation

For histological evaluation, three rabbits in each of the three groups were again anesthetized and the sciatic nerves were exposed using the same above described method. The previously drug-injected and identified sciatic nerves were severed and approximately a 1 cm length was removed in all three groups at 4 hours, 24 hours, and 1 week after injection respectively.

The severed nerves were prefixed with 2.5% glutaraldehyde solution in 1 mm strips and postfixed with 1% osmium tetroxide, dehydrated with a series of graded ethanol, changed with propylene oxide, and embedded in Epon 812 (Shell Chemical Co). The semithin sections(1 μm) were stained with toluidine blue and examined

with a light microscope. For electron microscopic studies, the ultrathin sections(60~90 nm) were done by a ultramicrotome(Sorvall MT 5,000) with a Dupont diamond knife and were stained with uranyl acetate[12] and lead citrate[13], and were examined with a Hitachi H-600 electron microscope.

RESULTS

1) Neurophysiological changes

After the drug injections, the distal part of the sciatic nerves were stimulated at an interval of 5 minutes by a nerve stimulator for 20 minutes. After recovery from anesthesia, the gait of the hind limbs was observed. The normal muscle twitching responses were clearly observed and the gait of the hind limb was normal in all the observation phases of the group injected with morphine(Group 1). However, in the groups injected with meperidine(Group 2) and pentazocine(Group 3), muscle twitching responses decreased gradually and finally disappeared after about 10 minutes. Complete motor paralysis of the hind limbs continued for approximately 60 minutes, and muscle reactions returned to normal approximately 90 minutes after injection. Except for these initial myoneural dysfunctions in group 2 and 3, other myoneural abnormalities were not noticed in all three groups during a one week period.

2) Neurohistological changes

Specimens of drug-injected sciatic nerves were severed at 4 hours, 24 hours, and 1 week respectively after injection. These specimens were prepared and examined under light as well as electron microscopy. The results are summarized in Table 1. Observations under the light microscope showed no changes in all the three groups; however, observations under the electron microscope showed slight differences in each groups. In the group injected with morphine(Group 1), there were no specific changes at the observation checking points of 4 hours and 24 hours respectively after injection, but there were rare vacuolizations in the axons one week after injection(Fig. 1, 2). In the group in-

Table 1. LM and EM Findings of Rabbit's Sciatic Nerve after Injection of Morphine, Meperidine and Pentazocine

Group / Time interval		Morphine	Meperidine	Pentazocine
LM	4 hrs			
	24 hrs	Unremarkable	Unremarkable	Unremarkable
	1 wk			Axon of MN: rare vacuolization
EM	4 hrs	Unremarkable	Axon of MN: rare vacuolization	
			UMN: myelin figure	UMN: moderate vacuolization
	24 hrs	Unremarkable	Axon of MN: rare residual vacuoles	
			UMN: restored to normal	
	1 wk	Axons of MN and UMN Unremarkable		Axon of MN: rare residual vacuoles
		: rare vacuolization		UMN: moderate vacuolization

LM: light microscopic
EM: electron microscopic
MN: myelinated nerve fiber
UMN: unmylinated nerve fiber

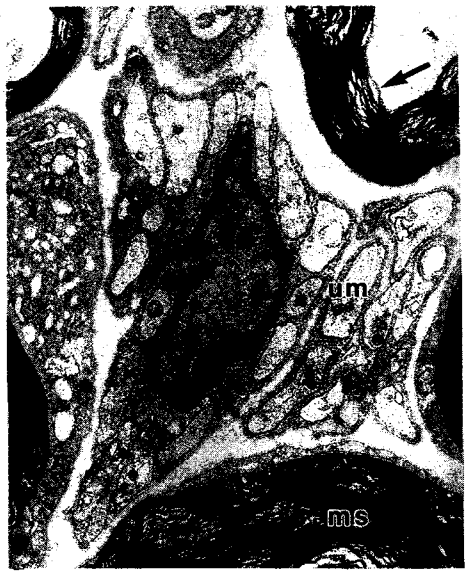

Fig. 1. Control. Rabbit sciatic nerve. Unmyelinated nerve(um) showing nothing of note. Myelinated nerve shows severe artefact of separation of myelin lamellae in myelin sheath (arrow)(×17,000)

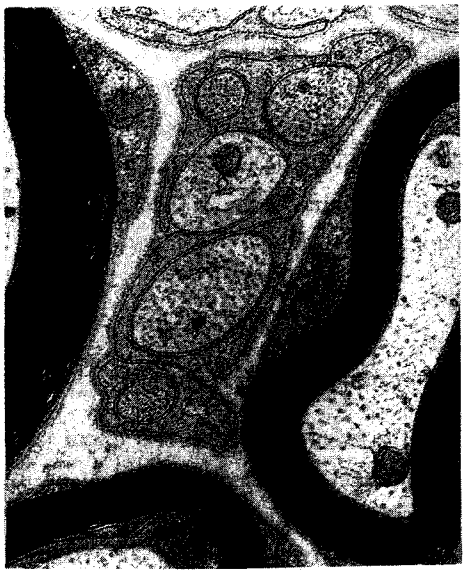

Fig. 2. Rabbit sciatic nerve, one week after morphine injection. The unmyelinated nerve (middle) to show a rare vacuolar change whereas the myelinated nerve(right) to be unremarkable(×17,000).

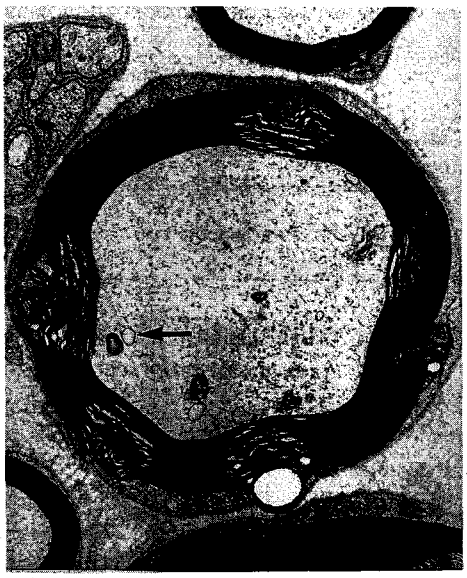

Fig. 3. Rabbit sciatic nerve, treated with meperidine and sacrificed 4 hours later, revealing myelinated nerve to show a rare vacuolization(arrow). The myelin sheath shows a severe artefact(×17,000).

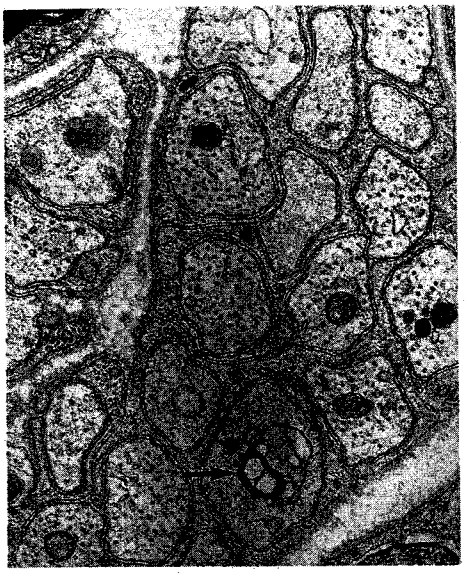

Fig. 4. The unmyelinated nerve of the sciatic nerve of the rabbit, treated with meperidine and sacrificed 4 hours later, showing one myelin figure(arrow)(×17,000).

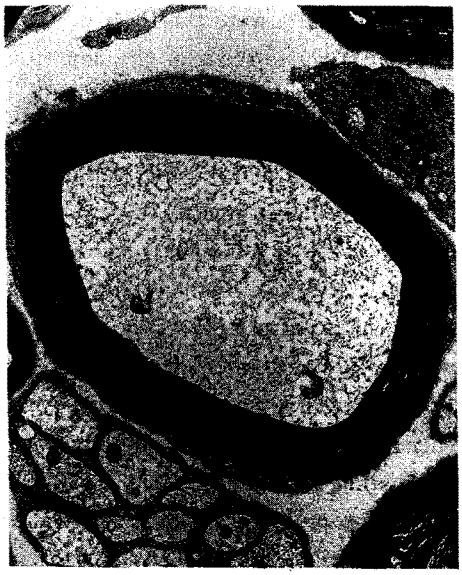

Fig. 5. Rabbit sciatic nerve, treated with meperidine and sacrificed 1 week later, showing the myelinated nerve to be unremarkable(× 17,000).

Fig. 6. Rabbit sciatic nerve, 24 hours after pentazocine injection. Axons of the unmyelinated nerve to reveal a moderate vacuolar changes(×34,000).

jected with meperidine(Group 2), there were rare vacuolizations in the myelinated axons at the observation checking point of 4 hours after injection and that lasted to the next observation checking point of 24 hours(Fig. 3). Myelin figures in the unmyelinated axons were revealed at the 4 hour checking point and restored to normal at the 24 hour checking point(Fig. 4), and at the 1 week checking pont there were no specific findings(Fig. 5). In the group injected with pentazocine(Group 3), there were rare to moderate vacuolizations in the myelinated axons at all three checking points, and there were moderate vacuolizations in the unmyelinated axons at the 24 hour and 1 week checking points(Fig. 6). Accordingly, the three groups under electron microscopic study had axonal vacuolizations in common. The axonal vacuolization was slight in the group injected with morphine and moderate in the group injected with pentazocine.

DISCUSSION

The discovery of opiate receptors in the brain and spinal cord-the three main types of receptors being μ, δ and κ-has significantly improved the management of chronic or acute pain. The reason for the success of intraspinal opiate is that high concentrations of opioid receptors present in the dorsal horn may readily be reached and stimulate analgesia from an opiate agonist which is administered. A small amount of intraspinal opiates produces analgesia without altering either the autonomic or neuromuscular function, proprioception and touch. There are, however, some exceptions in the case of neuromuscular function. Blacow and Mortinale[14] have reported the local anesthetic-like effect of meperidine, and Sandu et al[15] demonstrated the neuronal blocking effect after application of meperidine on neural tissues of frogs.

Clinically, the authors of these articles[5~9] have in the past reported meperidine as well as pentazocine as good anesthetic agents in various surgeries such as hysterectomy, hemorrhoidectomy, or as adjuvant anesthetics in open heart surgery. The reasons of local anesthetic-like effects of these opioids are due to the similarity of their structure as a phenylpyperidine derivative and its similarity in molecular weight with lidocaine.

In this study we confirmed the local anesthetic-effect of opioids when meperidine as well as pentazocine were applied to the peripheral sciatic nerves. Muscle twitching responses by the nerve stimulator gradually decreased and finally disappeared after about 10 minutes, complete motor paralysis continued for about 60 minutes, and muscle reaction returned to normal about 90 minutes after injection. These effects, however, were not observed in the group injected with morphine. Therefore, the local anesthetic-like effects according to the type of opioids are other factors considered in the selection of intraspinal opiate.

Although intraspinal narcotic administration is clinically well-established for pain management, the risks of neurotoxicity associated with intraspinal narcotic administration is not well-known yet. Toxic reactions such as demyelination[16] and necrosis[17] were reported after intrathecal narcotic administration. Further, Bromage[18] and Winnie[19] warned of the risks of neurotoxicity such as demyelination or intraspinal necrosis associated with intrathecal narcotic administration. However, their concern regarding neural injury was focused on the preservatives of the prepared drugs. Several mechanisms of neurotoxicity suggest a correlation with sodium metasulfite[18,19], apomorphine as a morphine metabolite[20], or changes of spinal cord blood flow[21].

Abouleish et al[22]. were the first to try an experimental study to confirm the neurotoxicity of intraspinal opioids. They did a double blind test with monkeys. A group of monkeys received intrathecal morphine in saline 0.07 mg/kg. This group was compared to a control group that received either a lumbar puncture alone or intrathecal saline. Neither morphine nor saline solutions contained preservatives. The monkeys were sacrificed at 6 and 42 days respectively. In the experimental group there was no evidence of meaningful pathological findings such as demyelination, arachnoiditis or necrosis. In the control group, on the other hand, one animal of following multiple lumbar punctures associated with paresthesia was revealed to have focal endoneurial fibrosis. Abouleish et al. concluded that a consistent deleterious effect of morphine on nervous tissue does not exist and that focal endoneurial fibrosis is correlated with the physical trauma associated with a lumbar puncture rather than an injected drug. Yaksh[23] conducted an experimental study in monkeys with the chronic administration of epidural narcotic, 15 to 122 times for 4 to 6 weeks. His study showed no histological toxicity in the spinal cord. Coombs et al[24]. conducted a postmortem examination in seven cancer patients who were given chronic intraspinal narcotic, due to chronic intractable pain. The examiners reported that two of the patients had clinically unsuspected posterior column degeneration. They hypothesized that the potential causes for posterior column degeneration is more likely associated with malignant disease rather than the intraspinal infusion of morphine.

In this study we found only rare to moderate vacuolization on the nerve axonal fiber(slight in the group injected with morphine and moderate in the group injected with pentazocine). However, these are not pathological findings.

The findings from our previous experiment in rats revealed severe fatty degeneration in the cytoplasm and severe vacuolar changes in the axon only in one case. We assume that the narcotics applied to the nerve tissue are not correlated with direct toxicity on the nervous tissue. Previous nerve damage might develop erroneously from the experimental manipulation or something else because this study showed no evidence of significant neurotoxicity in the nerve tissue injected with morphine, meperidine or pentazocine respectively.

Therefore, the neural complications with intraspinal narcotic administration may not develop. Their potential causes are commonly thought to be mechanical trauma from a needle or manual ineptitude, preservatives or contaminated drugs, but not with the opioid itself. However, it is possible that the kind of opioids or the method of administration or maintenance may be other factors which cause neurotoxicity in cases of long term intraspinal narcotic administration. Thus, to increase the safety in management of chronic pain, further neurohistological experiments and clinical evaluations are needed regarding chronic intraspinal narcotic administration.

SUMMARY

The sciatic nerves of anesthetized rabbits were exposed and stimulated by a nerve stimulator in order to observe the myoneural response. These rabbits were divided into three groups and respectively injected with morphine (Group 1), meperidine(Group 2) and pentazocine (Group 3). The sciatic nerves were stimulated periodically and gait changes were observed to see the myoneural activity after the injections. When the distal part of the sciatic nerves were stimulated by the nerve stimulator after the respective drug injections, the normal muscle twitch responses were observed in all the progressional stages of Group 1. However, in Group 2 and 3, the muscle twitch responses decreased gradually, finally disappearing after approximately 10 minutes in these two groups. Complete motor paralysis continued for about 60 minutes. The muscle reactions returned to normal approximately 90 minutes after injection.

Specimens drug-injected tissues were severed 4 hours, 24 hours and 1 week after injection respectively. These tissue were investigated under light as well as electron microscopy. The tissue revealed rare to moderate vacuolizations scattered in the axons of the myelinated and unmyelinated nerves of some of the specimens; however, there were no significant pathologic lesions. These results provide evidence that neurophysiologically, meperidine and pentazocine have a local anesthetic-like effect such as motor paralysis, but morphine does not. In addition, the results indicated that neurohistologically, the three narcotics have no significant toxic effects on the nerve tissue.

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