INTRODUCTION:
Lumbar radicular pain with lumbar disc herniation represents some of the symptoms most commonly treated by spinal surgeons. It is generally known that conservative therapy and surgery can alleviate lumbar radiculopathy, but some patients continue to experience pain that is resistant to conventional treatment. This intractable pain is thought to be associated with the neuropathic pain, which is an extremely complex pain state typically accompanied by tissue injury.

Recently, animal models of neuropathic pain following peripheral nerve injury have been developed in the search for underlying mechanisms. Using these models, several studies have found that sympathetic nerve fibers sprout in the dorsal root ganglion (DRG). Norepinephrine (NE) is the neurotransmitter, which is released from postganglionic neurons in the sympathetic nervous system. Some electrophysiological studies have shown that NE application induced hyperexcitability of DRG in the neuropathic pain models, including our root constriction model. However it has not been fully understood which adrenoceptor subtypes are involved in the mediation of the NE effects. The purpose of the present study is to examine the adrenergic sensitivity of DRG neurons in the root constriction model in combination with adrenergic antagonists.

MATERIALS & METHODS:
All experiment protocols were approved by the Sapporo Medical University Animal Care and Use Committee. We used a total of 15 adult male Sprague-Dawley rats weighing 150-200g at the beginning of the study. The left L5 spinal root was exposed and tightly ligated with 8-0 nylon suture. At postoperative days 10-14, the ipsilateral L5 DRG neurons were quickly excised and enzymatically digested with collagenase.

To evaluate the excitability of DRG neurons, we used two kind of protocol. One protocol (short stimulation) was that depolarizing currents of 0.2-4.0 nA (0.5ms duration) were injected in increments 0.2 nA until an action potential was evoked. We examined the threshold current, resting membrane potential (RMP), amplitude, afterhyperpolarization, threshold voltage, APD50, and dv/dt max. The other protocol (long stimulation) was depolarizing currents of 0.01-0.39 nA (1ms duration) in increments 0.02 nA, which was evoked by repetitive discharge. We counted the maximum values of number of the spike in each current (max spike count).

Drugs used in this study were NE, phenolamine (α-antagonist), prazosin (α1-antagonist), and yohimbine (α2-antagonist). NE stock solution (10 mM) was dissolved in distilled water with an equivalent amount of ascorbic acid. Phenolamine and yohimbine (10 mM) were dissolved in distilled water. Prazosin (5 mM) was made by dissolution in 10% ethanol in distilled water. Final concentration of all drugs was 10 µM. We examined the effects of NE by pretreatment with adrenergic antagonists. Five minutes after NE application, electrophysiological recording was performed.

Data were expressed as the mean ± SEM and analyzed statistically using paired t-test or analysis of variance (ANOVA). P<0.05 was the accepted level for statistical significance.

RESULTS:
5 DRG neurons were used in each antagonist. Before NE stimulation, there were no significant differences between three groups. Despite exposing α1-antagonist prazosin, NE application induced the rise of dv/dt max and increased the max spike count. The dv/dt max and the max spike count increased significantly from 10.8±2.2 to 17.8±0.8, respectively (Fig. 1, 2). On the other hand, the effects of NE were inhibited by pretreatment with α1-antagonist phenolamine or α2-antagonist yohimbine (Fig. 1, 3). The increase in dv/dt max induced by NE application indicates an increase in inward current, which may involve sodium ions. Further studies are needed in order to clarify the current properties for NE-induced hyperexcitability of DRG neurons in the root constriction model.

DISCUSSION:
The presence of adrenoceptors in DRG and spinal dorsal horn neurons has been demonstrated. The present study showed that the excitatory effects of NE on DRG neurons in the root constriction model were modulated by α1-adrenergic. It is known that the expression of adrenoceptors in DRG neurons alters after nerve injury. Accordingly, DRG neurons in the root constriction model apparently possessed an excess of α1-adrenoceptors, and responded more sensitively to the application of NE.

The increase in dv/dt max induced by NE application indicates an increase in inward current, which may involve sodium ions. Further studies are needed in order to clarify the current properties for NE-induced hyperexcitability of DRG neurons in the root constriction model.

REFERENCE: