INTRODUCTION:
Calcitonin is generally used in the treatment for back pain accompanying osteoporosis. Some clinical trials have shown it provides analgesic effects in patients with painful conditions including osteolytic metastases, diabetic neuropathy, complex regional pain syndrome, etc. However the pathomechanisms by which Calcitonin reduces pain remain unclear yet. Lumbar radicular pain associated with lumbar spinal canal stenosis or lumbar disc herniation is one of the most common complaints and many clinicians have difficulties in treating it. The purpose of this study is to investigate direct antinociceptive effects of Calcitonin in the rats with lumbar radicular pain and to clarify changes in sodium channel expression in DRG neurons by Calcitonin treatment.

METHODS:
The experimental protocol used in this study was approved by the Sapporo Medical University Animal Care and Use Committee. A total of 62 male Sprague-Dawley rats weighing 150-170g at the beginning of the experiments were used in this study. The right L5 spinal root was tightly ligated with 8-0 nylon suture proximal to the DRG as the root constriction group. In the root constriction (RC) group, a total of 42 rats (n=14 for behavioral studies, n=28 for real-time RT-PCR quantitation) underwent operations used as the lumbar radicular pain model. The right L5 spinal root and DRG were exposed without ligation as the sham-operated group. In the sham-operated (sham) group, a total of 20 rats (n=5 for behavioral studies, n=15 for real-time RT-PCR quantitation) underwent operations. These rats were assigned to one of three groups: 1) Calcitonin-treated RC group (n=21), 2) RC group (n=21), 3) sham group (n=20). On day 11 after the operation, Calcitonin or vehicle was administered for three weeks (20U/kg/day, s.c.; 5 times a week) to Calcitonin-treated RC rats. RC rats and sham rats were injected with vehicle for the same period.

A) Behavioral study: The mechanical withdrawal response was examined by a stimulus of 3.8g using Von Frey filaments. The right and left hind paw was probed with 10 tactile stimulation alternately and it was repeated 3 times. The mechanical withdrawal frequency was obtained as the number of responses from the left side subtracted from the number of responses from the right side. The thermal withdrawal response was measured by a radiant heat source. Each hind paw was tested five times and the mean withdrawal latency was calculated. The thermal withdrawal latency was defined as left-right asymmetry of the latency. Behavioral tests were performed the day before the operation and after operation on day 4, 7, 10, 13, 15, 19, 22, 26, 29, 33, 36, 40.

B) Real-time RT-PCR quantitation: The rats were sacrificed the day after administration of Calcitonin or vehicle. Each right L5 DRG neuron was harvested. Total cellular RNA was isolated and reverse transcription and quantitation were performed as described previously. Nav1.8, 1.9 (tetrodotoxin-resistant sodium channel) and Nav1.3 (tetrodotoxin-sensitive sodium channel) mRNA was assessed using real-time quantitative RT-PCR. GAPDH was used as an endogeneous internal control to normalize the average expression level of the sham group was the standard of comparison. Statistical analysis of the data was performed by unpaired Student’s t-test. P<0.05 was considered statistically significant.

RESULTS:
The root constriction produced mechanical allodynia and thermal hyperalgesia (Fig.1, Fig.2). The Calcitonin-treated RC group showed a gradual decrease in hypersensitive responses after administration of Calcitonin. It demonstrated a significant improvement in tactile sensitivity on day 5 of treatment and in thermal sensitivity on day 9 of treatment compared with the RC group. The effect of Calcitonin on radicular pain was seen in 7 days after treatment.

DISCUSSION:
The present study demonstrates the possibility that Calcitonin has direct antinociceptive effect on lumbar radicular pain. Also it is suggested that its effect of Calcitonin depends on the down-regulation of the sodium channels related to the hyperexcitability of DRG neurons. Further study is needed to clarify how Calcitonin can regulate the sodium channels.