INTRODUCTION

Neuropeptides produced in dorsal root ganglion are closely related to pain signal transmission. In patients with traumatic conditions such as fracture, burn, and whiplash, plasma levels of some neuropeptides have been reported to be significantly higher than those in normal individuals (1, 2). This suggests the role of plasma neuropeptides in objective pain assessment. However, compared to traumatic conditions, it is more difficult and important to objectively evaluate neuropathic pain. In the present study, we chose lumbar disc herniation (LDH) as a prototype of diseases causing neuropathic pain, and prospectively evaluated the levels of plasma neuropeptides in patients who underwent lumbar discectomy.

METHODS

Subjects

Inclusion criteria of patients are those who had (i) sciatica with visual analogue scale (VAS) of 5 or more, despite conservative treatment, (ii) positive straight leg raising (SLR) test, and (iii) LDH at a single corresponding level. Exclusion criteria are patients with other concomitant painful conditions and those receiving diuretic drugs. Eligible patients gave written consent to participate in the study and were elected for lumbar discectomy. Two patients who exhibited residual pain 3 weeks after surgery were then excluded. Consequently 16 males and 14 females were subjected the study (Tables 1 and 2). Average age was 43 years old (range, 18-61). Preoperative VAS was 70 +/- 27 mm (average +/- standard deviation). Surgery was performed using modified Love method.

Blood collection and processing

Patients had their blood samples drawn before surgery and 3 weeks after surgery. Blood samples were collected in EDTA tubes. The tubes were transported on ice and centrifuged at 3000 rpm for 10 min. The plasma samples were snapped and stored frozen at -80 degree centigrade until analysis. Samples were analyzed within 3 months from blood sampling.

Enzyme-linked immunosorbent assay (ELISA)

Using commercially available ELISA kits (Peninsula Laboratories, St. Helens, UK), concentration of calcitonin gene-related peptide (CGRP), galanin, neuropeptide Y (NPY) and substance P (SP) in the corrected plasma samples was measured. Assays were performed according to manufacture’s protocol. In brief, known amount of the respective peptides were incubated with biotinylated-labeled peptides. After removing unbound biotinylated peptides by washing, streptavidin-conjugated horseradish peroxidase (SA-HRP), was added. After washing away excess SA-HRP, Tetramethyl Benzidine Dihydrochloride was allowed to react with bound HRP and absorbance read at 450 nm. All samples were performed in duplicates and the counts bound divided by the total counts were expressed as a percent. These numbers were then fitted to the standard curve to derive numeric values.

Statistics

Results were presented as the mean +/- standard deviation. Statistical analysis was conducted using paired t-test to compare plasma neuropeptides concentration before surgery to after. A probability of less than 0.05 was considered to be statistically significant.

RESULTS

All 30 patients showed VAS of 0 mm 3 weeks after surgery. Table 3 summarizes the result of ELISA. As shown, plasma levels of galanin significantly decreased after lumbar discectomy in association with the disappearance of sciatica (p=0.008). In contrast, other plasma neuropeptides failed to show significant changes before and after surgery, even though CGRP and SP showed some decreases after discectomy.

DISCUSSION

In the present study, we designed a prospective analysis on patients with LDH to determine the effects of lumbar discectomy on sciatic pain and plasma neuropeptides. We found that, among 4 neuropeptides examined, plasma levels of galanin significantly decreased after lumbar discectomy in association with the disappearance of sciatica. Galanin is a neuropeptide consisting of 29 amino acids, and is known to be involved in feeding behavior, pain threshold, memory and cognition (3, 4). In our recent analysis, ligation of a rat lumbar nerve root resulted in increases of galanin mRNA in dorsal root ganglion as determined by cDNA chip analysis and quantitative RT-PCR. These findings suggest the role of galanin in neural inflammation and pain caused by LDH, and also in objective pain assessment of patients with this condition.