**INTRODUCTION:**

Synovitis is considered to contribute to the pathogenesis of osteoarthritis (OA) through the formation of pro-inflammatory mediators increasing cartilage degradation, including cytokines and neuropeptides. It has been shown that neuropeptides expressed in the inflamed synovium, such as substance P (SP) and calcitonin gene-related peptide (CGRP), contribute to the disease process of OA through the diverse actions (1). On the other hand, estrogen replacement therapy (ERT) in postmenopausal women has been reported to protect against OA, although its exact mechanism remains unclear. We hypothesized that estrogen regulates the intraarticular inflammation through the alteration of the expressions of SP and CGRP in the synovium of OA joints. The objective of this study was to examine whether the expressions of SP and CGRP in the synovium of experimental OA models in rats change with the plasma level of estradiol by quantitative immunohistochemistry.

**MATERIALS AND METHODS:**

Twenty-four Wistar female rats aged 12 weeks were used. All experimental procedures have been permitted by the Committee for Animal Research in our institute. By ovariectomy (OVX) and hormone replacement using the subcutaneous implantation of a sustained-release pellets (Innovative Research of America) containing estradiol-3-benzoate (E2) (dose; 0.5 mg/60 days), three groups of animal models were prepared: OVX group (n=8), OVX + E2 replacement group (E2 group; n=8), and sham operation group (sham group; n=8). Additionally, immediately after hormonal manipulation, all animals underwent transections of anterior cruciate ligament in their left knees.

After 30 days, blood samples were taken transcardially before sacrifice, and plasma concentration of estradiol was measured using enzyme immunoassay. Knee joints were rapidly frozen and undecalcified fresh-frozen sagittal sections were prepared with a thickness of 16 µm, according to a previously described method (2). The histological appearance of knee joints was evaluated using Mankin score (Grade 0-14), from hematoxylin-eosin (HE) and Safranin O staining. Following sections were used for the immunofluorescence staining of protein gene product 9.5 (PGP 9.5; pan-neuronal marker), SP, and CGRP. The sections were evaluated by a confocal laser scanning microscope (LSM 510 META, ZEISS). At least non-serial three sets of serial sections were randomly selected, and the density of PGP 9.5-, SP-, and CGRP-positive nerve fibers (/mm²) in the posterior synovium was calculated, respectively.

The data on the Mankin score and the density of immunoreactive nerve fibers were analyzed among three groups, using one-way ANOVA followed by a post hoc Tukey-Kramer test.

**RESULTS:**

The plasma concentration of estradiol in the OVX, E2, and sham group was 13.6 ± 1.7, 73.4 ± 5.4, and 29.9 ± 4.0 pg/ml, respectively.

In HE staining, synovial inflammation with the proliferation of microvessels was observed in all groups. The degree of synovial inflammation in the OVX group was higher than in the E2 and sham groups (Fig. 1). The changes in the early phase of OA, such as the irregularity of cartilage surface and the reduction in Safranin O staining, were observed in all groups. The Mankin score in the OVX group was significantly higher than in the E2 and sham groups. The score in the E2 group was reduced to the level similar to that in the sham group.

In immunofluorescence staining, PGP-, SP-, and CGRP-positive nerve fibers were observed mainly along the proliferating microvessels, as perivascular neural networks, in the synovium of posterior joint space in all groups (Fig. 2). No significant differences in the density of PGP 9.5-positive nerve fibers were observed among the three groups. On the other hand, the density of SP- or CGRP-positive nerve fibers in the OVX group was significantly higher than in the sham group (Fig. 3). The density in the E2 group was lower than in the OVX group, and approximately similar level to that in the sham group (Fig. 3).

**DISCUSSION:**

It has been reported that SP has various physiological actions, including vasodilation, vascular hyperpermeability, neurogenic inflammation, pain transmission, and cellular proliferation, and that CGRP induces the similar actions in conjunction with SP (1). In this study, we showed that both SP and CGRP were expressed mainly along the microvessels. This finding was consistent with several previous reports. SP and CGRP may contribute to the development of synovial inflammation, via the chemotaxis of proinflammatory agents from microvessels and endothelial cell proliferation in microvessels. Additionally, we demonstrated that, in the low-estradiol group (OVX group), the levels of synovial inflammation, cartilage degradation, and expression of SP and CGRP in the synovium were increased, while in the high-estradiol group (E2 group) they were reduced to the similar level to in the sham group. These findings raise the possibility that estrogen regulates the intraarticular neurogenic inflammation in OA joints by modulating the expressions of neuropeptides in the synovium, and that ERT protects articular cartilage from degradation in the early phase of OA.

**REFERENCES:**