INCREASED LEVELS OF SENSORY NEUROPEPTIDES IN TENDONS OF RATS WITH ADJUVANT ARTHRITIS.

INTRODUCTION
Accumulating data suggest that the nervous system is involved in the pathophysiology of rheumatoid arthritis (RA) as expressed by increased levels of neuronal mediators, so called neuropeptides in joints with adjuvant arthritis [2]. It is plausible that the nervous system is involved also in the development of extraarticular RA manifestations. Altered expression of neuropeptides in extraarticular tissues may prove to enhance the recruitment of inflammatory cells and the activation of cytokines so as to cause degenerative changes. In the present study we analyzed the expression of sensory neuropeptides in tendons from rats with adjuvant arthritis.

METHODS
Thirty-eight female Lewis rats 8 weeks of age (160-220g), were anesthetized with intraperitoneal Hypnorm® injection. Subsequently, the rats were injected subcutaneously in the base of the tail with killed Mycobacteria butyricum dispersed in paraffin oil (21 rats) or a pure paraffin oil preparation (17 rats). The rats injected with Mycobacterium developed inflammation with increased temperature, swelling and redness of the hind paws at day 16, while rats in the control group remained unaffected. Thirty-five days after injection the inflammation was considered to be chronic and the rats were killed by decapitation. The experiments were approved by the local animal ethics committee.

Radioimmunoassay (RIA): The Achilles tendons from 28 rats were dissected bilaterally, pooled as a single sample and immediately and frozen on dry ice and kept at -70°C until neuropeptide extraction. Frozen tissues were boiled for 10 min in 2 M acetic acid. After homogenization, the samples were sonicated and centrifuged at 3,000xg for 15 min. The supernatant was lyophilized and redissolved in phosphate buffer [1]. The concentrations of substance P (SP) and calcitonin gene-related peptide (CGRP) were expressed as pmol/g wet weight tissue. The tracer peptide for SP and CGRP were subjectively assessed in the loose connective tissue, paratenon and bone tendinous junction.

For each rat, three sections from different parts of the tendon were stained with PT and CGRP. A Nikon epifluorescense microscope (Eclipse E800 Yokohama, Japan) was used to examine the sections.

RESULTS
RIA: Detectable amounts of the neuropeptides analyzed were obtained from all samples. The concentration of both SP (p<0.05) and CGRP (p<0.001) were higher in the Achilles tendons of rats with adjuvant arthritis compared to controls (Fig. 1A-B). The concentration of SP and CGRP increased 21.8% and 71.1%, respectively.

IHC: Nerve fibers immunoreactive to SP and CGRP were identified in all parts of the Achilles tendon, both in artihritic and healthy rats. However, there was a clear increase in neuropeptide immunoreactivity in rats with adjuvant arthritis confirming the results of RIA (Fig. 2A-B). The increase in neuropeptide expression was most pronounced in the bone tendinous junction but was also seen in the paratenon, while there was no increase in the surrounding loose connective tissue. Closer analysis disclosed that SP and CGRP predominantly occurred in free nerve endings both in the paratenon and bone tendinous junction, while in the loose connective tissue they were mostly vessel related.

ACKNOWLEDGMENTS
This study was supported by grants from Swedish Medical Research Council (12X-08652-09B).

REFERENCES

48th Annual Meeting of the Orthopaedic Research Society
Poster No: 0688