A model for experimentally induced temporomandibular joint arthritis in rats: effects of carrageenan on neuropeptide-like immunoreactivity

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Summary Substance P (SP)-, neurokinin A (NKA)-, calcitonin gene-related peptide (CGRP)- and neuropeptide Y (NPY)-like immunoreactivity (-LI) was studied in rats' cerebrospinal fluid (CSF), plasma and perfusates (PF) from the temporomandibular joints (TMJs) at 2, 6 and 24 h following 0.01 ml injection of 2% carrageenan (CAR) into the right TMJ. SP-, NKA-, CGRP- and NPY-LI were significantly increased in both TMJ perfusates of the treated groups compared to controls. Generally an injection with CAR into the right TMJ induced a similar influence of the concentration of SP-, NKA-, CGRP- and NPY-LI in the CSF, plasma and PF at 2, 6 and 24 h following injection. However, the most pronounced changes in neuropeptide-LI occurred intra-articularly in the joint fluid, which indicates that both the sensory and sympathetic nervous system are activated in this joint following osteoarthritis induction by carrageenan.

INTRODUCTION

Carrageenan (CAR)-induced inflammation in animals is a commonly used model in the study of localized inflammatory processes. Histamine, serotonin, various kinins, leukotrienes and prostaglandins have been shown to be involved in CAR-induced oedema. A decrease of proteoglycan synthesis in articular cartilage have been shown in CAR-induced arthritis in rabbits. It has been suggested that the cartilage changes during CAR-induced arthritis are comparable to osteoarthritis in humans. Specific immune mechanisms do not seem to be involved in CAR-induced inflammation. One way of assessing the role of the sensory and sympathetic nervous system in inflammatory processes is by measuring the presence of various neuropeptides released from nerve fibers. In recent studies we have measured temporomandibular joint (TMJ) fluid and its neuropeptide content in patients with osteoarthritis, joint and rheumatoid arthritis. Changes in the presence of neuropeptide Y and calcitonin gene-related peptide were shown to be related to the intra-articular temperature of the arthritic TMJ. Furthermore, intra-articular glucocorticoid treatment of the arthritic TMJ significantly reduced the joint fluid content of NPY. The aim of the present study was to set up a model...
for experimentally induced temperomandibular joint osteoarthritis to elucidate the neurogenic component in experimentally induced CAR inflammation. We examined early changes in SP-, NKA-, CGRP- and NPY-LI in rats' CSF, plasma and PF at 2, 6 and 24 h following injections.

MATERIALS AND METHODS

The study was carried out on 60 male albino Sprague-Dawley rats, weighing 250–300 g, who were allowed to become habituated to the laboratory for at least 7 days before experimentation. All rats were maintained under identical conditions, which included alternate cycles of 12 h light and 12 h darkness, environmental temperature of 24°C, 60% relative humidity, food and water ad libitum. On the day of the experiment the rats were anaesthetized with chloralhydrate (0.4 g/kg) intraperitoneally. The skin overlying the TMJs was shaved and intra-articular injection was carried out using a 27 gauge needle. The rats were grouped as follows: 30 rats received 0.01 ml carrageenan 2% in the right TMJ and saline into the left (CAR rats); 30 rats were given 0.01 ml saline bilaterally into the TMJs (control group). After 2, 6 or 24 h following the first injection the rats were again anaesthetized with chloralhydrate intraperitoneally. A 2 cm longitudinal skin incision was made bilaterally, exposing the TMJs. A 27 gauge needle was inserted into each joint. Both joints were simultaneously perfused with 0.9% saline through the 27 gauge needle using a push and pull technique. Collection was carried out for 1.5 h and 0.1 ml perfusate was collected for each joint. For collection of CSF, following TMJ perfusion, the rats were placed in a stereotaxic frame. The atlanto-occipital membrane was exposed by retracting the overlying muscles and skin and the TMJs were removed and frozen. All samples were rapidly cooled and centrifuged and plasma was removed and frozen. All samples were rapidly cooled and stored at 80°C for analysis. Samples from the CSF, plasma and PF were extracted using a reverse-phase C18 cartridge (Sep Pak, Waters) and analyzed using competitive radioimmunoassays.12

Radioimmunoassay of substance P(SP-LI) was analyzed using antiserum SP21 which reacts with SP and SP sulfoxide, but not with other tachykinsins. Intra- and interassay coefficients of variation were 7 and 11%, respectively. Neurokinin A (NKA-LI) was analyzed using antiserum K12 which reacts with NKA (100%), NKA (3–10) (48%), NKA (4–10) (45%), neurokinin B (26%), neuropeptide K (61%) and edeolisin (30%), but not with SP. Intra- and interassay coefficients of variation were 7 and 12%, respectively. Calcitonin gene-related peptide (CGRP-LI) was analyzed using antiserum CGPR812 raised against conjugated rat CGRP. HPLC-purified 125I-Histidyl rat CGRP was used as radioligand and rat CGRP as standard. The cross-reactivity of the assay to SP, neurokinin A, neurokinin B, neuropeptide K, gastrin, neurotensin, bombesin, neuropeptide Y and calcium was less than 0.01%.

RESULTS

Cerebrospinal fluid

SP-LI and NKA-LI in the CSF of rats injected with CAR increased significantly in the CAR group at 6 and 24 h. CGRP-LI was significantly increased at 2 and 6 h and NPY-LI at 24 h in the CAR group. Thereafter, NPY-LI did not differ significantly between the saline and CAR group (Table 1).

Plasma

SP-LI, NKA-LI, CGRP-LI and NPY-LI did not change significantly in the CAR group (Table 1).

Temperomandibular joint perfusates

This was a significant increase in neuropeptide content (SP-, NKA-, CGRP- and NPY-LI) in the synovial fluid after induction of monoarthritis. The increase of SP-LI and NKA-LI in the CAR group at 6 and 24 h was significantly increased in the CAR group at 2 and 6 h. CGRP-LI was significantly increased at 2 and 6 and NPY-LI at 24 h in the CAR group. Thereafter, NPY-LI did not differ significantly between the saline and CAR group (Table 1).

DISCUSSION

Three different phases in the oedema response are reported to occur after injection of CAR into the plantar surface of rats' paws.1 A release of serotonin and histamine has been suggested to be involved in the initial phase (1.5h). The second phase is mediated by kinins (1.5–2.5 h) and the third by the release of prostaglandins (2.5–6 h). Furthermore, it has been observed that the maximal oedematous swelling is seen 3–5 h after CAR injection. According to Gamse et al.,17 injury or irritation of the skin or mucous membranes activates C-fibers.
Arthritis in rats: effects of carrageenan on neuropeptide-like immunoreactivity

Cerebrospinal fluid (CSF)

In acute CAR-induced monoarthritis in the cat, SP-LI and NKA-LI increased in the superficial layers of the spinal cord.\textsuperscript{22,23} In the present study, SP- and NKA-LI was unchanged in the CSF at 2 h following injection of CAR. The release of SP and NKA was, however, significant at 6 and 24 h following injection. This is a somewhat longer time interval than that reported by others using CAR intra-articularly.\textsuperscript{22,23} They, however, measured peptide release in the spinal cord with intramedular microprobes. The longer time presently observed may thus reflect the time taken for diffusion to CSF as well as transport from spinal cord to the sampling location more centrally. CGRP-LI also increased in the CSF compared with controls but this increase was temporary, without any significant differences at 24 h following injection. CGRP and SP released in CSF may interact in a collaborative way. Simultaneously applied SP and CGRP have been found to exert a potentiating effect on flexor reflex excitability in rats.\textsuperscript{24} Furthermore, CAR-induced hyperalgesia was inhibited by an intrathecal injection of CGRP antibodies.\textsuperscript{25} These data indicate that release of CGRP in the spinal cord is involved in CAR-induced hyperalgesia. NPY-LI did not increase in the CAR group. To what extent sympathetic activity is increased in the central nervous system of rats subjected to CAR is unclear.

Plasma

Neural SP is rapidly inactivated in plasma\textsuperscript{15} and the mean source of circulating SP in plasma seems to be intestinal.\textsuperscript{26} SP in rats’ plasma originates mainly from perivascular nerves.\textsuperscript{27} NPY circulating in plasma has also been shown to be of neural origin.\textsuperscript{26,29} Neuropeptide release from perivascular nerves in plasma possibly reflects only the general activation of the nervous system. However, enzymatic systems may also possibly be involved in the neuropeptide metabolism.\textsuperscript{26,31} CGRP-LI circulating in human plasma has been shown to be rapidly metabolized.\textsuperscript{32} In the present study the levels of neuropeptides in the CAR group plasma did not change during the 24 h period.
Temperomandibular joint perfusates

Increased concentrations of neuropeptide-LI were detected in the joints and the increase was bilateral in almost all perfusates, indicating a localized peripheral release. Generally the bilateral increase in neuropeptide-LI released persisted for at least 24 h.

The neuropeptides SP, NKA, CGRP and NPY have been found to be increased in the human TMJ in patients with arthritis. These findings suggest that there is a significant neurogenic component in the development and maintenance of TMJ arthritis. These results support the hypothesis that the injection of CAR into one TMJ, studied over a 24 h period, was found to alter the neuropeptide-LI in both TMJs and in CSF but to a lesser extent. It may be concluded that the sensory and sympathetic nervous system is activated in the TMJ following CAR-induced monoarthritis. The bilateral changes in neuropeptide-LI in the TMJs following the unilateral injection of CAR in the present study and the increase in neuropeptide content in the TMJ fluid in arthritic patients in our previous studies support the involvement of nervous system's mechanisms in arthritis. Furthermore, the similarities in the peptide content in the CAR-induced TMJ arthritis and the arthritic patients TMS fluid suggest that CAR-induced monoarthritis may be used as an animal model.

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REFERENCES


