EFFECTS OF CAPSAICIN IN TEMPOROMANDIBULAR JOINT ARTHRITIS IN RATS

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Summary—Temporomandibular joint (TMJ) arthritis was induced in female Lewis rats by unilateral injection of a suspension of heat-killed Mycobacterium butyricum in paraffin oil into the TMJ. Control rats received paraffin oil by the same route. Arthritic and control rats were pretreated either with capsaicin or denervation of the mandibular branch of the trigeminal nerve. Tissues were collected for neuropeptide extraction and analysed by radioimmunoassay and reverse-phase high-performance liquid chromatography. In all groups, the levels of substance P- (SP), calcitonin gene-related peptide- (CGRP) and neuropeptide Y- (NPY) like immunoreactivity (LI) were higher in the trigeminal ganglia than in the TMJs. In control rats, capsaicin significantly lowered the levels of SP-LI in the trigeminal ganglia and TMJ, but not CGRP-LI and NPY-LI. In the arthritic rats, capsaicin pretreatment significantly lowered the SP-LI and CGRP-LI in the trigeminal ganglia and TMJ, but not the NPY-LI. In the trigeminal ganglia the unilateral denervation significantly lowered SP-LI in control rats, and in arthritic rats SP-LI and CGRP-LI. On the denervated side of the arthritic TMJ, NPY-LI, SP-LI and CGRP-LI were significantly lowered as compared to the arthritic control rats and to the contralateral side. In this rat model, pretreatment with capsaicin and surgical denervation decreased the neuropeptide content in the trigeminal ganglia and the TMJ. The results clearly demonstrate a close interaction between increased neuropeptide release from sensory and sympathetic neurones after induction of arthritis in the rat. © 1997 Elsevier Science Ltd. All rights reserved

Key words: arthritis, capsaicin treatment, surgical denervation, substance P, calcitonin gene-related peptide, neuropeptide Y, temporomandibular joint, trigeminal ganglia.

INTRODUCTION

The nervous system contributes to the development of joint inflammation in rats (Levine et al., 1988). Increased concentrations of the sensory neurotransmitters substance P and CGRP (Marshall et al., 1989) and neurotransmitter found in sympathetic nerves, neuropeptide Y, have been found in synovial fluid from patients with various forms of inflammatory joint diseases (Larsson et al., 1991). This agrees with previous reports showing an increase in the concentrations of the sensory neuropeptides substance P and CGRP in dorsal root ganglia of rats with adjuvant arthritis (Donaldson et al., 1992). Yet controversy prevails about the involvement of substance P and CGRP in inflammatory joint disease, as both an increase and a decrease in the number of peptidergic nerve fibres have been reported in rats with arthritis (Mapp et al., 1990). Also, there is a suggestion that the autonomic nervous system participates in the development of arthritis (Appelgren et al., 1991, 1995). This is supported by the finding that sympathectomy attenuates adjuvant-induced arthritis in the rat (Levine et al., 1985). Like substance P, CGRP is found in C-nociceptive afferents (Lundberg et al., 1985), and CGRP may co-operate with substance P in mediating local reflex reactions. In addition, CGRP has a strong vasodilatory effect in joints and muscles, and it is several times more potent as a vasodilator than substance P (Brain et al., 1988). Clinically, CGRP was found in higher concentrations in arthritic knee joints than in controls (Larsson et al., 1991) and in concentrations much above that in the plasma in rheumatoid arthritis of the temporomandibular joint (Appelgren et al., 1991). Neuropeptide Y was also suggested to be a modulator of arthritis. Low intra-articular temperature and accordingly impaired blood flow occurred frequently in the temporomandibular joint in human chronic rheumatoid arthritis and were associated with high levels of neuropeptide Y-like immunoreactivity in joint aspirates (Appelgren et al., 1993). Such immunoreactivity has also been associated with pain in the temporomandibular joint.
The rats were divided into three groups. In the first group, six rats were injected with \textit{M. butyricum} into the temporomandibular joint and six rats served as controls and received paraffin by the same route. In the second group, 12 rats were pretreated with capsaicin subcutaneously; 4 days later six of them were injected with \textit{M. butyricum} and six were injected with paraffin oil. The rats were killed by decapitation under ether anaesthesia. Temporomandibular joints and trigeminal ganglia were dissected from both sides and immediately frozen on dry ice and kept at $-70^\circ$C until neuropeptide extraction.

Capsaicin (50 mg/kg body wt, dissolved in 10% ethanol and 10% Tween-80 in isotonic saline) was injected bilaterally subcutaneously into the temporomandibular joints of 12 rats (deeply anaesthetized with ether) for four consecutive days (total dose 200 mg/kg). To reduce respiratory symptoms, the animals were injected with theophylline (5 ml/kg body wt) intraperitoneally before each capsaicin injection. Six rats were inoculated with mycobacteria 1 week after capsaicin administration had been completed. In the capsaicin group, three rats died during inoculation of capsaicin, but another three were then subjected to inoculation, so that the series still consisted of 12 rats. Six rats received a control solution for capsaicin treatment and were injected with paraffin oil.

Arthritis was induced in 18 rats by intra-articular injection (0.01 ml) into the right temporomandibular joints of a suspension of heat-killed mycobacteria in paraffin oil (10 mg/ml). In 12 rats the unilateral ligation of the auriculotemporal and muscetric nerve involved both arthritic and normal temporomandibular joints. The six intact control rats (control for arthritic rats) received 0.01 ml paraffin oil by the same routes as the arthritic rats. The six capsaicin-pretreated rats (controls for capsaicin-treated rats) received 0.01 ml paraffin oil by the same route. The six denervated control rats received 0.01 ml paraffin oil by the same route.

The rats were killed by decapitation under ether anaesthesia 29 days after inoculation with mycobacteria. Both temporomandibular joints, including the capsule and the synovial membrane, were dissected separately. The trigeminal ganglia were excised, and thus each rat yielded two samples. Each frozen tissue sample was weighed before extraction. The neuropeptides were extracted and quantified as recently described for bone and joint tissue (Ahmed \textit{et al}., 1994). Before the extraction the tissues were cut into small pieces, boiled for 10 min in 2 mol/l acetic acid in 4% EDTA, homogenized in a Polytron (15 s), sonicated (30 s) and centrifuged at 3000 rev/min x 9 g for 15 min. The supernatants were lyophilized and diluted in 2 ml radioimmunoassay buffer. These samples were further diluted.
Capsaicin and temporomandibular joint arthritis

1:10 and kept at −20°C until analysis. The analysis was performed with the following antisera. CGRP was analysed using antiserum CGRP8 raised in a rabbit against conjugated rat CGRP. HPLC-purified [125I]histidyl rat CGRP was used as radioligand and rat CGRP as standard. The detection limit of the assay for rat CGRP was 1 pmol/l and cross-reactivity of the assay to substance P, neurokinin A, neurokinin B, neuropeptide K, gastrin, neurotensin, bombesin, neuropeptide Y and calcitonin was less than 0.01%. Cross-reactivity with human CGRP-α and -β was 93 and 24%, respectively, and with rat CGRP-α and -β 100 and 120%, respectively. Intra- and interassay coefficients of variation were 8 and 14%, respectively. Substance P was assessed using antiserum SP2 (Brodin et al., 1986) raised in a rabbit against bovine serum albumin-conjugated rat substance P. The antiserum reacts with substance P and substance P sulphoxide, but not with other tachykinins in the concentration range 7.8–3200 pmol/l. LC-purified, rat [125I]tyrosine substance P was used as radioligand and rat substance P as standard. The detection limit was 1 pmol/l. Intra- and interassay coefficients of variation were 7 and 11%, respectively. Neuropeptide Y was analysed using antiserum NI, which cross-reacts at a level of 0.1% with the avian pancreatic polypeptide but not with other peptides (Theodorsson-Norheim et al., 1987). The detection limit of the assay was 11 pmol/l. Intra- and interassay coefficients of variation were 7 and 12%, respectively.

Reverse-phase HPLC was applied to extracts of normal as well as arthritic temporomandibular joints to characterize the neuropeptides studied. Extraction in 2 M acetic acid in 4% EDTA was found to provide an optimal yield of both sensory and autonomic neuropeptides. A Waters Delta Pak C18 300 A 3.9 mm × 150 mm column was used and elution was performed with a 40-min linear gradient of acetonitrile in water containing 0.1% trifluoroacetic acid. Two Pharmacia P3500 HPLC pumps were controlled by a Pharmacia GP250 gradient programmer. A gradient of 20–40% acetonitrile was used for substance P, and gradient of 20–50% for CGRP and neuropeptide Y. These samples were passed through Millipore GS filters (0.45 μm) before chromatography to remove particulate matter, and 200 μl of each sample was injected into the column. Fractions of 0.5 ml were collected at an elution rate of 1.0 ml/min. Each fraction was lyophilized and reconstituted in 100 μl distilled water before analysis. The fractions were assayed for immunoreactivity in the same tubes used for their collection. HPLC analysis of the immunoreactive material from joint samples with regard to substance P, CGRP and neuropeptide Y consistently resulted in a main peak eluting in the position of the corresponding synthetic peptide. Thus, no evidence of multiple immunoreactivities was noted for substance P, CGRP or neuropeptide Y.

Statistical analysis was carried out with SPSS software (release 6). An ANOVA one-way test (post hoc Bonferroni test within and between treatments) was used to test the differences expressed in percentage changes, and Spearman’s rank correlation coefficient was used to analyse correlations between variables; p values < 0.05 were considered significant.

RESULTS

In the arthritic trigeminal ganglia, substance P was increased by 39% and CGRP by 98% as compared to the arthritic control rats (Fig. 1). All neuropeptide concentrations were increased in the arthritic temporomandibular joint—substance P by 50%, CGRP by 65% and neuropeptide Y by 47% (Fig. 2). Following capsaicin treatment, substance P

![Fig. 1. Contents of substance P (SP), calcitonin gene-related peptide (CGRP) and neuropeptide Y (NPY) like immunoreactivity (LI) are presented in pmol/g tissue wt as the mean ± SEM in the trigeminal ganglia (TG). *Denotes difference from the control rats inside the group (p < 0.05); + denotes difference from the arthritic control and arthritic rats, respectively p < 0.05; § denotes difference between the capsaicin and the ligated rats, respectively (p < 0.05).](image-url)
was decreased by 38% in the trigeminal ganglia of the capsaicin-treated control rats as compared to the arthritic control rats (Fig. 1); substance P was decreased by 50% in the temporomandibular joint as compared to the capsaicin control rats (Fig. 2). In capsaicin-pretreated arthritic rats, substance P was decreased by 25% and CGRP by 45% in the trigeminal ganglia as compared to the arthritic control rats (Fig. 1); in the temporomandibular joint in capsaicin-pretreated controls there was a decrease by 33% in the substance P content and by 32% in CGRP as compared to the arthritic controls (Fig. 2). After denervation of the right temporomandibular joint: (i) substance P decreased in the trigeminal ganglia by 64% as compared to control arthritic rats in the trigeminal ganglia (Fig. 1); (ii) in the temporomandibular joint substance P was decreased by 50% as compared to control arthritic rats (Fig. 2); (iii) substance P was decreased by 74% and CGRP by 47% as compared to the intact arthritic rats in the trigeminal ganglia (Fig. 1); (iv) substance P decreased by 67%, CGRP by 43% and neuropeptide Y by 48% in the trigeminal ganglia as compared to the arthritic control rats (Fig. 1); (v) substance P decreased by 57% and CGRP by 32% in the control temporomandibular joint on the denervated side as compared to the contralateral side (Fig. 3); and (vi) substance P decreased by 63%, CGRP by 45% and neuropeptide Y by 48% in the arthritic temporomandibular joint as compared to the contralateral side (Fig. 3). On comparing the decrease in the denervated and the capsaicin-pretreated rats, substance P was decreased in the arthritic control and arthritic rats by 26 and 49%, respectively, but neuropeptide Y increased by 33% in arthritic denervated control rats as compared to the capsaicin-pretreated control rats (Fig. 1); but none of these changes in neuropeptide content was statistically significant. In the denervated arthritic rats, substance P decreased by 33% and neuropeptide Y by 40% as compared to the capsaicin-pretreated rats (Fig. 2). In the trigeminal ganglia and in the temporomandibular joint the correlation between substance P and CGRP was $r = 0.53$ ($p < 0.001$) and $r = 0.75$ ($p < 0.0001$), respectively. Neuropeptide Y immunoreactivity was not correlated between the trigeminal ganglia and temporomandibular joint.

**DISCUSSION**

Our results show that, in all experimental groups, the concentrations of substance P, CGRP and neuropeptide Y were higher in the trigeminal ganglia than in the temporomandibular joints. In arthritic rats all neuropeptides were increased in the trigeminal ganglia and in the temporomandibular joint, but not significantly for neuropeptide Y in the trigeminal ganglia. In intact rats, capsaicin significantly lowered the concentrations of substance P in the trigeminal ganglia, but not CGRP and neuropeptide Y. There was a significant change of substance P content in the temporomandibular joint following capsaicin treatment in the control rats. In the arthritic rats, capsaicin pretreatment signifi-
stantly lowered the substance P and CGRP in the trigeminal ganglia. In the temporomandibular joint, significant changes were seen for substance P and CGRP only. The unilateral denervation significantly lowered substance P in the trigeminal ganglia in intact rats. In the arthritic rats, surgical denervation significantly lowered the substance P and CGRP levels in the trigeminal ganglia. In the temporomandibular joint on the denervated side, substance P, CGRP and neuropeptide Y were significantly lowered, as compared to the arthritic intact rats and to the contralateral side. Pretreatment with capsaicin and surgical denervation attenuated the increase of neuropeptide concentrations in the trigeminal ganglia and in the temporomandibular joint in a manner similar to that following arthritis.

We have recently reported that experimentally induced acute arthritis of the temporomandibular joint results in pronounced changes in neuropeptide-like immunoreactivity in the synovial fluid of rats (Carlsson et al., 1996a-c; Lundeborg et al., 1996). Furthermore, it has been shown that there is a relation between neuropeptides in the arthritic temporomandibular joint fluid and joint pain, reduced mandibular mobility, and signs of tissue destruction in patients with rheumatoid arthritis (Appelgren et al., 1995). The majority of studies have focused on the proximal effects, i.e. those in the dorsal horn and dorsal root ganglia. Only occasional reports have dealt with the distal effects, notably in nerve, skin and muscle (Franco-Cereceda et al., 1991). Work on neuropeptide immunoreactivities has almost exclusively been confined to nerve fibres (Ahmed et al., 1993; Bjerholm et al., 1988). Considering that neuropeptide concentrations in the blood are very low, the contribution of neuropeptides from the circulation of the temporomandibular joint fluid and joint pain, reduced mandibular mobility, and signs of tissue destruction in patients with rheumatoid arthritis (Appelgren et al., 1995). The significance of assessing neuropeptides in joint tissue relates to previous observations suggesting an involvement of the nervous system in the pathophysiology of inflammatory joint disease (Kuraishi et al., 1989). Other studies (Levine et al., 1986) support the hypothesis that arthritis in the temporomandibular joint is modulated by the peripheral nervous system, including both activation of an axon reflex in the nerve terminals as well as activation of the sympathetic system with release of neuropeptide Y.

The arthritic trigeminal ganglia and temporomandibular joint

In patients with inflammatory joint disease, elevated amounts of substance P have been demonstrated in the synovial fluid of affected joints (Hernanz et al., 1993). It is well known that substance P has a number of pro-inflammatory actions (Lotz et al., 1988), which may be elicited following an increased release of neuronal substance P in arthritis. The mechanism behind this increase is unknown. A decrease of substance P- and CGRP-immunoreactive nerve fibres in the synovium in adjuvant-induced arthritis and rheumatoid arthritis was demonstrated by Mapp et al., (1990). It was claimed that the decreased nerve immunoreactivity was due to increased release followed by depletion of neuropeptides from nerve terminals in the synovial membrane. Previous observations (Goedert et al., 1984; Levi-Montalcini et al., 1996) suggest that nerve growth factor is involved in the increased expression of substance P and CGRP in arthritis. In our normal rats, capsaicin significantly lowered the concentrations of substance P in the temporomandibular joint and in the trigeminal ganglia; in the arthritic rats, it significantly lowered the concentrations of substance P and CGRP in the temporomandibular joint and in the trigeminal ganglia. Capsaicin is known to elicit neurotransmitter release from small-sized neurones (C-fibre) of dorsal root ganglia (Kashiba et al., 1990) and their projections of unmyelinated C and thinly myelinated A-δ fibres (Papka et al., 1984). Thus, the small-sized neurones, producing most of the substance P found in dorsal root ganglia, are more vulnerable to capsaicin than the large-sized neurones producing mainly CGRP, and unmyelinated fibres are more sensitive to capsaicin than are myelinated fibres (Kashiba et al., 1990; Wimalawansa, 1990). In a study of adult guinea pigs, capsaicin was found to impair the retrograde transport of nerve growth factor to the dorsal root ganglia (Goedert et al., 1981). This was presumed to cause the decreased synthesis of substance P noted.

These observations show that capsaicin has a down-regulatory effect on nerve growth factor, whereas arthritis has an up-regulatory effect. As it is well known that sensory nerves mediate afferent impulses to the central nervous system, they may also have an effenter role by mediating a local response to exogenous and endogenous agents (Ansel et al., 1996). A decrease may be due to inflammatory damage of the nerve fibres, while increased release of neuropeptides may occur (Ahmed et al., 1995). In an immunohistochemical study of joint tissues from patients with rheumatoid arthritis by Mapp et al., (1990), a decrease in neuropeptide Y-positive fibres was noted in the synovial membrane. Seemingly contradictory findings were reported in a study of rheumatoid arthritis by Larsson et al., (1991) and Appelgren et al. (1991), who found a significant increase in neuropeptide Y in arthritic synovial fluid. Hence, it remains unclear whether there is an up- or down-regulation of autonomic neuropeptides in arthritis. Moreover, it is still unclear whether the change in release of autonomic neuropeptides in arthritis occurs independently of or in interaction with the sensory nervous system, which is suggested to be involved in the pathophysiology of the disease.
The therapeutic effect of sympathectomy in arthritis may reflect an interaction between the sensory and autonomic nervous systems. Nerve growth factor plays a significant part in maintaining the function of sensory and sympathetic neurons (Barde, 1989). Furthermore, the concentration of nerve growth factor is increased in the synovial fluid and synovial membrane with arthritis (Aloe et al., 1993). Assuming that the simultaneous up-regulation of sensory and autonomic neuropeptides in arthritis occurs independently, it seems likely that the stimulation of both systems is related to the same factor, possibly nerve growth factor (Donnerer et al. 1992). In the paper by Donnerer et al. (1992) it was reported that a unilateral injection of a pro-inflammatory substance into one hind paw resulted in a unilateral increase of substance P and CGRP in the dorsal root ganglia and dorsal horn, but no increase was detected in the peripheral tissue. In the present study we report that there was an increase in the trigeminal ganglia and in the peripheral tissue (temporomandibular joint). This discrepancy is probably due to the longer duration of inflammation, i.e. 28 days as opposed to the 5 days of induction in the study of Donnerer et al., allowing for an increase of neuropeptide synthesis in the periphery. This in turn implies that neuronal therapy in arthritis should be targeted against both the sensory and autonomic systems.

Effects of unilateral surgical denervation of temporomandibular joint

In both untreated and arthritic rats, the unilateral surgical denervation caused a significant reduction in substance P in the ipsilateral temporomandibular joint and in the ipsilateral trigeminal ganglia, and CGRP was decreased in the untreated arthritic rats in the ipsilateral temporomandibular joint. The reduction of neuropeptide Y was more evident peripherally in the temporomandibular joint than centrally in the trigeminal ganglia. In this study, section of the mandibular part of the trigeminal nerve caused a significant reduction in sensory neuropeptides in temporomandibular joints, but it could not prevent the development of arthritis. Others have shown that ipsilateral sciatic nerve section causes complete disappearance of substance P-positive nerve fibres in the synovial membrane in a normal ankle joint and a marked reduction in the number of CGRP-positive fibres (Ahmed et al., 1995). In normal rats, Villar et al. (1989) reported a slight decrease in substance P of the contralateral dorsal root ganglia after sciatic nerve section. Also, degeneration of the contralateral dorsal horn following sciatic nerve section has been described (Jessell et al., 1979). A probable explanation of why nerve section did not fully abolish the substance P and CGRP in temporomandibular joints could be the presence of non-neuronal sources of sensory neuropeptides such as macrophages, lymphocytes and leukocytes (Jakab et al., 1993).

Effects of capsaicin in arthritic temporomandibular joint and trigeminal ganglia

It is reasonable to assume that the capsaicin-induced reduction of substance P and CGRP in inflamed joints can alleviate the hyperalgesia and swelling associated with arthritis. However, the remaining content of substance P and CGRP may still contribute to joint inflammation, possibly in conjunction with, or in addition to, other factors such as from the immune system (Ahmed et al., 1995). Surgical and chemical sympathectomy reduced the severity of pain and inflammation in patients with rheumatoid arthritis and reflex sympathetic dystrophy (Hannington-Kiff, 1990), but the mechanisms underlying the therapeutic effect of sympathectomy remain unclear. Recently, an increased expression of neuropeptide Y mRNA was observed in dorsal horn neurones of the spinal cord during inflammation (Ji et al., 1994).

Although we believe this is the first study of neuropeptide Y in temporomandibular joints of arthritic rats given capsaicin, the results are in close agreement with those reported from similar studies of other tissues (Evangelista et al., 1992; Guarna et al., 1991; Nilsson et al., 1990; Barde, 1989). Overall, it seems that chemical and surgical denervation causes essentially the same degree of sensory neuropeptide depletion, which is encouraging when considering a pharmacological treatment option. The peripheral sensory and autonomic nervous systems are involved in the development of temporomandibular joint arthritis. Capsaicin treatment decreased the neuropeptide content. It is possible that this approach may be used in the future treatment of temporomandibular joint arthritis.

REFERENCES


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