Capsaicin effects on substance P and CGRP in rat adjuvant arthritis

Mahmood Ahmed a,*; Anders Bjurholm a; Gopala Rao Srinivasan b; Tomas Lundeberg c; Elvar Theodorsson b; Marianne Schultzberg d; Andris Kreicbergs a

a Department of Orthopaedics, Karolinska Hospital, S-171 76 Stockholm, Sweden
b Department of Clinical Chemistry, Karolinska Hospital, S-171 76 Stockholm, Sweden
c Department of Physiology, Karolinska Institute, S-171 77 Stockholm, Sweden
d Department of Clinical Neuroscience and Family Medicine, Geriatric Medicine, Novum, KFC, S-141 86 Huddinge, Sweden

Received 25 February 1994; revised version received and accepted 30 September 1994

Abstract

The effects of capsaicin on the sensory neuropeptides substance P and calcitonin gene-related peptide were analyzed in the ankle joints and dorsal root ganglia (L2-L6) of adult female Lewis rats. The study included 23 normal rats and 23 arthritic rats, all injected subcutaneously with capsaicin (total dose 200 mg/kg bw). Another two groups of animals from a previous study, i.e., 23 normal rats and 23 arthritic rats not given capsaicin served as controls. Adjuvant arthritis was induced by inoculation with heat-killed mycobacteria. The morphological distribution of sensory neuropeptides was assessed by immunohistochemistry and the tissue concentrations were determined by radioimmunoassay. In normal rats, capsaicin significantly reduced the concentrations of substance P and calcitonin gene-related peptide in ankle joints (54 and 36%, respectively) as well as dorsal root ganglia (40 and 54%, respectively). In arthritic rats those pretreated with capsaicin had significantly lower concentrations of substance P and calcitonin gene-related peptide in ankle joints (19 and 42%, respectively) compared to the arthritic controls. In the ankle joints, however, only the SP concentration was reduced (42%). Notably, this was accompanied by a 40% reduction in inflammatory response as assessed by comparing the ankle joint weights of the experimental groups. In general, there was a good correlation between the neuropeptide concentrations in ipsilateral ankle joints and the corresponding dorsal root ganglia as assessed in individual rats. The present study of adjuvant induced arthritis shows that capsaicin administration reduces the otherwise up-regulated levels of sensory neuropeptides in dorsal root ganglia and ankle joints. However, capsaicin at the dose given can only mitigate, not completely prevent the development of joint inflammation. Nonetheless, the findings suggest that antineuronal therapy targeted against specific neurotransmitters may prove useful in inflammatory joint disease.

Keywords: Ankle joint; Dorsal root ganglia; Mycobacterium; Neuropeptide

* Corresponding author. Fax: +46 8 7295778.

0167-0115/95/$9.50 © 1995 Elsevier Science B.V. All rights reserved
SSDI 0167-0115(94)00095-6
1. Introduction

Capsaicin, naturally occurring in red pepper, is a well known neurotoxin for sensory neurons. It specifically elicits neurotransmitter release from small sized neurons of dorsal root ganglia and their projections [1-4,7,8,38]. Several studies have shown that capsaicin depletes sensory neuropeptides, i.e., substance P (SP) and calcitonin gene-related peptide (CGRP) from cells in dorsal root ganglia and from terminals in the dorsal horn and the periphery [1-6,44]. In dorsal root ganglia, the site of SP and CGRP synthesis, capsaicin specifically depletes small sized cells [1,2,6,7]. Capsaicin administration to neonatal rats causes degeneration of small size primary sensory neurons [1,8-11], whereas in adult rats it results in a reversible depletion of sensory neuropeptides [12,13]. The effects of capsaicin on dorsal root ganglia are not confined to SP and CGRP. Thus, capsaicin has been reported to deplete somatostatin, vasoactive intestinal polypeptide (VIP) and neurokinin A (NKA) from dorsal root ganglia and dorsal horn [2,14,15]. Notably, SP fibres of sensory origin in autonomic ganglia are also affected by capsaicin [16,17].

Capsaicin administration has been shown to attenuate adjuvant arthritis in rats [18,19]. In patients with osteoarthritis of finger joints, topical capsaicin treatment was recently reported to reduce the signs of inflammation [20,21]. These, as well as other clinical and experimental observations, strongly indicate that the nervous system, notably the sensory, has a pathophysiological role in inflammatory joint disease [18,19,22-26]. It has been shown that the expression of sensory neuropeptides are altered in arthritis [24-30]. In a recent study of adjuvant rat arthritis based on immunohistochemistry and radioimmunoassay (RIA) we found a significant increase in SP-like immunoreactivity (LI) and CGRP-LI in ankle joints and the corresponding dorsal root ganglia [31]. Although capsaicin has been reported to mitigate the classical signs of arthritis, most likely by an antineuronal mechanism, its effect on sensory neuropeptides in arthritic joints has not been clarified.

In the present study, the effect of capsaicin on sensory neuropeptides in ankle joints and the corresponding dorsal root ganglia was investigated in both normal and arthritic rats by immunohistochemistry and RIA.

2. Materials and methods

The study included 46 female Lewis rats (bw 230-250 g) treated with capsaicin. The series was divided into 2 experimental groups, 23 rats in each. One group received capsaicin only, whereas the other was also inoculated with heat-killed mycobacteria (Mycobacterium butyricum) to induce arthritis. Another two groups reported previously [31] served as controls; one comprised 23 untreated normal rats, and the other included 23 rats with adjuvant arthritis induced by the mycobacteria. The animals were housed 5/cage at 21 °C in a 12 h light/dark cycle with water and pellets ad libitum according to the Karolinska Institute protocol.

Capsaicin (50 mg/kg bw, dissolved in 10% ethanol and 10% Tween-80 in isotonic saline) was injected subcutaneously in all 46 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 mg/kg) intraperitoneally (i.p.) prior to each capsaicin injection. 23 of the animals were also inoculated with mycobacteria 1 week after capsaicin administration was completed, i.e., on day 11.

Arthritis was induced by intradermal injection (0.05 ml) of a suspension of heat-killed mycobacteria in paraffin oil (10 mg/ml) into the base of the tail [32]. The 23 normal controls received 0.05 ml paraffin oil via the same route.

Radiography of the ankle joints (not shown) was performed 11 and 29 days post inoculation in 10 arthritic rats and 4 normal controls. The weights of the dissected ankle joints were determined as a mea-
Table 1
Comparison of ankle joint weights (mg)

<table>
<thead>
<tr>
<th></th>
<th>Control ratsa median: UQ-LQ</th>
<th>Arthritic ratsa median: UQ-LQ</th>
<th>Capsaicin + arthritic ratsa median: UQ-LQ</th>
</tr>
</thead>
</table>

Comparison of ankle joint weights are made between the control, untreated arthritic and capsaicin pretreated arthritic rats. Each value (mg) corresponds to the median and upper-lower quartiles (UQ–LQ), n = 18. Differences are expressed by asterisks according to the level of significance: *** P< 0.001, Rt = right; Lt = left. a The date on control and arthritic rats are taken from Ref. [31].

sure of the degree of inflammation to permit comparison between the experimental groups: i.e., control rats, arthritic rats and capsaicin pretreated arthritic rats (Table 1). Histological analysis (hematoxylin-eosin) of ankle joints from these three groups was also performed to assess inflammatory changes (Fig. 1A–C).

The end-point of the experiment was set at day 29 after the mycobacteria inoculation. Thus, in the experimental group receiving pretreatment with capsaicin for 4 days followed by an interval of 7 days before inoculation with mycobacteria, the whole experimental period covered 40 days, i.e., 4 + 7 + 29 days. For meaningful comparison, the animals given capsaicin only, were also killed 40 days after the first capsaicin injection.

In each experimental group, 18 rats were taken for RIA and 5 rats for immunohistochemistry. In each rat, the dissection comprised both ankle joints and the lumbar dorsal root ganglia (L2-L6). The specimens for RIA were prepared and analyzed separately with the exception of the dorsal root ganglia. Thus, the latter were pooled as left and right specimens from the L2-L6 levels in each rat, i.e., 5 ganglia in each pool yielding a total of 18 right and 18 left samples from each experimental group. This permitted correlative studies of the neuropeptide concentrations in a given ankle joint and the corresponding dorsal root ganglia.

2.1. Radioimmunoassay

The rats were killed by decapitation during ether anaesthesia 29 days post inoculation with mycobacteria. The ankle joint including the tarsal joints was dissected as one specimen, bilaterally. Thus, the specimen consisted of capsular/synovial tissue as well as the distal tibial epiphysis, the whole of talus and calcaneus, and the tarsal bones. The dorsal root ganglia (L2-L6) were excised bilaterally, and pooled as left and right side specimens. Thus, each rat yielded two samples, each consisting of 5 ganglia. All of the dissected tissues were immediately frozen on dry ice and kept at –70°C until neuropeptide extraction.

Each frozen sample was weighed before extraction. The neuropeptides were extracted and quantified as described recently for bone and joint tissue [33,34]. Briefly, the samples were cut into small pieces, boiled for 10 min in 10 volumes of 2 mol/l acetic acid in 4% ethylene diaminotetraacetic acid (EDTA), homogenized in a Polytron (15 s), sonicated (30 s) and centrifuged 3000 g for 15 min. The supernatants were diluted in 10 ml RIA buffer and kept at –20°C until analysis.

The analysis was performed using the following antisera:

Substance P-like immunoreactivity (SP-LI) was assessed using antiserum SP2 [35] raised in a rab-
The antiserum reacts with SP and SP sulfoxide, but not with other tachykinins. High performance liquid chromatography (HPLC) purified $^{125}$I-tyrosine rat SP was used as radioligand and rat SP was used as standard. The detection limit was 3 pmol/l. Intra- and interassay coefficients of variation were 7 and 11%, respectively.

Calcitonin gene-related peptide-like immunoreactivity (CGRP-LI) was analyzed using antiserum CGRP8 raised in a rabbit against conjugated rat CGRP. HPLC purified $^{125}$I-histidyl rat CGRP was used as radioligand and rat CGRP as standard. The detection limit of the assay for rat CGRP was 9 pmol/l and cross-reactivity of the assay to SP, neurokinin A, neurokinin B, neuropeptide K, gastrin, neuropeptide Y and calcitonin was less than 0.01%. Cross-reactivity with human CGRP $\alpha$ and $\beta$ was 93 and 24%, respectively, and with rat CGRP $\alpha$ and $\beta$, 100 and 120%, respectively. Intra- and interassay coefficients of variation were 8 and 14%, respectively.

2.2. High performance liquid chromatography

In two previous studies [31,33], reverse phase HPLC was applied to extracts of normal knee joints as well as normal and arthritic ankle joints to characterize the neuropeptides of interest. Extraction in 2 mol/l acetic acid in 4% EDTA was found to provide optimum yield of both sensory and autonomic neuropeptides. HPLC analysis of the immunoreactive material from joint samples with regard to SP and CGRP consistently resulted in a main peak eluting in the position of the corresponding synthetic peptide. Thus, no evidence of multiple immunoreactivities was noted for SP and CGRP. As the same extraction procedure and antisera were used in the present study, the HPLC analysis was not repeated.

(A). S = synovium, C = joint cavity, Ta = talus, Ti = distal tibia. The original magnification is $87 \times$ in all micrographs.
2.3. Immunohistochemistry

The rats were anaesthetized by i.p. injection of sodium pentobarbitone (60 mg/kg bw). Intra-arterial perfusion with phosphate-buffered saline (PBS) preceded perfusion with the fixative consisting of 4% paraformaldehyde in 0.2 mol/l Sörensen phosphate buffer, pH 7.3, containing 0.2% picric acid.

The dissection of the ankle joints and dorsal root ganglia was performed as described for the RIA. All samples were immersed in the same fixative for two days at +4°C. The joint specimens containing bone were subjected to demineralization in a 4% EDTA solution at pH 7.3 for approximately 3 weeks as described previously [36], whereas the dorsal root ganglia did not require this step.

All specimens were rinsed for at least 2 days in 20% sucrose in 0.1 mol/l Sörensen phosphate buffer, pH 7.2, containing sodium azide and bacitracin (Sigma, St. Louis). The tissues were cut on a Leitz cryostat to a section thickness of 15 μm. The sections were mounted on chrome alum-gelatin coated slides and processed for an indirect immunofluorescence method as described by Coons [37]. Briefly, the sections were rinsed for 15 min in 0.01 mol/l PBS, pH 7.3, and then incubated overnight in a humid atmosphere at +4°C with antiserum to SP, CGRP (dilution 1:200) and Protein Gene Product 9.5 (PGP 9.5) (dilution 1:400).

The SP antiserum was raised in rabbits against the synthetic undecapeptide (Peninsula Laboratories Europe Ltd., St. Helens, UK). The CGRP antiserum was raised in rabbits against the synthetic 37 amino acid peptide of rat CGRP (Peninsula Laboratories Europe Ltd., St. Helens, UK). The PGP 9.5 antiserum was obtained from Ultraclone Ltd., Cambridge, UK. The characteristics of this antiserum have been reported previously [38].

After incubation with the primary antisera, the sections were rinsed in PBS for 2 × 10 min, and then incubated with fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit antibodies (diluted 1:10, Amersham Sweden, Solna) for 30 min at +37°C. A final rinse in PBS for 15 min preceded mounting in a mixture of glycerol p-phenylenediamine [39]. Controls were performed by omitting the primary antiserum. Addition of 50 μg/ml peptide to the corresponding antiserum prior to application on tissue sections served as another control. A Nikon epifluorescence microscope was used to analyze the sections, and T-Max black and white film (Kodak, Rochester) was used for photography.

2.4. Statistics

The median and upper/lower quartiles were used as measures of central tendency and variation. Data were tested for normality using the Anderson-Darling test [36]. The Mann-Whitney test was used to assess differences between two groups. Tukey multiple comparison test was used to assess differences between the four groups. Spearman’s rank coefficient was used to analyze the correlation between groups. P values < 0.05 were considered significant.

3. Results

3.1. Arthritis

In the control group of 23 rats inoculated with mycobacteria, signs of arthritis as reported previously [27], appeared in the ankle joints 9 to 13 days after injection. There was bilateral hind paw swelling, warmth and redness, which persisted until the end of the experiment. There were no radiographical abnormalities on day 11. However, on day 29 there were bony erosions, periosteal thickening and joint space narrowing. In the 23 rats pretreated with capsaicin before inoculation with mycobacteria, there were similar inflammatory signs, although they were less pronounced. The control rats showed no macroscopical, histological (Fig. 1A) or radiographical signs of joint inflammation [31]. Comparison of ankle joint weights (left and right sides combined)
showed significantly higher values (109% increase) in arthritic rats compared to control rats (Table 1). In arthritic rats pretreated with capsaicin, the increase in weight was 44% compared to control rats. Notably, there was a significant reduction (40%; \( P<0.001 \)) in ankle joint weights of capsaicin pretreated arthritic rats compared to that of untreated arthritic rats (Table 1). Histological analysis of ankle joints (hematoxylin-eosin staining) from arthritic rats showed synovial hypertrophy and chronic inflammatory cell infiltration (Fig. 1B). In arthritic rats pretreated with capsaicin, there were similar inflammatory changes, although they were less pronounced (Fig. 1C).

Table 2a
Tissue concentrations of SP-LI in control and capsaicin treated rats

<table>
<thead>
<tr>
<th>Control vs. Capsaicin</th>
<th>median: UQ-LQ</th>
<th>median: UQ-LQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankle joints, Rt</td>
<td>0.47:0.55-0.31</td>
<td>0.22:0.27-0.18***</td>
</tr>
<tr>
<td>Ankle joints, Lt</td>
<td>0.50:0.55-0.38</td>
<td>0.23:0.27-0.18***</td>
</tr>
<tr>
<td>DRG-Rt</td>
<td>3.59:4.43-3.15</td>
<td>2.00:2.34-1.45***</td>
</tr>
<tr>
<td>DRG-Lt</td>
<td>4.02:4.50-3.72</td>
<td>2.55:2.79-1.95***</td>
</tr>
</tbody>
</table>

Table 2b
Tissue concentrations of SP-LI in arthritic and capsaicin pre-treated arthritic rats

<table>
<thead>
<tr>
<th>Arthritis vs. Capsaicin + arthritic</th>
<th>median: UQ-LQ</th>
<th>median: UQ-LQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankle joints-Rt</td>
<td>0.80:0.90-0.77</td>
<td>0.48:0.61-0.45***</td>
</tr>
<tr>
<td>Ankle joints-Lt</td>
<td>0.81:0.86-0.75</td>
<td>0.46:0.58-0.41***</td>
</tr>
<tr>
<td>DRG-Rt</td>
<td>5.80:6.38-5.04</td>
<td>5.13:5.34-4.71***</td>
</tr>
<tr>
<td>DRG-Lt</td>
<td>5.48:5.95-5.03</td>
<td>4.15:4.60-3.62***</td>
</tr>
</tbody>
</table>

The concentrations were obtained from RIA measurements in tissue extracts from individual animals as described in Materials and methods. Each value (pmol/g) corresponds to the median and upper-lower quartiles (UQ-LQ), \( n = 18 \). Differences are expressed by asterisks according to the level of significance: *** \( P<0.0001 \), Rt = right, Lt = left, DRG = dorsal root ganglia, vs. = versus. * The data on arthritic rats are taken from Ref. [31].

3.2. Radioimmunoassay

The concentrations of CGRP-LI in the different tissues were consistently higher than the SP-LI concentrations. In all experimental groups, the concentrations of SP-LI and CGRP-LI were higher in the ankle joints than in the ankle joints (Tables 2 and 3).

In normal rats, capsaicin administration significantly lowered the concentrations of SP-LI and CGRP-LI in ankle joints and dorsal root ganglia (Tables 2A and 3A). The mean decrease (left and right sides combined) in SP-LI and CGRP-LI in ankle joints was 54 and 36%, respectively. In dorsal root ganglia, the corresponding figures were 40 and 54%.

In arthritic rats, those pretreated with capsaicin had lower concentrations of SP-LI in both ankle joints and dorsal root ganglia than the rats not pretreated with capsaicin. The mean decrease (left and right sides combined) in SP-LI was 42% in ankle joints and 19% in dorsal root ganglia (Table 2B). The CGRP-LI concentration was reduced only in the dorsal root ganglia (mean 42%) (Table 3B).

Comparative analysis of ipsilateral ankle joints and the corresponding dorsal root ganglia in individual animals disclosed consistent correlations with regard to both SP and CGRP concentrations (Table 4A,B).

3.3. Immunohistochemistry

In the arthritic control rats, as reported previously [31], there was a clear increase in SP- and CGRP-LI in the synovium of ankle joints (cf. Fig. 2A and B and Fig. 4A and B) and dorsal root ganglia (cf. Fig. 3A and C and Fig. 5A and C).

In capsaicin treated rats, there was a clear reduction in SP and CGRP immunoreactivity in all tissues analyzed. The effect of capsaicin seemed more pronounced for SP than CGRP. The effect was also more obvious in normal than in arthritic rats.
Table 3a
Tissue concentrations of CGRP-LI in control and capsaicin treated rats

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control median: UQ-LQ</th>
<th>Capsaicin median: UQ-LQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankle joints, Rt</td>
<td>1.34:1.82-1.27</td>
<td>1.09:1.22-0.97***</td>
</tr>
<tr>
<td>Ankle joints, Lt</td>
<td>1.80:2.15-1.54</td>
<td>0.91:0.81-1.11***</td>
</tr>
</tbody>
</table>

Table 3b
Tissue concentrations of CGRP-LI in arthritic and capsaicin pretreated arthritic rats

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Arthritic median: UQ-LQ</th>
<th>Capsaicin + arthritic median: UQ-LQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankle joints, Rt</td>
<td>2.53:2.61-2.12</td>
<td>2.47:2.61-1.90 NS</td>
</tr>
<tr>
<td>Ankle joints, Lt</td>
<td>2.77:2.81-1.80</td>
<td>2.42:2.81-1.75 NS</td>
</tr>
<tr>
<td>DRG-Rt</td>
<td>37.53:50.85-24.65</td>
<td>20.02:27.68-12.86***</td>
</tr>
<tr>
<td>DRG-Lt</td>
<td>25.35:39.35-29.99</td>
<td>17.34:20.88-11.65***</td>
</tr>
</tbody>
</table>

The concentrations were obtained from RIA measurements in tissue extracts from individual animals as described in Materials and methods. Each value (pmol/g) corresponds to the median and upper-lower quartiles (UQ-LQ), n = 18. Differences are expressed by asterisks according to the level of significance: *** P<0.001, Rt = right, Lt = left, DRG = dorsal root ganglia, NS = non-significant, vs. = versus. The data on arthritic rats are taken from Ref. [31].

**SP**

In normal rats, SP immunoreactive nerve fibres were almost completely absent in the ankle joint synovium (cf. Fig. 2A and C) and only occasional SP-positive cells were seen in the dorsal root ganglia (cf. Fig. 3A and B) after capsaicin treatment. SP-positive fibres could not be detected in bone, periosteum and bone marrow of capsaicin treated rats.

In arthritic rats pretreated with capsaicin, some SP immunoreactive fibres could still be found in the synovium (cf. Fig. 2B and D), but no SP-positive fibres were seen in bone, periosteum or bone marrow. In the dorsal root ganglia, SP-LI was only found in a few cells (cf. Fig. 3C and D).

**CGRP**

In normal rats, CGRP-LI was clearly reduced but still discernible in the ankle joint synovium (cf. Fig. 4A and C) and the dorsal root ganglia after capsaicin treatment. The CGRP-positive fibres in the synovium were predominantly non-vascular. A few immunoreactive fibres were also seen in the periosteum, bone and bone marrow. In the dorsal root ganglia, a number of CGRP-positive cells could still be found (cf. Fig. 5A and B).

In arthritic rats, those pretreated with capsaicin showed a clear reduction in CGRP immunoreactive fibres in the ankle joint synovium (cf. Fig. 4B and E). The remaining fibres were both vascular and non-vascular. A substantial number of CGRP-positive fibres were still observed in the periosteum (Fig. 6E), bone (Fig. 4D) and bone marrow despite the capsaicin pretreatment. In the dorsal root ganglia, a large number of CGRP-positive cells could still be seen (cf. Fig. 5C and D).

**PGP 9.5**

The distribution of PGP 9.5 immunoreactive fibres in normal as well as arthritic rats pretreated with capsaicin was similar to that of SP- and CGRP-positive fibres. The number of PGP 9.5 immunoreactive fibres in the synovium was clearly reduced by capsaicin in both normal and arthritic rats (cf. Fig. 6A and D, B and C).

4. Discussion

In a previous study of adjuvant induced arthritis in the rat, we observed a significant up-regulation of SP-LI and CGRP-LI in ankle joints and dorsal root ganglia [31]. In the present study, pretreatment with capsaicin was found to mitigate this upregulation, thus complying with previous reports showing that the neurotoxin capsaicin reduces the expression of sensory neuropeptides, which have been implicated in adjuvant arthritis [18,19]. However, the preventive effect of capsaicin on adjuvant arthritis was only
Table 4a
Correlations of SP-LI between paired tissues (right and left side) and between ipsilateral ankle joints and dorsal root ganglia

<table>
<thead>
<tr>
<th></th>
<th>Control*</th>
<th>Capsaicin</th>
<th>Arthritic*</th>
<th>Capsaicin + arthritic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankle joints Rt, Lt</td>
<td>0.52*</td>
<td>0.85***</td>
<td>0.65**</td>
<td>0.45*</td>
</tr>
<tr>
<td>DRG Rt, Lt</td>
<td>0.47*</td>
<td>0.32 NS</td>
<td>0.59**</td>
<td>0.25 NS</td>
</tr>
<tr>
<td>Ankle joints Rt, DRG Rt</td>
<td>0.59**</td>
<td>0.50*</td>
<td>0.82***</td>
<td>0.43*</td>
</tr>
<tr>
<td>Ankle joints Lt, DRG lt</td>
<td>0.49*</td>
<td>0.70***</td>
<td>0.60**</td>
<td>0.50*</td>
</tr>
</tbody>
</table>

Table 4b
Correlations of CGRP-LI between paired tissues (right and left side) and between ipsilateral ankle joints and dorsal root ganglia

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Capsaicin</th>
<th>Arthritic*</th>
<th>Capsaicin + arthritic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankle joints Rt, Lt</td>
<td>0.66*</td>
<td>0.57**</td>
<td>0.75***</td>
<td>0.59**</td>
</tr>
<tr>
<td>DRG Rt, Lt</td>
<td>0.46*</td>
<td>0.46*</td>
<td>0.73***</td>
<td>0.19 NS</td>
</tr>
<tr>
<td>Ankle joints Rt, DRG Lt</td>
<td>0.84***</td>
<td>0.57**</td>
<td>0.73***</td>
<td>0.42*</td>
</tr>
<tr>
<td>Ankle joints Lt, DRG Lt</td>
<td>0.57**</td>
<td>0.48*</td>
<td>0.80***</td>
<td>0.40*</td>
</tr>
</tbody>
</table>

Values were given as Spearman’s rank coefficients. Each represents 18 paired comparisons. The level of significance is represented by asterisks: * P< 0.05, ** P< 0.01, *** P< 0.001, NS = non-significant, Rt = right, Lt = left, DRG = dorsal root ganglia. The data on normal and arthritic rats are taken from Ref. [31].

Partial as reflected by persisting inflammatory signs (c.f. [41]) and increased ankle joint weights. In arthritic rats, the capsaicin induced reduction in ankle joint weights and sensory neuropeptide levels was essentially of the same magnitude.

Capsaicin is known to elicit neurotransmitter release from small sized neurons (type B) of dorsal root ganglia [1-4,42] and their projections of unmyelinated C and thinly myelinated A δ fibres [1,2,7,8,42]. The majority of these neurons synthesize both SP and CGRP [43-44]. A substantial proportion of CGRP, in contrast to SP, is also synthesized in large sized cells (type A) [43-47], from which myelinated fibres arise [48]. It has been shown that capsaicin administration to neonatal rats reduces SP-LI in dorsal root ganglia by 74% [2,42] and CGRP-LI by 50–60% [4,5]. Thus, the small sized neurons, producing most of the SP found in dorsal root ganglia, are more vulnerable to capsaicin than the large sized neurons mainly producing CGRP. Moreover, it has been reported that unmyelinated fibres are more sensitive to capsaicin than myelinated fibres [8-12,42]. Differential effects of capsaicin on various other neuropeptides in dorsal root ganglia have also been described [2].

The present immunohistochemical findings in normal rats would seem to comply with a higher sensitivity to capsaicin of the small type cells than of the large type cells. Thus, there was an almost complete disappearance of SP-LI in the synovium and the dorsal root ganglia after capsaicin treatment, whereas CGRP-positive structures were still observed, conceivably representing projections of large sized cells, known to be resistant to capsaicin. However, our immunohistochemical findings suggesting a difference between the response of SP and CGRP to capsaicin are not entirely in agreement with our RIA results. Firstly, measurable levels of SP were still present in the tissues after capsaicin treatment. Secondly, the capsaicin induced reductions of SP and CGRP in normal ankle joints and dorsal root ganglia were of approximately the same magnitude [36–
Fig. 3.
Presumably, the immunohistochemical findings suggesting a difference between SP and CGRP in response to capsaicin are due to the difference in tissue concentrations. Thus, a reduction by half of SP, which normally occurs at very low tissue concentrations, seems to leave too small amounts for detection by immunohistochemistry. In contrast, CGRP, which normally occurs at high tissue concentrations, is still easily detected by immunohistochemistry despite a reduction by half.

These observations would seem to reflect the difference in the detection limit of immunohistochemistry and RIA [49]. Although immunohistochemistry provides valid information about the morphological distribution of neuropeptides, it is not as reliable as RIA in detecting small amounts. Admittedly, our RIA analysis may entail some non-neuronal SP and CGRP, e.g., in interstitial tissue and blood. However, the immunohistochemical analysis of normal tissues demonstrated that the SP and CGRP immunoreactivities indeed occurred in neuronal structures. In addition, staining with antiserum to the neuronal marker PGP 9.5 revealed a similarity in appearance of PGP 9.5 immunoreactive nerve fibres and the SP and CGRP-positive fibres. From the present study based on both immunohistochemistry and RIA, it appears that capsaicin at the dose employed in adult rats only partly depletes SP and CGRP from sensory neurons, as also described in other studies [2]. Moreover, there does not seem to be any difference in capsaicin response between SP and CGRP according to RIA.

The partial effect of capsaicin observed in normal rats may explain that treatment with capsaicin before inoculation with mycobacteria could not prevent the development of arthritis, nor an up-regulation of sensory neuropeptides from subnormal levels. It seems that the neuropeptide depleting effect of capsaicin at the given dose is incomplete, reversible and counteracted by the induction of arthritis. The latter caused an up-regulation from subnormal levels to levels that seemed to vary according to site...
and neuropeptide. In ankle joints, the SP concentration reached the normal level, whereas that of CGRP clearly exceeded the normal level (+55%). Conversely, in dorsal root ganglia the SP concentration exceeded the normal level (+22%), whereas that of CGRP reached a level below the normal (-14%). However, these figures in relation to normal levels should be interpreted with caution, since the actual starting levels are unknown. Thus, they represent the net effect of an initial downregulation by capsaicin and a subsequent upregulation by induction of arthritis. The specific mechanisms behind the differential effects of capsaicin on SP-LI and CGRP-LI at different sites in arthritic rats remain unknown. Although the differences were moderate, it may be speculated that arthritis induces expression of sensory neuropeptides in capsaicin resistant neurons, which are not normally of the SP or CGRP phenotype.

An important factor, which most likely influences the levels of SP and CGRP in normal as well as inflamed tissues is nerve growth factor (NGF). Under normal conditions, it is well known that NGF plays a significant role in maintaining the function of sensory and sympathetic neurons [50,51]. In adult rats, it has been shown that NGF treatment induces increased synthesis of SP and CGRP in the dorsal root ganglia [52,53]. In addition, NGF deprivation has been shown to cause a downregulation of SP [54,55]. In a study of adult guinea pigs, capsaicin treatment was found to impair the retrograde transport of NGF to the dorsal root ganglia. This was presumed to cause the decreased synthesis of SP noted [56]. Notably, NGF administration to guinea pigs and rats pretreated with capsaicin was found to reverse the effect of capsaicin. In fact, SP was found to be upregulated in the dorsal root ganglia [56,57]. In arthritis, it has been shown that the concentration of NGF is increased in synovial fluid and synovial membrane [58–60]. In a study on paw inflammation, there was a parallel upregulation of NGF and sensory neuropeptides in the sciatic nerve [61]. In all, these observations show that capsaicin has a downregulatory effect on NGF, whereas arthritis has an upregulatory effect. Whether the effect of arthritis is stronger than that of capsaicin, as indicated by our results, has yet to be demonstrated.

It seems reasonable to summarize from the present investigation of the capsaicin effect in arthritis that SP and CGRP levels end up approximately intermediate between those noted in rats given capsaicin only and those in rats given mycobacteria only. It may prove that a complete depletion of SP and CGRP in adult rats can be achieved by higher doses of capsaicin than those employed in the present study (200 mg/kg bw). Whether higher doses of capsaicin would completely prevent the development of arthritis has yet to be demonstrated. In a study on adult rats by Gamse and co-workers [2], capsaicin elicited a dose-dependent depletion of SP in dorsal root ganglia (33% by 125 mg/kg and 67% by 950 mg/kg), although the highest dose did not result in a complete depletion of SP. In the dorsal horn and saphenous nerve there was no further depletion by higher doses. In neonatal rats, a lower dose (50 mg/kg bw) of capsaicin decreased SP by 74% in the dorsal root ganglia [2,42].

The present study also specifically explored the effects of capsaicin on sensory neuropeptides in individual ankle joints and corresponding dorsal root ganglia. Comparative analysis disclosed a consistent correlation between the concentrations of sensory neuropeptides in ipsilateral dorsal root ganglia and ankle joints. However, it remains unknown whether the correlations observed reflect depletion at both anatomical sites or only peripherally.

It may be summarized that capsaicin significantly reduces SP- and CGRP-LI in dorsal root ganglia and ankle joints of normal rats. Pretreatment with capsaicin mitigates, but does not prevent the development of adjuvant arthritis. It is reasonable to assume that capsaicin induced reduction of SP and CGRP in inflamed joints can alleviate hyperalgesia and swelling associated with arthritis. However, the remaining SP and CGRP may still contribute to joint inflammation, possibly in conjunction with, or
in addition to, other factors, e.g., from the immune system.

Acknowledgements

This study was supported by grants from the Royal 80 Year Funds of King Gustaf V, Ugglä’s Foundation, Åke Wibergs Foundation, the Karolinska Institute Research Funds, Lundbergs Foundation, the Swedish Association Against Rheumatic Disease, the Swedish Medical Research Council (12X-08652-01, 12X-07464) and the Swedish Society for Medical Research.

References

[21] Deal, C.L., Schnitzer, T.J., Lipstein, E., Seibold, J.R.,
munoreactivities in dorsal spinal cord and loss of CGRP-related peptide (CGRP), substance P and enkephalin im-

Pradelles, P., Elevated levels of tachykinin-like immunore-
activity in joint fluids from patients with rhematoid in-

Ahmed, M., Bjurholm, A., Srinivasan, G.P., Theodorsson, E. and Kreiebergs, A., Extraction of neuropeptides from

Ahmed, M., Bjurholm, A., Srinivasan, G.P., Theodorsson, E. and Kreiebergs, A., Extraction and quantitation of neu-


Bjurholm, A., Kreiebergs, A. and Schultzberg, M., Fixation and demineralization of bone tissue for immunohistoche-

Coons, A.H., Fluorescent antibody method. In F. Danielli (Ed.), General Cytochemical Methods, Academic Press,

visualising whole uterine innervation and pregnancy-
duced developmental changes in the guinea pig. His-

Johnson, D.G. and De Nogueira-Araujo, G.M., A simple method of reducing the fading of immunofluorescence dur-

Theodorsson-Norheim E., BASIC computer program to summarize data using non-parametric statistics including


Gibbins, I.L., Furness, J.B., Costa, M., MacIntyre, I., Hill-
yard, C.J. and Girgis, S., Co-localization of calcitonin gene-

Lee, Y., Takami, Y., Kawai, Y., Girgis, S., Hillyard, C.J., MacIntyre, I., Emson, P.C. and Tohyama, M., Distribution of calcitonin gene-related peptide in the rat peripheral ner-

Wiesenfeld-Hallin, Z., Hökfelt, T., Lundberg, J.M., Fos-
man, W.G., Reinecke, M., Tschopp, F.A. and Fisher, J.A., Immunoreactive calcitonin gene-related peptide and sub-


