Neuropeptide gene expression and capsaicin-sensitive primary afferents: maintenance and spread of adjuvant arthritis in the rat

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1. Many experimental and clinical arthritides are characterized by their bilateral nature. There is strong evidence to suggest that this bilateral spread may be mediated by a neuronal mechanism. We have previously shown early and sustained induction of mRNAs encoding preprotachykinin (PPT) and calcitonin gene-related peptide (CGRP) in dorsal root ganglion (DRG) neurons innervating an inflamed, arthritic joint. We have now investigated the involvement of capsaicin-sensitive primary afferents and the expression of neuropeptide mRNAs in the maintenance and bilateral spread of mild adjuvant-induced arthritis in the rat.

2. Capsaicin was applied perineurally to either the left (Cap-L) or right (Cap-R) sciatic nerve of halothane-anaesthetized male Han Wistar rats. Two weeks after capsaicin lesioning, arthritis was induced by injection of Freund's complete adjuvant (FCA) around the left ankle at a dose that caused inflammation of the left ankle joint, and a delayed (14 days) contralateral (right) ankle arthritis. Arthritis was monitored for 15 days after injection, when animals were killed and the lumbar DRG dissected. PPT, CGRP, somatostatin (SS), and vasoactive intestinal polypeptide (VIP) mRNA expression was determined in L5 DRG using *in situ* hybridization.

3. Spread of inflammation/arthritis to the right limb was associated with bilateral rises in PPT and CGRP mRNA expression in L5 DRG. SS mRNA expression in right DRG was unaffected by spread of inflammation. FCA-L + Cap-L reduced left joint swelling and prevented spread of arthritis to the right joint when assessed by joint swelling. This inhibition of spread of arthritis was associated with significant reductions in all left L5 DRG neuropeptide mRNAs compared with controls, and the rise in right L5 DRG PPT mRNA expression seen in FCA-L-alone animals was blocked. FCA-L + Cap-R also reduced left joint swelling and prevented the spread of inflammation to the right ankle. This lesion prevented the rise in PPT and CGRP mRNA expression seen in right DRG with FCA-L alone.

4. These findings suggest a role for capsaicin-sensitive primary afferents and the primary afferent neuropeptides encoded by PPT and CGRP mRNA in the maintenance and spread of arthritis.

The importance of the peripheral sensory nervous system in inflammation was first described by Lewis (1942) in the cutaneous 'triple response'. Such neurogenic inflammation is dependent upon an intact sensory innervation and can be prevented by surgical denervation or specific sensory neurotoxic lesions (Jancsó, 1992). In experimental inflammatory arthritis, sensory deafferentation by either axotomy (Courtright & Kuzell, 1965) or chemical lesioning attenuates joint damage and contralateral swelling and hyperalgesia (Levine, Dardick, Roizen, Helms & Basbaum, 1986), which provides evidence supporting the hypothesis of a neurogenic component in experimental arthritis. Furthermore, the bilateral nature of many clinical and experimental arthritides strongly implicates peripheral neural involvement in the pathogenesis and/or spread of arthritis (Kidd *et al.* 1989). While various classes of primary afferent nerves have been extensively investigated in the pathogenesis of inflammatory arthritis, there has been no systematic determination of the cellular and molecular mechanisms involved.

Neuropeptides such as substance P and calcitonin gene-related peptide (CGRP), which mediate the vasodilatation and plasma extravasation seen in neurogenic inflammation (Brain & Williams, 1985), have also been widely investigated in relation to arthritic disease. Vasoactive intestinal polypeptide (VIP) is not highly expressed in lumbar dorsal root ganglion (DRG) neurons under physiological conditions, but, because it is markedly induced following neuronal damage (Atkinson & Shehab, 1986), is thought to be involved in neuronal repair or reorganization. Neuropeptides are known to exert potent effects on both the vascular system and the immune system (Holzer, 1988). Substance P, CGRP and somatostatin (SS) are also implicated in the central modulation of nociception, since they are released in the dorsal horn by peripheral noxious stimulation (Morton, Hutchison, Hendry & Duggan, 1989; Schaible, Jarrot, Hope & Duggan, 1990). Levels of substance P and CGRP are known to be increased in primary afferent and dorsal horn neurons innervating arthritic joints (Colpaert, Donnerer & Lembeck, 1983; Kuraishi, Nanayama, Ohno, Minami & Satoh, 1989; Smith, Harmar, McQueen & Seckl, 1992; Hanesch, Pfrommer, Grubb & Schaible, 1993; Sluka & Westlund, 1993). There is conflicting evidence concerning levels of SS which have been reported either to increase (Ohno, Kuraishi, Nanayama, Minami, Kawamura & Satoh, 1990) or remain unchanged (Smith *et al.* 1992) in arthritic rats. These changes probably reflect alterations in neuropeptide synthesis since peripheral noxious stimuli increase expression of the gene encoding substance P in dorsal horn (Minami *et al.* 1989) and DRG (Noguchi, Morita, Kiyama, Ono & Tohyama, 1988).

Capsaicin, a sensory neurotoxin, causes preferential degeneration of unmyelinated primary afferents when applied directly to the nerve trunk, and permanently abolishes neurogenic inflammation in the innervated skin (Jancsó, 1992). These capsaicin-sensitive afferents have been widely studied in neurogenic inflammation and are known to contain many different neuropeptides. Perineural capsaicin causes an initial irreversible impairment of C fibre function followed by a reduction in the number of C fibres to one-third of control values after 2 weeks (Pini & Lynn, 1990). This represents a 70–80% reduction in polymodal nociceptor afferents, leaving other C and A fibres unaffected (Pini, Baranowski & Lynn, 1990). Capsaicin-sensitive nerve fibres have been shown to be of importance in both acute synovitis (Inman, Chiu, Rabinovich & Marshall, 1989) and arthritis (Levine *et al.* 1986).

We have developed a model of adjuvant arthritis in the rat, in which an intradermal injection of small doses of Freund's complete adjuvant (FCA) around one tibio-tarsal joint (ankle) produces a stable mild monoarthritis, without apparent systemic effects. In this model it has been shown that the inflammatory lesion is arthritic, rather than dermal, and that delayed inflammation of the contralateral tarsal joint can be achieved by a small increase in the dose of adjuvant used (Donaldson, Seckl & McQueen, 1993). We have previously shown in this model that increased expression of substance P and CGRP mRNAs occurs selectively in DRG neurons innervating the joint. This is a very early event in the inflammatory response, whereas SS mRNA expression only changes once arthritis is established after 14 days, and VIP gene expression remains undetectable (Donaldson, Harmar, McQueen & Seckl, 1992; L. F. Donaldson, unpublished results). We have now investigated whether the contralateral spread of inflammation is associated with a concurrent change in contralateral primary afferent neuropeptide gene expression. In addition, we also studied the effects of capsaicin (applied topically to the sciatic nerve) on the induction and spread of arthritis and the associated changes in neuropeptide mRNA expression in DRG neurons.

METHODS

All chemicals were obtained from Sigma unless otherwise specified. Reagents used for *in situ* hybridization were of molecular biology grade.

Experimental groups and induction of arthritis

Male rats (Han Wistar, 150 g) were assigned to the following groups. Control rats ($n = 5$) were totally untreated and were used in *in situ* hybridization studies to determine basal expression of neuropeptide mRNAs. Ten animals were injected with FCA around the left ankle joint under halothane anaesthesia (see below) and were used after 15 days to assess the joint swelling, histological features and mRNA expression in bilateral arthritis (termed FCA-L alone). Three additional groups of animals underwent surgery before induction of arthritis. Exposure of the sciatic nerve served as a surgical control (FCA-L + nerve exposure; $n = 5$) for topical capsaicin lesioning of the left (FCA-L + Cap-L; $n = 4$) or right (FCA-L + Cap-R; $n = 9$) sciatic nerves. These three groups were used to analyse the effects of surgery and capsaicin on the spread of arthritis. All three of these groups had arthritis induced 2 weeks after surgery, as described by Donaldson *et al.* (1993). Animals were briefly re-anaesthetized with halothane (4% in O_2), and arthritis was induced by an intradermal injection, at two sites around the left tibio-tarsal joint, of a total of 250 μg attenuated *Mycobacterium tuberculosis* (*M. tub*; Ministry of Agriculture, Fisheries and Food) suspended in 0·05 ml paraffin oil. FCA-L-alone rats showed near-identical changes in joint circumferences to FCA-L + nerve exposure. These groups were therefore pooled for analysis of *in situ* hybridization data and are referred to as 'FCA-L'.

Capsaicin lesioning

Capsaicin (Cap) was applied to either the left or right sciatic nerves as described below; this procedure will be referred to as Cap-L or Cap-R, respectively, throughout. Cap was applied perineurally to the exposed sciatic nerve, using the method described by Ainsworth and co-workers (Ainsworth *et al.* 1981). Male rats were anaesthetized with halothane (4% in O_2) and either the right or the left sciatic nerve was exposed via an incision in the mid-thigh. Cotton wool soaked in 1·5% Cap solution (15 mg ml^{-1} in 10% ethanol, 10% Tween 80 and 80% normal saline) was applied to the nerve trunk for 15 min while anaesthesia was maintained. Exposed muscle was covered in saline-soaked gauze. Cotton wool

and excess solution were then removed, the muscle and skin closed, and the animals allowed to recover until induction of arthritis.

Monitoring of inflammation and tissue preparation

Animals were weighed, tarsal joints scored for inflammation (index of inflammation: 0, normal; 1, mild redness; 2, moderate redness and swelling; 3, severe swelling/lesions over the joint), and change in tarsal joint circumferences were measured before induction of arthritis and twice weekly thereafter. Animals were killed by decapitation 15 days post-induction, and right and left L4/5 DRG rapidly removed, frozen on dry ice and stored at −80 °C until processed for either radioimmunoassay or *in situ* hybridization. Both hindlimbs were also removed by section of the tibia and fibula, post-fixed in 10% formal saline (10% formaldehyde in 0·9% saline) for 2 weeks and decalcified in Gooding and Stewart's solution (15% formic acid, 5% formaldehyde in distilled water). Sections (20 μm) were cut, counterstained with Haematoxylin and Eosin and the histological inflammation was scored blind (classified as: 0, normal; 1, subdermal inflammatory infiltrate; 2, mild synovitis; 3, moderate synovitis/arthritis; 4, severe arthritis; scores $\geqslant 3$ were taken as indicative of arthritis. Particular features scored as being indicative of arthritis were: synovial hyperplasia and oedema, pannus formation and erosion of subchondral bone and new bone formation).

Neuropeptide radioimmunoassays

The efficacy of the capsaicin lesion was determined with an additional group of Cap-lesioned animals that had not been injected with FCA. Cap lesioning of the left sciatic nerve was carried out as described above; animals were killed by decapitation 2 weeks later and DRG were dissected. DRG from control animals were also taken. Radioimmunoassay was carried out as previously described (Smith *et al.* 1992); briefly L4 and L5 left or right DRG were pooled and homogenized in 2 M acetic acid on ice, centrifuged at 14000 r.p.m. for 10 min and the supernatant assayed using specific antisera for substance P, calcitonin gene-related peptide and somatostatin. Cross-reactivity between specific antibodies and the other two peptides under investigation was always $< 0{\cdot}01\%$; inter-assay coefficient of variation (c.v.) was $< 20\%$ and intra-assay c.v. $< 10\%$ for all three radioimmunoassays. Neuropeptide levels are shown as picograms per ganglion as small amounts of peripheral nerve or dorsal root are often left attached to DRG on dissection, invalidating expression by weight.

In situ hybridization

In situ hybridization was performed on 10 μm sections of DRG using ^{35}S-labelled riboprobes complementary to preprotachykinin (PPT), CGRP, SS and VIP mRNAs, as previously described (Donaldson *et al.* 1992). In brief, ^{35}S-labelled cRNA probes were transcribed from cDNAs encoding β-PPT (441 base pairs), α-CGRP (450 base pairs), SS (450 base pairs) and VIP (350 base pairs). Sections were post-fixed in 4% paraformaldehyde and rinsed in 2×SSC (standard saline citrate: 0·3 M NaCl, 0·03 M trisodium citrate). cRNA probes were added to the hybridization mix (50% deionized formamide, 0·6 M NaCl, 10 mM Tris-Cl (pH 7·5), 0·02% Ficoll, 0·02% polyvinylpyrrolidone, 0·1% bovine serum albumin, 1 mM EDTA, 0·1 mg denatured salmon sperm DNA, 0·05 mg ml^{-1} yeast tRNA, 10% dextran sulphate and 10 mM dithiothreitol) to give 10×10^6 counts ml^{-1}. Hybridization mix (200 μl) was added to each slide and hybridization carried out overnight at 50 °C. After hybridization slides were rinsed in 2×SSC, treated with RNase A and washed to a maximun stringency of 0·1×SSC at 50 °C for 60 min. Sections were dehydrated and dipped in K5 nuclear emulsion (Ilford, UK), exposed for 2 weeks, developed and counterstained with Haematoxylin and Eosin. Control sections were treated with RNase A (100 μg ml^{-1}) prior to hybridization. Sections through the hypothalamic suprachiasmatic nucleus were used as positive controls for VIP expression. Expression of mRNA was compared using quantitative computer image analysis (Seescan, Cambridge, UK) to count silver grains overlying neuronal cell bodies of less than 30 μm diameter. This software uses a processed image and objectively determines silver grain number in situations where clumps of grains are formed due to high mRNA expression, by division of total area of clumps by the mean grain size. For each animal, four to ten cells in each of three separate non-serial sections of each DRG were counted and background subtracted. Mean neuropeptide mRNA expression in individual neurons was then calculated for each animal, and these data subsequently used to calculate the mean expression for each group of animals. (Some Cap-lesioned animals showed extremely low numbers of neurons expressing the mRNA under study, which is the reason for low numbers of cells being analysed in some cases. This was particularly apparent for SS mRNA when all expressing cells were analysed in lesioned animals.) All quantification was performed blind to the experimental group. L5 DRG from all animals injected with FCA-L were compared with L5 DRG from controls. FCA-L + Cap-L/R animals were also compared with FCA-L alone.

Statistics

Changes in joint circumference and *in situ* hybridization data were assessed by ANOVA followed by Duncan's *post hoc* test. Radioimmunoassay data from control and Cap-lesioned DRG were compared using Student's paired or unpaired t tests, as appropriate. Inflammation and histology scores were compared using Mann–Whitney U tests. Data are expressed as means ± S.E.M. unless otherwise stated. The null hypothesis was rejected at $P < 0{\cdot}05$.

RESULTS

No animal showed any abnormal motor effects following capsaicin application. FCA-L-alone animals showed a significant increase in left ankle joint circumference within 24 h of adjuvant injection, and this increase was maintained throughout the 15 days of the study (Fig. 1*A*). These animals also showed right ankle joint involvement after 14 days, as is evident from a significant increase in joint circumference (Fig. 1*B*) and histological features of inflammation and arthritis (Table 1).

Efficacy of perineural capsaicin lesions: effects on DRG neuropeptide content

The efficacy of the capsaicin lesions was determined in animals killed 2 weeks after Cap-L/R without FCA-L injection, to verify that the treatment did affect primary afferent neuropeptides in L4/5 DRG. Cap-L caused a significant reduction in ganglionic content of substance P, CGRP and SS on the lesioned side only. Substance P showed the greatest decrease (62% fall compared with controls), CGRP fell by 37% and SS by 35%. Contralateral neuropeptide content was not different from control values (Table 2).

Table 1. Inflammation and histological scores for left and right ankle joints in bilaterally arthritic (FCA-L) and capsaicin-lesioned animals (FCA-L + Cap-L; FCA-L + Cap-R) 15 days after adjuvant injection

	Inflammation score			Histological score		
	Left ankle	Right ankle	*n*	Left ankle	Right ankle	*n*
FCA-L	3 (0–4)	1 (0–3)	10	3 (2–4)	2 (2–4)	5
FCA-L + Cap-L	1·5 (1–3)	0	9	2·5 (1–4)	1·5 (1–2)	4
FCA-L + Cap-R	2 (1–3)	0 (0–1)	4	2·5 (1–4)	0·5 (0–2)*	4

Values are medians (range). *$P < 0·05$ *vs.* FCA-L.

Effect of perineural capsaicin on left and right joint inflammation

Arthritis + capsaicin application to the left sciatic nerve (FCA-L + Cap-L)

FCA-L + Cap-L significantly reduced swelling in both left (days 11 and 15) and right (days 14 and 15) joints when compared with FCA-L alone (Figs 1 and 2). The reduction in right joint swelling was accompanied by an attenuation of joint involvement as assessed by histological score, but the inflammation score was not significantly affected (Table 1). The reduction in left joint swelling was not reflected in a significant difference in the inflammation score or histology between FCA-L-alone and FCA-L + Cap-L animals.

Arthritis + capsaicin application to the right sciatic nerve (FCA-L + Cap-R)

FCA-L + Cap-R caused a significant reduction in right joint swelling compared with FCA-L alone only at day 14

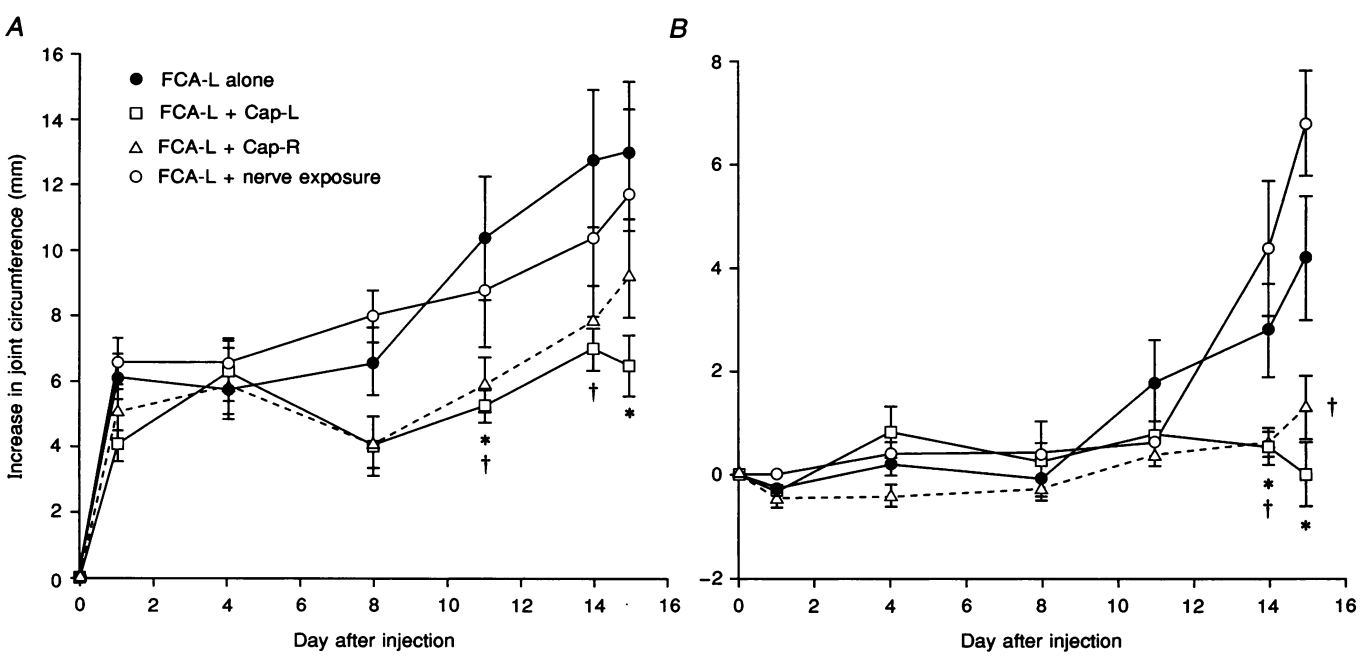

Figure 1. Increase in joint circumference in arthritic and capsaicin-lesioned rats

A, increase in left ankle joint circumferences of FCA-L-alone, FCA-L + nerve exposure, and FCA-L + Cap-L/R rats after adjuvant injection ($n = 10$, 5, 4 and 9, respectively). FCA-L + Cap-L and FCA-L + Cap-R both caused a significant reduction in the left joint swelling as denoted. *$P < 0·05$, FCA-L + Cap-L; †$P < 0·05$, FCA-L + Cap-R *vs.* FCA-L/FCA-L + nerve exposure. Values are means ± S.E.M. *B*, increase in right ankle joint circumferences in FCA-L-alone, FCA-L + nerve exposure and FCA-L + Cap-L/R rats after adjuvant injection (numbers as in *A*). FCA-L + Cap-L and FCA-L + Cap-R both inhibited the right joint swelling seen in FCA-L-alone or FCA-L + nerve exposure rats after 15 days. *$P < 0·05$, FCA-L + Cap-L; †$P < 0·05$, FCA-L + Cap-R *vs.* FCA-L/FCA-L + nerve exposure. Values are means ± S.E.M. Note the different *y*-axis from *A*.

Table 2. Substance P, CGRP and somatostatin peptide content of L4–L5 DRG from control animals and Cap-L animals (without arthritis)

	Substance P (pg ganglion^{-1})		CGRP (pg ganglion^{-1})		Somatostatin (pg ganglion^{-1})	
	Left DRG	Right DRG	Left DRG	Right DRG	Left DRG	Right DRG
Control	186 ± 26	192 ± 32	1178 ± 110	1171 ± 116	46 ± 2	46 ± 3
Cap-L	70 ± 24*	164 ± 9	748 ± 75*	1217 ± 39	30 ± 3*	40 ± 2

Values are means ± S.E.M.; $n = 5$ per group. Paired and unpaired t tests were used to compare lesioned DRG peptide content with the contralateral DRG, and with control DRG, respectively. *$P < 0.05$.

after adjuvant injection, which is when spread of arthritis to the right joint is normally apparent (Fig. 1*B*). This reduction in swelling was reflected in attenuated histological features of arthritis in the right joint (Table 1). FCA-L + Cap-R also altered left (adjuvant-injected) joint parameters, causing a significant reduction in left joint swelling at 11 and 14 days, but not at 15 days after adjuvant injection (Fig. 1*A*).

Effect of perineural capsaicin on neuropeptide mRNA expression

FCA-induced arthritis

In FCA-L rats, expression of PPT, CGRP and SS mRNAs was increased in left (FCA-injected) L5 DRG neurons 15 days after induction of arthritis, in agreement with our previous results in monoarthritis (Donaldson *et al.* 1992). At this time, when spread to the right joint became

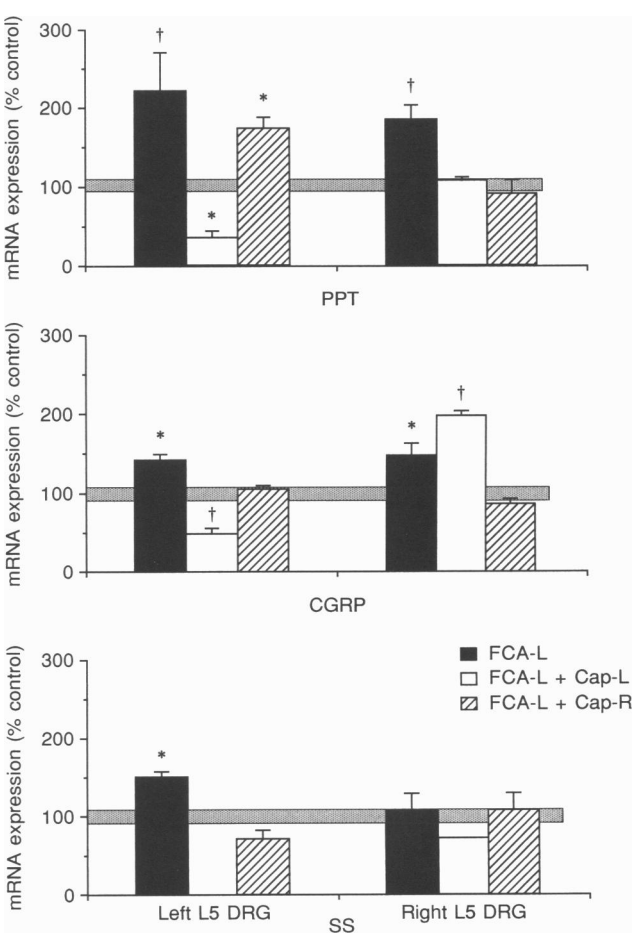

Figure 2. Neuropeptide mRNA expression in L5 DRG neurons from bilaterally arthritic and capsaicin-lesioned rats

Mean neuropeptide mRNA expression in individual neurons from L5 DRG in FCA-L, FCA-L + Cap-L and FCA-L + Cap-R rats ($n = 15$, 4 and 9, respectively), quantified by *in situ* hybridization 15 days after adjuvant injection. The horizontal stippled bars in each histogram represent the mean basal expression of each mRNA in control rats (± S.E.M.). All values are means ± S.E.M. and are expressed as a percentage of the control mean. * $P < 0.05$, † $P < 0.01$ compared with controls. SS mRNA was undetectable in left DRG following FCA-L + Cap-L, and the error bar in right DRG is too small to be seen.

apparent, PPT and CGRP, but not SS mRNA expression was also increased in right L5 DRG neurons when compared with control values (Fig. 2).

VIP mRNA was not detected in any section of DRG from either control, FCA-L-alone or FCA-L + Cap animals, but was always detected in positive control sections through the suprachiasmatic nucleus.

FCA-L + Cap-L

FCA-L + Cap-L caused a significant reduction in PPT, CGRP and SS mRNA levels in left L5 DRG neurons compared with control rats, and this was associated with a small attenuation in joint swelling but no alteration in histological score. FCA-L + Cap-L also prevented the rise in neuronal PPT mRNA expression in right L5 DRG, but did not affect the rise in CGRP mRNA seen in L5 DRG of FCA-L-alone animals. SS mRNA expression remained at control levels in right DRG neurons (Fig. 2).

FCA-L + Cap-R

FCA-L + Cap-R prevented the rise in PPT and CGRP mRNA expression seen in right L5 DRG neurons in FCA-L animals, and did not alter SS mRNA expression. FCA-L + Cap-R prevented the rise of CGRP and SS mRNA expression in left L5 DRG. However, the elevation of PPT mRNA expression in the left DRG (the side injected with adjuvant) was maintained (Fig. 2), as was inflammation and arthritis.

DISCUSSION

We have used an easily manipulated model for studying the bilateral spread of arthritis from an initial adjuvant monoarthritis of the rat tarsal joint, and have found increases in specific neuropeptide mRNA expression in DRG innervating the inflamed joint on development of overt arthritis, both ipsilateral to the initial inflammatory focus and contralaterally. Perineural capsaicin lesions of the sciatic nerve inhibited the spread of arthritis and attenuated the arthritis on the side which received the injection of FCA. These lesions also resulted in an attenuation of the increases in primary afferent neuropeptide mRNA expression seen in arthritic rats, suggesting a possible association between arthritis, its spread, and the induction of DRG neuropeptide mRNA expression.

Bilateral changes in joint swelling and neuropeptide gene expression

In FCA-L rats, bilateral arthritis was seen 15 days after injection of adjuvant and was associated with bilateral changes in mRNAs encoding β-PPT (the precursor for substance P and neurokinin A) and CGRP, but not SS. This agrees with previous studies that demonstrated increased substance P and CGRP in DRG and peripheral nerves in response to inflammation (Donnerer, Schuligoi & Stein, 1992) or arthritis (Colpaert *et al.* 1983; Kuraishi *et al.* 1989). Others have described bilateral increases in SS in established adjuvant polyarthritis (Ohno *et al.* 1990). We examined mRNA changes immediately after the appearance of contralateral inflammation, so it would seem that SS biosynthesis does not increase in the early phase of the inflammatory arthritic process. This conclusion is further supported by our recent data showing that ipsilateral SS mRNA levels do not change in the first 8 h after adjuvant injection (Donaldson, McQueen & Seckl, 1994). We cannot determine whether induction of PPT and CGRP mRNA expression initiates the contralateral spread of inflammation or is a consequence of it, as we have examined mRNA expression in animals with overt contralateral disease. However, expression of both neuropeptide genes shows increases concurrent with the onset of bilateral disease, suggesting a close or causal relationship between the spread of inflammation and the induction of synthesis of these peptides.

Perineural capsaicin lesion of the left sciatic nerve and arthritis (FCA-L + Cap-L)

We have shown that the Cap lesions employed here caused a significant decrease in DRG neuropeptide content after 2 weeks, only in the DRG ipsilateral to the Cap application. This agrees with the results of Pini and colleagues (Pini *et al.* 1990), who showed a reduction in the number of DRG neurons expressing neuropeptides. FCA-L + Cap-L resulted in reduced expression of all neuropeptide mRNAs estimated in the remaining neurons compared with control animals. This effect on neurons remaining after capsaicin lesioning may reflect a persistent sub-population, but as it was not observed when capsaicin was administered to the contralateral (right) sciatic nerve it may also indicate variation in the lesioning technique. We feel, however, that this is unlikely to reflect differences in the degree of the initial capsaicin-induced lesion between the sides, since all lesioning was performed by the same operator.

FCA-L + Cap-L did not completely reduce swelling of the ipsilateral joint but attenuated it by $\sim$50%, suggesting that neurogenic mechanisms contribute to, but are not essential for, the initial inflammation. This reduction in the initial inflammatory focus may be the underlying cause of the attenuation of contralateral arthritis following FCA-L + Cap-L, supporting the hypothesis that the dose dependency of the spread of arthritis is a threshold effect and only occurs when there is a sufficiently strong ipsilateral inflammatory stimulus. As the histological scores of the ipsilateral limb in FCA-L + Cap-L animals were similar to those of the FCA-L-alone animals, it is possible that capsaicin-sensitive neurons primarily mediate the oedema associated with this inflammation, and do not directly cause joint destruction. This would be a reasonable assumption, as substance P and CGRP can cause oedema formation through vasodilatation and increases in vascular permeability (Brain & Williams, 1985). Substance P has,

however, also been shown to be involved in joint destruction, and infusion of substance P into joints not normally affected in adjuvant disease will result in a greater degree of joint damage (Levine, Clark, Devor, Helms, Moskowitz & Basbaum, 1984). However, FCA is a profound immune stimulant and this potent local effect may only be slightly modulated by the influence of neuropeptides.

Perineural capsaicin treatment of the right sciatic nerve and arthritis (FCA-L + Cap-R)

The inhibition of the spread of arthritis to the right joint and the reduction in left ankle histological score in FCA-L + Cap-R animals further support the hypothesis that the distant spread of arthritis following adjuvant injection is mediated through capsaicin-sensitive primary afferents. Lesion of the right sciatic nerve completely prevented the spread of arthritis to the right joint, and the induction of CGRP and PPT mRNAs in the right L5 DRG. This lesion also significantly retarded the development of left tarsal joint arthritis on days 11 and 14, but when neuropeptide mRNA expression was examined (day 15), had not caused a significant attenuation of left joint swelling. The finding that FCA-L + Cap-R affected left joint swelling is not without precedent; Levine and colleagues (Levine, Dardick, Basbaum & Scipio, 1985) found a similar effect with perineural capsaicin when studying swelling following mild paw injury. This effect remains unexplained but, as discussed below, is probably atttributable to transneuronal signalling between damaged neurons and their contralateral homologues (Kolston, Lisney, Mullholland & Passant, 1991). FCA-L + Cap-L significantly reduced the levels of all three neuropeptide mRNAs expressed in left DRG (Fig. 2), whereas FCA-L + Cap-R did not reduce expression in right DRG below control values. This inconsistency may reflect the combined effect of the Cap-R capsaicin lesion plus a transneuronal signal from the left, inflamed side on the right DRG neurons, resulting in neuropeptide mRNAs being maintained at control levels rather than being reduced. A transneuronal mechanism possibly also mediates the disparate changes in neuropeptide mRNA expression seen contralateral to the capsaicin lesions, such as an attenuation of the increase in CGRP mRNA in left DRG from FCA-L + Cap-R animals in the face of ongoing local arthritis. In contrast, the increase in PPT mRNA ipsilateral to adjuvant injection was maintained following Cap-R, as was arthritis, consistent with the hypothesis that this gene and its products are of importance in neurogenic inflammation.

Possible mechanisms mediating spread of arthritis and block by capsaicin lesioning

Our data suggest that the contralateral spread of joint disease from the initial inflammatory focus is mediated, at least in part, through capsaicin-sensitive components of the nervous system. Interestingly, many reports of effects contralateral to either a nerve injury or unilateral inflammation are now appearing in the literature. However, the mechanism (s) underlying these contralateral effects and particularly the spread of arthritis and induction of neuropeptide mRNA expression in this model are unclear.

The effect of nerve injury on contralateral motoneurons in rodents was initially reported by Rotschenker & Tal (1985), who suggested that motoneurons contralateral to an axotomized motor nerve sprouted in response to a transneuronal signal from the injured neurons mediated through the spinal cord. More recently, in models of unilateral nerve injury and neuropathic pain, bilateral changes have been reported in nociceptive thresholds (Kim & Chung, 1992), protein kinase C levels and spinal cord metabolic activity, as assessed by [^{14}C]2-deoxyglucose uptake (Mao, Mayer, Hayes & Price, 1993), and opioid receptor binding (Stevens, Kajander, Bennett & Seybold, 1991). Primary afferents also show bilateral changes in both ability to cause neurogenic inflammation (Allnatt, Dickson & Lisney, 1990) and neurochemistry (Verge, Wiesenfeld-Hallin & Hökfelt, 1993) following unilateral axotomy. These effects on primary afferents appear to be restricted to the homologous contralateral nerve (Kolston, *et al.* 1991) and therefore may also be mediated via a similar mechanism to that proposed by Rotschenker & Tal (1985).

In inflammatory conditions, similar effects have also been noted. Mechanical nociceptive thresholds are altered bilaterally after unilateral inflammation induced by carrageenan (Kayser & Guilbaud, 1987) and FCA (Millan & Colpaert, 1991), although this is variable (Traub, Solodkin & Gebhart, 1994). Contralateral intra-articular release of substance P and CGRP has been shown in a monoarthritis of the rat knee (Bileviciute, Lundeberg, Ekblom & Theodorsson, 1993) and this is thought to be due to a neural mechanism rather than a systemic release (Bileviciute, Lundeberg, Ekblom & Theodorsson, 1994). At a molecular level, ipsilateral inflammation may also induce contralateral mRNA and protein changes. Carrageenan caused bilateral changes of NADPH-diaphorase-like staining in both DRG and spinal cord of rats (Traub *et al.* 1994). Other workers have shown bilateral changes in dorsal horn neuropeptide immunoreactivity during the early development of monoarthritis (Mapp *et al.* 1993), suggesting that a contralateral sensitization of these neurons occurs within days of the establishment of unilateral inflammation. Unilateral noxious or electrical stimulation also induces contralateral expression of immediate early gene/transcription factors in dorsal horn neurons, including the AP1 components c-*fos* and c-*jun* (Herdegen, Tolle, Bravo, Zieglgansberger & Zimmermann, 1991) and the nerve growth factor (NGF)-induced factor NGFI-A (krox24/zif268; Herdegen, Walker, Leah, Bravo & Zimmermann, 1990). Physiologically, deep dorsal horn neurons, both wide dynamic range and nociceptive specific, have bilateral

receptive fields which become more responsive to both ipsilateral and contralateral stimuli on induction of monoarthritis in the knee. The receptive fields of these neurons also expand both ipsilateral and contralateral to the inflammatory focus (Grubb, Stiller & Schaible, 1993). Interestingly, a possible spinal mechanism could be inferred by the work of Millan & Colpaert (1991), who showed that chronic naloxone administration, in a dose that discriminates between μ- and κ-receptors, will also block the spread of inflammation to the opposite limb in a similar model to that used here. They postulated that this could be due to an action on either the immune system, the sympathetic nervous sytem or the central nervous system. κ-Opioid receptors and dynorphin expression in spinal neurons may be involved in the central mechanism of distant spread of arthritis in this model, as endogenous dynorphin is thought to contribute to enhanced neuronal excitability in superficial dorsal horn neurons in inflammatory conditions (Hylden, Nahin, Traub & Dubner, 1991), though κ-receptor antagonists increase ongoing activity in the majority of spinal neurons receiving input from a unilaterally inflamed ankle joint (Stiller, Grubb & Schaible, 1993). Following perineural capsaicin application, transganglionic degeneration of primary afferents is apparent within 14 days (Jancsó, 1992), and this treatment alters afferent inputs to dorsal horn neurons both ipsilateral and contralateral to the injury (Fitzgerald, 1982). Thus there may be plastic changes within the dorsal horn resulting in altered primary afferent reactions to inflammation, and this plasticity may conceivably also contribute to the block of spread of arthritis. Our data suggest that the contralateral spread of joint disease from the initial inflammatory focus is mediated, at least in part, through capsaicin-sensitive components of the peripheral nervous system. The spinal mechanism(s) underlying the contralateral spread of arthritis and induction of neuropeptide mRNA expression may involve the spinal opioidergic systems, but clearly require further study.

While the data presented here are strongly suggestive of a fundamental role for the peripheral sensory nervous system and the neuropeptides expressed in this condition, it is not intended to imply that there is not a large immune component in this disease. Rather, this would suggest an extremely close relationship between the immune and sensory nervous systems in inflammatory processes. The arthritogenicity of *Mycobacterium* has been attributed to activation of T-cell subtypes that cross-react with articular tissues, thus causing joint destruction and amplification of a local immune response (Wooley, 1991). It would therefore appear that an intimate interaction between the immune and nervous systems is probably of great importance in the spread of disease. We propose that activation of the sensory nervous system and release of neuropeptides play an important role in the establishment, maintenance and spread of inflammation and arthritis.

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