Increase in substance P and CGRP, but not somatostatin content of innervating dorsal root ganglia in adjuvant monoarthritis in the rat

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Neuropeptides, synthesized in dorsal root ganglia (DRG), are implicated in noicception and neurogenic inflammation. Alterations in DRG neuropeptide levels have been described in polyarthritic rats, but these models are associated with widespread systemic disease. Using mild adjuvant-mediated monoarthritis of the left carpal joint we found significant increases in substance P (+69%) and calcitonin gene-related peptide (CGRP; +204%), but not somatostatin in ipsilateral C6/7 DRG. Peptide levels in contralateral DRG and other ipsilateral DRG were unaltered. Substance P and CGRP in DRG may be of importance in the pathogenesis and maintenance of adjuvant arthritis.

Joints and synovia are innervated by primary afferent (sensory) fibres projecting from cell bodies in the dorsal root ganglia (DRG); these neurons also project to the dorsal horn of the spinal cord. The fibres contain neuropeptide neurotransmitters which are involved in pain sensation and mediate neurogenic inflammation [14], implicated in the pathogenesis of experimental and clinical inflammatory joint disease [2, 3, 5]. Section of these nerves [13] or treatment with capsaicin which destroys primary afferents [1] attenuates arthritis and hyperalgesia and reduces joint swelling in experimental arthritis. In man, neuropeptidergic afferents innervate the normal and inflamed synovium [17], neuropeptide levels are elevated in synovial fluid from affected joints [9] and denervated joints are resistant to inflammatory arthritis. However, the precise role of each neuropeptide and their site(s) of involvement in pain transmission and neurogenic inflammation have yet to be fully determined.

Of the neuropeptides thought responsible for neurogenic inflammation in arthritis, most attention has focused on substance P (SP) which initiates vasodilation and oedema formation [11]. Substance P is synthesized in a subpopulation of DRG neurons and substance P-immunoreactive fibres project to the synovium and dorsal horn. In experimental arthritis, substance P levels are increased in affected joints, in fibres innervating those joints [10] and in the spinal cord and infusion of substance P into joints increases inflammation and joint destruction [12]. Substance P is released in spinal dorsal horn during nociception [6] and in polyarthritis [22], where the peptide excites sensory neurons [24] and elicits aversive behaviour reminiscent of pain [26].

Calcitonin gene-related peptide (CGRP) is also found in DRG neurons, co-localised with either substance P [30] or somatostatin. CGRP-immunoreactive terminals densely innervate the dorsal horn. Intrathecal CGRP lowers the nociceptive threshold to mechanical stimulation [21] and substance P [31] and enhances substance P release in the spinal cord [21]. Painful stimuli [18] or arthritis [19] lead to release of endogenous CGRP and substance P into the dorsal horn where CGRP binding sites are found [27]. Intrathecal immunoneutralization of CGRP attenuates hyperalgesia in polyarthritis [8], systemic immunoneutralization reduces arthritic inflammation [15]. CGRP is a potent vasodilator and may contribute to peripheral changes in neurogenic inflammation, both directly and by potentiating substance P-mediated increases in capillary permeability.

We have recently demonstrated distinct neuropeptide distributions in cervical DRG; CGRP and substance P vary in parallel with DRG size, but somatostatin shows an independent pattern [28]. We have also developed a rat model of adjuvant-mediated unilateral arthritis (mo-
noarthrosis) [4], which shows no evidence of contralateral or generalised joint disease. This model employs much lower doses of adjuvant and avoids many of the confounding factors [23] of polyarthritic models (severe and extensive disease, systemic illness, profound weight loss) with the advantage that the contralateral (unaffected) side serves as an internal control. We have now investigated the effects of monoarthrosis on DRG neuropeptide content.

Male rats (Ham Wistar, 250–300 g, n=5), under halothane anaesthesia, were injected subdermally with 0.05 ml of Freund’s complete adjuvant (FCA; 1 mg/ml M. tuberculosis; Sigma) at two separate locations around the left carpal joint. Controls (n=5) received no treatment; a preliminary experiment demonstrated that treatment of controls with Freund’s incomplete adjuvant led to prolonged (>3 wk) unilateral joint swelling, albeit less severe than produced by FCA. Body weight and joint circumference were recorded throughout. Fifteen days after induction of arthritis the animals were killed by decapitation, trunk blood collected, plasma separated and corticosterone levels determined by radioimmunoassay. DRG were collected from ipsilateral and contralateral sides from C2 to T6, pooled in pairs (C2, C3, etc.), snap frozen and stored at –20°C prior to determination of neuropeptide content by radioimmunoassay [28]. Comparisons were made by ANOVA followed by Duncan’s multiple range test or paired t-test. Significance was set at P<0.05. Values are means ± S.E.M.

Following FCA injection the ipsilateral (injected) carpal joint was significantly swollen after one day (2.61±0.46 cm) compared with untreated animals (1.86±0.19 cm; P<0.01). The swelling persisted throughout the experiment. By contrast, the circumference of the contralateral (uninjected) forelimb joint did not vary in either group. Animals tended to protect the affected paw but were otherwise mobile. FCA led to a slight, but significant fall in body weight after 15 days (278±7 g) compared with control (295±6 g). Plasma corticosterone was greater in arthritic rats (828±153 nmol/l) than controls (398±89 nmol/l; P<0.05).

In untreated controls, SP content of DRG increased from a nadir at C2-3 (32.9±4.5 pg/ganglion) to a maximum at C6-7 (55.9±4.7) and then decreased to T5-6 (26.0±2.5). FCA treatment was associated with significantly increased SP content of the ipsilateral arthritic C6-7 DRG (79.7±3.5; P<0.01) compared with the contralateral side (47.2±7.2) and untreated controls. There were no differences between ipsilateral, contralateral and control DRG at any other level (Fig. 1). The CGRP content in DRG of untreated animals was greater at C4-5 (2358±302 pg/ganglion), C6-7 (2748±336) and T1-2 (3561±489) than at T5-6 (1410±168). FCA injection caused a marked increase in CGRP levels in the ipsilateral C5-7 DRG (7220±1064; P<0.01) compared with the contralateral C5-7 ganglia (2374±255) and untreated C6-7 DRG (2831±324). No other differences were observed (Fig. 1). In controls, somatostatin content of DRG was greatest at C2-3 (68.9±16.2 pg/ganglion) and least at C6-7 (43.6±3.6) and T5-6 (52.2±5.0). The somatostatin content of DRG was unaffected by arthritis (Fig. 1).

These data demonstrate a unilateral increase in SP and CGRP, but not somatostatin content of DRG in experimental monoarthrosis. Previous studies of sensory neuropeptides in experimental arthritis have mainly employed polyarthritic models. In general the data derived have shown increases in SP [10], CGRP [7] and perhaps somatostatin [20], changes which are not due to increased
numbers of cells expressing SP and CGRP [29]. However, these models produce not only severe polyarthritis but also widespread systemic changes including profound weight loss, mucosal ulceration, debilitation, greatly reduced locomotor activity and high mortality [1]. Thus it is difficult to ascribe a causal relationship between the changes seen in neuropeptide expression per se and the arthritic process. Interestingly, our original intention was to treat control animals with incomplete adjuvant (vehicle without bacterial peptidoglycans). However, this also caused chronic inflammation (representing the effects of vehicle; L. Donaldson et al., unpublished observations) and untreated controls were therefore employed.

More recently a range of models of monoarthritis have been examined as these may reveal site-specific processes and monoarthritis occurs in clinical inflammatory joint disease. Although unilateral injection of inert spheres leads to bilateral joint swelling [5], unilateral changes are found following adjuvant injection into one paw and this is associated with localised ipsilateral increases in enkephalin and dynorphin immunoreactivities in spinal dorsal horn [29]. Using a similar model we find evidence of joint swelling and inflammation, persisting for at least 3 weeks, only on the arthritic side. This model is not associated with anorexia or profound weight loss and animals were mobile at all stages. However, monoarthritis presumably still represents a chronic, though mild stress since plasma corticosterone was elevated, albeit submaximally. Nevertheless the data show a clear and marked elevation of SP and CGRP in C67 DRG presumed to innervate the affected joint. This suggests a close relationship between these neuropeptides and the inflammatory process itself. Whether increased DRG neuropeptide content represents increased biosynthesis, reduced degradation and/or altered axonal transport is unknown although previous studies with colchicine suggest altered axonal transport is unlikely to be responsible [7].

The importance of elevated plasma corticosterone levels in adjuvant arthritis is unclear. Glucocorticoids are effective therapy for inflammatory arthritis in man and have analgesic effects. Although corticosteroids have little or no effect on substance P, CGRP or somatostatin levels in various brain regions [25], glucocorticoids do regulate axonal transport of substance P [16]. In addition, we have recently demonstrated that adrenalectomy leads to increased substance P and CGRP, but decreased somatostatin content of cervical DRG [28], effects reversed by dexamethasone. Since DRG neurons express both corticosteroid receptor mRNAs (Seckl et al., unpublished observations), corticosteroids might influence the changes in neuropeptides seen in DRG in arthritis. Our preliminary observations suggest that adrenalectomy prolongs the duration of swelling and potentiates both joint inflammation and the elevation of CGRP and substance P in innervating DRG in adjuvant monoarthritis (Smith, McQueen, Seckl and Harmar; in preparation). Since much evidence suggests that increased release of SP and CGRP — both centrally and peripherally — is proinflammatory, the 'stress-like' levels of corticosterone might tend to ameliorate the changes in neuropeptide expression in DRG in adjuvant arthritis.

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