Joint inflammation is reduced by dorsal rhizotomy and not by sympathectomy or spinal cord transection

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Abstract

Objectives—To investigate the role of primary afferents, sympathetic postganglionic effereents and descending systems on the central control of peripheral inflammation.

Methods—Acute inflammation was induced by intra-articular injection of kaolin and carrageenan into the knee joint cavity of the rat. Before the induction of the arthritis, a unilateral dorsal rhizotomy, a chemical (phenolamine) and/or surgical sympathectomy, or a spinal transection was performed. Joint inflammation (joint circumference and thermographic readings) and behavioural signs were assessed.

Results—Only arthritic animals with a dorsal rhizotomy showed a significant reduction of the inflammatory response compared with control arthritic animals. No significant differences in the inflammatory response occurred following sympathectomy or spinal transection. The animals who received sympathectomy showed similar behavioural manifestations to the arthritic animals.

Conclusions—The central terminals of primary afferents are important in the development of acute joint inflammation since dorsal rhizotomy attenuated the inflammatory response in the knee joint. The sympathetic nervous system is not involved in the acute inflammatory phase of this arthritis model. The central processes controlling acute inflammation involve a local spinal circuit since spinal cord transection at T9 has no effect on the inflammation.

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The existence of an experimentally inducible arthritis in animals, with behavioural manifestations that mimic those in humans, provides an excellent model for investigating the involvement of the nervous system in inflammation. There are five cardinal signs of inflammation: heat, redness, swelling, pain and loss of function. The present study addresses these parameters in an animal model of arthritis. Injection of kaolin and carrageenan into the knee joint cavity of experimental rats induces acute arthritis that develops within one to three hours. This arthritis model produces localised inflammation, behavioural signs of heat hyperalgesia and guarding of the limb. A recent study by Sluka and Westlund demonstrated a central spinal cord involvement in the control of peripheral joint inflammation. Specifically, spinaly administered non-NMDA (non-N-methyl-D-aspartate) glutamate¹ or GABA₅ (gamma-aminobutyric acid)² receptor antagonists significantly decrease the extent of peripheral joint inflammation compared with untreated animals with arthritis. These findings stimulus the investigation of the influence of primary afferents, sympathetic efferents and descending systems on the dorsal horn events controlling peripheral inflammation.

Primary afferents have been shown to be involved in neurogenic inflammation. The classic axon reflex experiments of Lewis³ have demonstrated that the peripheral terminals of primary nociceptors are the source of inflammatory mediators that are released by activation of primary afferent neurons. It is evident that the primary afferent neurons serve a dual function; they transmit neural responses centrally and release neuromodulators of inflammation peripherally into the inflaming tissue. Moreover, it has been demonstrated that denervation of the joint, intra-articular injection of capsaicin or substance P antagonists inhibit both acute and chronic inflammation in the rat.⁴⁻⁷

Evidence suggests a role for the postganglionic sympathetic effereents in enhancing joint injury in adjuvant induced arthritis.⁸⁻⁹ Activity in these effereents is considerably increased by spinal cord reflexes elicited by activation of nociceptive afferents. Moreover, the interaction between primary afferents and sympathetic efferents increases local tissue production of prostaglandins which are potent mediators of inflammation.¹⁰ The sympathetic nervous system can therefore contribute to physiological changes associated with inflammation.

On the other hand, it is well established that spinal nociceptive pathways in vertebrates are generally influenced by descending pathways originating in the cortex or brainstem and projecting to the spinal cord. Previous studies have shown that most spinal cord cells responding to stimulation of joint afferents are tonically active descending inhibitory control. These spinal neurons driven by joint afferents become more responsive under conditions of experimental acute arthritis and increase their
spontaneous firing during application of spinal cord block.11 12

In view of the evidence documenting the involvement of primary afferent fibres, post-ganglionic sympathetic efferents and supraspinal descending control in the development of inflammation and pain associated with arthritis, the present study examines the effects of these neural mechanisms on acute arthritis produced by injection of kaolin and carrageenan in the rat knee joint. This study will test inflammatory changes following dorsal rhizotomy, sympathectomy and spinal cord transection. The specific hypothesis to be tested is that the central terminals of primary afferents contribute to acute joint inflammation, that is, heat, redness, swelling, pain and loss of function.

Methods
Experiments were performed on 32 male Sprague-Dawley rats weighing between 250–350 g. The animals were kept at 12/12 hour dark/light cycle and had free access to food and water before the experiments. Animals undergoing surgical manipulations (dorsal rhizotomy, sympathectomy, spinal cord transection) were anaesthetised with sodiumpentobarbital (50 mg/kg, ip).

DORSAL RHIZOTOMY
Six rats received a unilateral dorsal rhizotomy (from L2 to S1 inclusive). Dorsal laminectomy of the appropriate vertebrae was performed. Lateral removal of bone was necessary to provide access to the dorsal roots without damage to the cord. The dorsal roots were identified and cut just inside the intervertebral foramen proximal to the dorsal root ganglia with fine scissors under a Zeiss operating microscope. Neurological examination confirmed that the limb that had been rhizotomised was completely deafferented but was not paralysed. A recovery period of two days was allowed before induction of arthritis. After the end of each experiment, rats were dissected to confirm: (1) complete dorsal rhizotomy; (2) level of cut dorsal roots, that is, L2 to S1; and (3) intact dorsal root ganglia. Only animals that met all three criteria were included in this study. It is well established that sectioning proximal to the dorsal root ganglia (dorsal rhizotomy) does not result in cell death in the ganglia or degeneration distal to the ganglia, that is, peripheral nerve.14–16 In one animal two days following dorsal rhizotomy, the cut end of a dorsal root was placed on a platinum hook electrode. Action potentials were recorded after both noxious and innocuous cutaneous stimulation of the hindlimb. This indicates that the peripheral nerves are intact and both large and small primary afferent fibres respond functionally to natural stimuli.

SYMPATHETECTOMY
Surgical (n = 10) and/or chemical (n = 8) sympathectomy was performed for a total of 12 rats. Unilateral abdominal sympathectomy was aseptically performed in 10 anaesthetised rats. With an operating microscope, the left sympathetic chain and ganglia were readily identified on the wall of the aorta. The sympathetic chain and ganglia were excised from the level of the renal vein to the origin of the external iliac artery (L2–S2). The abdominal incision was sutured and the rats permitted to recover for an interval of at least six days before the induction of arthritis. In six surgically sympathectomised animals, the α-adrenergic blocker, phentolamine (1 mg/kg, n = 2; 4 mg/kg, n = 4, i.p.), was given one hour before the induction of arthritis. In two animals, phentolamine at 1 mg/kg was given one hour before the induction of arthritis. The effects of phentolamine have been shown to last at least one day following administration.13 Confirmation of surgical sympathectomy (n = 4) was carried out by immunohistochemical staining of the peripheral blood vessels for tyrosine hydroxylase (TH; Chemicon 1:1000) or perfusion with glyoxylic acid (2%). An absence of TH terminals was noted on the inflamed side.

SPINAL CORD TRANSECTION
Ten rats were subjected to a sterile laminectomy between T8 and T10. After removing the dura at the site, the exposed cord was completely transected at T9. Complete spinal cord transection was verified by slightly retracting the proximal and distal stumps with tissue forceps. Before and after transection, the cord was bathed in ice cold physiological saline for 10 minutes to reduce tissue metabolic activity, facilitate cord cutting and promote haemostasis. After haemostasis, a strip of gelfoam was placed on the dorsal surface of the cord at the site of transection. The incision was then closed with wound clips. Postoperative care was critical to successful survival and consisted of the following protocol: (1) temperature control cages during the three days of recovery; (2) change of cage bedding (diapers) twice daily; (3) monitoring and correcting fluid intake and output; and (4) monitoring physical appearance daily. Ringer’s lactated solution (1 ml/100 gr, ip) was administered immediately after surgery. In addition, penicillin G (5000 IU, im) and gentamicin sulphate (0.1 mg/100 gr, im) were given daily for three days. The rats urinary bladder was expressed twice daily under running warm water. Drinking water was limited to 10 ml during the night to prevent overdrinking and bladder rupture.

INDUCTION OF ARTHRITIS
A group of untreated arthritic animals (n = 10) was used as a control. Arthritis was induced in all animals by intra-articular injection of 3% kaolin and 3% carrageenan (Sigma, 0.1 cc). The knee joints in the control, sympathectomised dorsal rhizotomised animals were injected while the rat was anaesthetised with the short acting barbiturate, sodium methohexital (50 mg/kg, ip).
Dorsal rhizotomy reduces inflammation

JOINT CIRCUMFERENCE AND THERMOGRAPHIC ASSESSMENTS
The degree of joint inflammation was assessed by knee joint circumference measurements and thermographic readings. Knee joint circumference was measured with a flexible plastic coated tape measure around the centre of the knee joint. Joint circumferences were assessed before the induction of arthritis, at four hours and at eight hours post induction. Thermographic readings were taken eight hours post induction of arthritis using a liquid crystal tablet (Flexitherm; 29-5–33-4°C) applied to the ventral surface of the anaesthetised animals. The position assessed surface heat of the anteromedial aspect of both knees while the hindlimb was outwardly rotated.

BEHAVIOURAL ASSESSMENTS
Behavioural changes were used as another measure to assess the role of the sympathetic nervous system in peripheral inflammation. Behavioural changes in animals with and without sympathectomy were determined by paw withdrawal latencies (PWL) to radiant heat before induction of arthritis and at four hours and eight hours post induction.20 Before testing, rats were placed in clear plastic cages and allowed to acclimatise for 20 minutes. Radiant heat was provided by a metal box focusing a high intensity light beam through an aperture (1-0 cm x 0-8 cm) attached to an on/off switch. The light beam was shown through the glass onto the plantar surface of the hindpaws. Both paws were tested independently, five trials at least 10 minutes apart were conducted on each hindpaw. The latencies of the five trials were averaged to give a mean withdrawal latency for each hindpaw. To further quantify behavioural changes, the animals were graded by a subjective pain rating scale (0–5) modified from Guilbaud and colleagues17 where: 0 is normal; 1 is curling of toes; 2 is eversion of foot; 3 is partial weight bearing; 4 is non-weight bearing and guarding; and 5 is avoidance of any contact with the limb.

STATISTICAL ANALYSIS
Differences from baseline in joint circumference, thermographic readings, and pain-related behaviour ratings were compared with a one-way analysis of variance (ANOVA). Post-hoc testing with a Scheffé test (p < 0.05) compared differences between groups (untreated, sympathectomised, dorsal rhizotomised and transected). Paired t tests compared PWL scores from baseline to four hours and eight hours following induction of arthritis.

Results
KNEE JOINT CIRCUMFERENCE MEASUREMENTS
Assessment of joint inflammation (circumference and thermography) revealed that the severity of the arthritis was reduced only by previous dorsal rhizotomy and not by sympathectomy or spinal cord transection. There was a mean (SEM) increase from baseline of 1-6 (0.24) cm at four hours and of 1-64 (0.10) cm at eight hours for control arthritic animals. The mean (SEM) increase in circumference at both four hours [1-4 (0.09) cm, sympathectomy; 1-3 (0.12) cm, spinal cord transection] and eight hours [1-6 (0.07) cm, sympathectomy; 1-9 (0.16) cm, spinal cord transection] in the sympathectomised and spinal cord transected arthritic animals was similar to control arthritic animals. In contrast, joint circumferences, mean (SEM), of dorsal rhizotomised arthritic animals only increased by 0.82 (0.04) cm at four hours and 0.97 (0.05) cm at eight hours following induction of arthritis.

An overall effect for time was observed for the increase in joint circumference (F3,32 = 14.16; p = 0.001) and temperature (F3,30 = 12.04; p = 0.001) eight hours following induction of arthritis. The joint circumferences in animals that had had a previous dorsal rhizotomy were significantly less than those of control, sympathectomised, and spinal cord transected arthritic animals (p < 0.05) (fig 1). In animals that had had a previous sympathectomy or spinal transection the increase in joint circumference was not significantly different from control arthritic animals. In one spinal transected animal, the joint circumference measured at 12 hours and 24 hours after induction of arthritis remained unchanged from the eight hour time period.

THERMOGRAPHIC READINGS
Thermographic readings, mean (SEM), taken eight hours after induction of arthritis revealed that a major temperature increase of 3.25 (0.23)°C occurring in the inflamed knee joint is typical for arthritic rats. This increase was significantly reduced (p < 0.05) in

Knee joint circumference of inflamed hindpaws after 8 hours of arthritis

Figure 1 The joint circumferences are represented as the average circumference of the inflamed knee joint measured before and at eight hours post induction of arthritis for all groups of animals: control arthritic (ARTh), dorsal rhizotomy (DRHIZ), sympathectomy (SYMP), and transection (TRANS). A significant decrease occurred for the arthritic animals with dorsal rhizotomy compared with all other groups. *p < 0.05.
animals that underwent dorsal rhizotomy [1.32 (0.31)°C] (fig 2). The temperature increases in the knee joint of transected [3.52 (0.19)°C] and sympathectomised [3.17 (0.25)°C] rats were similar to those in control arthritic rats.

**BEHAVIOURAL ASSESSMENTS**

Sympathectomy had no effect on the arthritis-induced decrease in the paw withdrawal latency (PWL) that occurs following four hours (p = 0.001) and eight hours (p = 0.001) of inflammation. The PWL was significantly reduced at both four hours (p = 0.001) and eight hours (p = 0.001) in both sympathectomised and control arthritic animals (fig 3) and no significant differences were observed between the two groups. Additionally, the pain-related behaviour ratings were similar in both the sympathectomised and the control arthritic animals. Mean (SEM) ratings from control arthritic animals were 4.3 (0.35) at four hours and 4.6 (0.15) at eight hours. Similarly, ratings from sympathectomised arthritic animals were 3.5 (0.33) at four hours and 4.3 (0.47) at eight hours. No significant differences between the two groups were observed.

**Discussion**

The results of the present study have clearly shown that dorsal rhizotomy performed before the induction of arthritis significantly reduces the degree of joint inflammation in this acute arthritis model. On the other hand, these data indicate that the postganglionic sympathetic efferents and the supraspinal descending pathways do not contribute significantly to the control of peripheral inflammation associated with this experimental model of arthritis. Although the dorsal root ganglia and presumably the peripheral terminals are intact, the inflammation is thus still greatly reduced following dorsal rhizotomy. The neurogenic component of acute peripheral inflammation therefore requires intact dorsal roots and does not result from either increased activity in the ganglia, interaction of the peripheral terminals with the sympathetic efferents, or influence of descending systems. These data suggest that the central control of peripheral inflammation occurs through a local neural circuit at the spinal cord level.

**DORSAL RHIZOTOMY**

The attenuation of acute arthritis observed in our experiments after dorsal rhizotomy agrees with another study showing that peripheral denervation or pretreatment with capsaicin significantly reduces the severity of inflammation. In a study by Ferrell and Russell,16 it was found that antidromic electrical stimulation of the cat knee joint C-fibre produces a significant plasma extravasation into the synovial cavity of the knee suggesting that C-fibre afferents are the principle mediators of neurogenic inflammation. In our study, sectioning of the central processes of the primary afferents prevented the development of inflammation. This indicates that it is the connectivity of the primary afferents with the dorsal horn circuitry that is important for induction of the acute joint inflammation.

In contrast to our study, Levine et al.10 observed that dorsal rhizotomy considerably increases the severity of adjuvant-induced arthritis on the deafferented side. This may represent differences between acute and chronic models. It is known that an increase in spontaneous discharges occurs in a cut peripheral nerve beginning at three days and reaching a maximum by two weeks.13 This spontaneous neuronal activity generated in a damaged nerve and/or neuroma could result in an increased release of neurogenic inflammatory mediators in the knee joint13 and the development of neuropathic pain.13 17 20 To avoid the possibility of neuroma formation and spontaneous neuronal activity in the present experiment, arthritis was induced two days following dorsal rhizotomy rather than one week as in the studies by Levine et al.10
Dorsal rhizotomy reduces inflammation

These data support the hypothesis that central control of peripheral inflammation occurs through the activation of primary afferent terminals at the spinal cord level. Moreover, recent studies from this laboratory provided additional evidence to support this hypothesis. Administration of either NMDA or GABA_A receptor antagonists into the spinal cord significantly decreases joint inflammation,2 similar to the decreases observed following dorsal rhizotomy in the present study.

SYMPATHECTOMY

Conversely, no significant difference in joint inflammation or behaviour signs was observed between the control and sympathectomised arthritic animals. These results demonstrated that combined surgical and chemical sympathectomy had no effect on the acute inflammatory response. It is therefore clear that the pain and hyperalgesia to heat stimuli and the degree of inflammation that occurs following induction of acute arthritis does not require an intact sympathetic nervous system.

Studies by Lam and Ferrell21 support the view that acute inflammation of a joint does not involve the sympathetic nervous system. These researchers demonstrated that acute arthritis was unaffected by pretreatment with reserpine. The extent of peripheral inflammation caused by antidromic stimulation of the posterior articular nerve was not blocked by adrenergic blockers.18 These experiments as well as the present data contrast with the findings of several other studies that observed a decreased severity in the inflammatory response22 and behavioural manifestations23 following chemical sympathectomy. In addition to depleting norepinephrine, guanethidine also results in a depletion of serotonin from both central and peripheral sites. The effects of guanethidine in inflammation23 are not as easily interpreted. The studies by Aloe et al.,22 also use the injection of carageenan to induce acute arthritis. Newborn rats received chemical sympathectomy (6-hydroxydopamine) and arthritis was induced in the adult rat. Since 6-hydroxydopamine may also have central effects on the newborn rat, the differences in results noted between the work of Aloe et al.22 and the present data are difficult to compare directly.

SPINAL CORD TRANSECTION

Additionally, we observed that complete spinal cord transection at the thoracic level had little or no influence on the severity of acute arthritis. This was also surprising since other investigators have found dorsal horn neurons are more responsive under conditions of acute arthritis. Electrophysiological studies by Schaible and Schmidt11 demonstrated that most spinal cord cells responding to stimulation of joint afferents were subjected to tonic descending inhibitory control from the brainstem. Since the effect of spinal cord transection on the inflammatory response was less than expected, peripheral inflammation must occur through central mechanisms separate from those involving transmission of nociceptive information. Previous studies from this laboratory support differential spinal cord control of nociception and inflammation. Specifically, pretreatment of the spinal cord with an NMDA glutamate receptor antagonist prevented the development of heat hyperalgesia but had no effect on the peripheral inflammation.1

In conclusion, the present study provides new evidence to support the hypothesis that the central terminals of primary afferent fibres contribute significantly to the inflammatory response of experimental acute arthritis in rats. Moreover, our data suggest that the central terminals of sensory afferents are involved in peripheral joint inflammation. The data presented here demonstrate that neither the sympathetic postganglionic efferents nor the supraspinal sympathetic neural pathways are responsible for the neurogenic component of this acute inflammatory model. Central terminal of acute peripheral joint inflammation is therefore dependent on both a neuronal circuit confined to the spinal cord and on intact dorsal roots, that is, central terminals of primary afferents.

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