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Intraneuronal Substance P Contributes to the Severity of Experimental Arthritis

Abstract. *There is evidence that substance P is a peptide neurotransmitter of some unmyelinated primary afferent nociceptors and that its release from the peripheral terminals of primary afferent fibers mediates neurogenic inflammation. The investigators examined whether substance P also contributes to the severity of adjuvant-induced arthritis, an inflammatory disease in rats. They found that, in the rat, joints that developed more severe arthritis (ankles) were more densely innervated by substance P–containing primary afferent neurons than were joints that developed less severe arthritis (knees). Infusion of substance P into the knee increased the severity of arthritis; injection of a substance P receptor antagonist did not. These results suggest a significant physiological difference between joints that develop mild and severe arthritis and indicate that release of intraneuronal substance P in joints contributes to the severity of the arthritis.*

Recent studies have implicated the peripheral nervous system in the inflammation seen in arthritis. For example, in rats with experimental arthritis, the concentration of the undecapeptide substance P (SP) increases in peripheral nerves that have branches innervating inflamed joints (*1*). Furthermore, if capsaicin, a neurotoxin that is relatively selective for unmyelinated sensory neurons (*2*), is administered to rats before or after the onset of arthritis, paw swelling and tenderness is diminished (*3*). The hypothesis that the nervous system contributes to arthritis is supported by studies demonstrating that the inflammation and tissue destruction seen clinically in rheumatoid arthritis and experimentally in adjuvant- or collagen-induced arthritis more frequently and more severely involve the more densely innervated, distal joints of an extremity (*4*). We report here further evidence that the nervous system contributes to arthritic inflammation, and, more specifically, that the severity of arthritis can be attributed, at least in part, to actions of SP in the affected joint.

Our initial experiments established that the joints most severely affected are indeed the most densely innervated and have the highest SP concentration. It is difficult to directly measure the innervation density of joints. We therefore used two indirect measures, the nociceptive threshold of the joint capsule, which should be inversely correlated with innervation density (*5*), and the magnitude of the spinal projection of the afferent fibers.

Vocalization in response to a noxious stimulus was measured in rats lightly anesthetized with pentobarbital. A pair of stimulating electrodes, separated by 1 mm, were placed against the surgically exposed joint capsule. Increasingly intense 1-second trains of monopolar pulses (100 μsec; 8 Hz) were delivered to evoke vocalization (*6*).

Nociceptive thresholds of the left and right ankle joints of normal rats were similar, as were the thresholds of left and right knee joints (Fig. 1). The threshold of a given ankle or knee joint was also constant throughout the experiment, and thresholds at different points on the same joint capsule were similar. Consistent with its having a less dense innervation, however, the knee joint (n = 8) showed a mean threshold three times higher than that of the ankle joint (n = 8) (3.5 ± 0.5 versus 0.56 ± 0.25 W, respectively) ($P < 0.01$, Student's t-test).

To examine the central projection of joint afferent neurons, we injected, under fluoroscopic guidance, a 1 to 4 percent solution of wheat germ agglutinin coupled to horseradish peroxidase (WGA-HRP; Sigma) into ankle and knee joints on opposite sides. Forty-eight hours later the rats were perfused with a mixed-aldehyde fixative. Frozen sections (50 μm) of the third lumbar to second sacral spinal segments were cut and reacted for anterogradely and transganglionically transported WGA-HRP (*7*).

Injection of 4 percent WGA-HRP revealed that the ankle has a significant afferent projection to the dorsal horn of the spinal cord. No projection from the knee joint capsule could be found. Since it could be argued that HRP is more dilute in the knee than in the ankle because of the larger joint space and that this may contribute to differential uptake of HRP by knee and ankle joint afferent fibers, a series of animals was studied in which increasingly higher concentrations of WGA-HRP were injected into the knee. Five rats were injected in opposite ankle and knee joints in dose ratios as high as 20:1 (25 μl of 4 percent solution in the knee and 5 μl of 18 percent solution in the ankle). Even at the highest dose of WGA-HRP, only minimal reaction product was recorded in the dorsal horn ipsilateral to the knee injection. In all rats, however, a concentrated area of reaction product was seen in sections of the fourth lumbar segment of the spinal cord ipsilateral to the injected ankle. The densest afferent projection was in a wedge shape that covered lamina I and the substantia gelatinosa (Fig. 1). Since many small nociceptive primary afferents terminate in the superficial dorsal horn, it appears that the ankle receives a major nociceptive innervation.

To assess whether SP is found in joint capsules and whether it is derived from joint innervation, we collected joint capsules (including both fibrous capsule and synovial membrane) from normal rats and from rats whose sciatic and femoral nerves had been bilaterally transected 6 days earlier. Substance P was measured by radioimmunoassay (*8*). Immunoreactivity in tissue extract had a retention time identical to that of synthetic SP when separated by high-performance liquid chromatography and measured by

radioimmunoassay. Samples of homogenate were taken for protein determination.

The mean concentration of SP in the normal ankle capsule (37.9 ± 2.7 fmol per milligram of protein; $n = 4$) was more than twice that in the knee (15.1 ± 2.2 fmol; $n = 4$) ($P < 0.0005$). Neurectomized rats had a markedly lower SP content in both the ankle (10.1 ± 1.5 fmol; $n = 4$) ($P < 0.0005$) and knee (7.0 ± 1.6 fmol; $n = 4$) ($P = 0.01$).

Thus, affected ankles, although smaller than knees, have a greater content of intraneuronal SP, a lower nociceptive threshold, and more extensive projection of afferent fibers to the spinal cord. While these results are consistent with the view that severity of arthritis reflects innervation of joints by SP-containing afferent fibers, the data are only correlative. Therefore, we next examined the effect of infusing SP into the joint capsule on the inflammation seen in experimental arthritis.

To best appreciate any increased severity that may be produced by infusion of SP, we studied the knee joint, which, as described above, is normally only mildly affected. Rats were anesthetized with pentobarbital and the knee joint was cannulated with a 30-gauge hypodermic needle. For 48 hours SP (30 ng/min; Peninsula Laboratories) was continuously infused into the left knee joint. One hour after the start of the infusion, adjuvant was injected into the tail in accordance with the standard protocol for generating arthritis (*9*). Another group of rats received the putative SP receptor antagonist $Pro^2,Trp^{7,9}$-SP (*10*). Since the effectiveness of substituted SP's as SP antagonists has been questioned (*11*), we did not try to reverse the effects of SP with them. However, these compounds are the most appropriate control agents since they have similar charge and molecular weight.

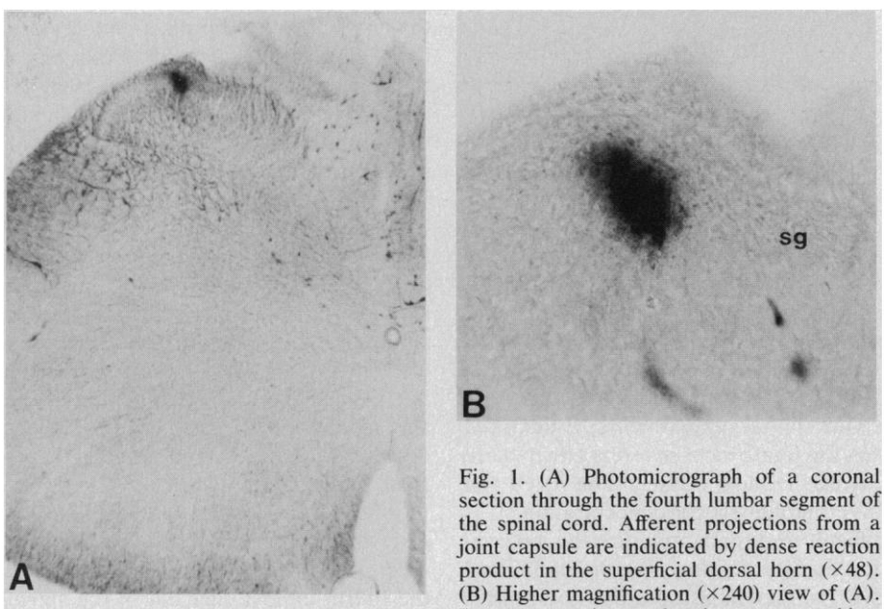

Fig. 1. (A) Photomicrograph of a coronal section through the fourth lumbar segment of the spinal cord. Afferent projections from a joint capsule are indicated by dense reaction product in the superficial dorsal horn (×48). (B) Higher magnification (×240) view of (A). Punctate reaction product is concentrated in a small wedge of the substantia gelatinosa (sg). This staining pattern suggests that smaller fibers (probably nociceptive) arise from the joint capsule.

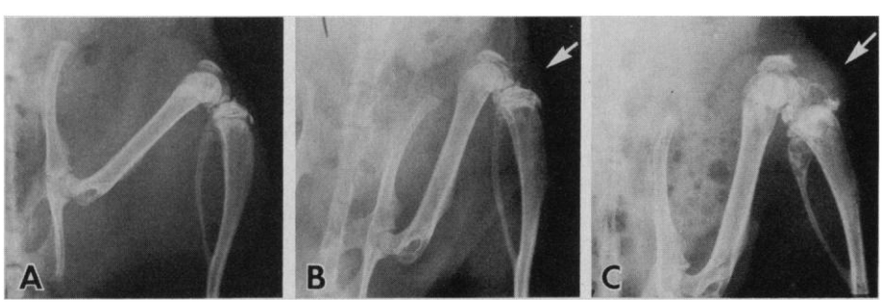

Fig. 2. X-ray photographs of the knee from (A) a nonarthritic control rat, (B) an arthritic rat injected intra-articularly with SP antagonist, and (C) an arthritic rat injected with SP. No abnormalities were detected in the x-ray of the control rat (score, 0). The knee of the rat injected with the SP antagonist shows moderate soft tissue swelling (arrow) and osteoporosis (score, 1). The knee of the rat injected with SP shows severe soft tissue swelling (arrow), osteoporosis, cartilage loss, and erosions (score, 3). Enhancement techniques were used to simultaneously demonstrate soft tissue and bony detail.

The severity of the arthritis in the knee joints was evaluated with radiographs taken 28 days after injection of antigen; arthritis typically develops 10 to 14 days after the injection. Radiographs were evaluated under double-blind conditions by C.H. using the grading scale of Ackerman *et al.* (*12*). The following radiographic parameters were evaluated: soft tissue swelling, decreased bone density (osteoporosis), joint space narrowing (cartilage loss), destruction of bone (erosions), and periosteal formation of new bone. A scale of 1 to 3 was used, with 0 indicating normal; 1, mild effects; 2, moderate effects; and 3, severe effects (Fig. 2). Infusion of SP resulted in significantly more severe arthritis (median score, 3; $n = 6$) than the SP antagonist (median score, 1; $n = 6$) ($P < 0.05$, Mann-Whitney U test). Substance P–treated rats had severe destruction of the joint and periarticular bone in addition to soft tissue swelling (Fig. 2C). The knee joints of rats given SP antagonist, however, were similar to those of normals, except for moderate soft tissue swelling and osteoporosis (Fig. 2B).

While many investigators have concentrated on the contribution of SP to the central transmission of nociceptive information, 90 percent of the SP in primary afferents is transported peripherally (*13*) and can be released by "antidromic" activation of peripheral nerves. Furthermore, electrical stimulation at intensities that release SP in peripheral tissues produces many of the physiological changes of acute inflammation (*14*). Our results indicate that severely affected joints are more densely innervated by SP-containing fibers than more mildly affected joints and that the severity of joint inflammation in experimental arthritis reflects the local release of SP by peripheral afferent fibers.

In addition to having an afferent, peptidergic innervation, the joint capsule also receives a significant sympathetic innervation. We have found that immunosympathectomy significantly attenuates adjuvant-induced arthritis, even in high-risk joints. Since sympathetic efferents modulate activity in primary afferent nociceptors (*15*), it is desirable to ascertain whether the effect of sympathectomy on arthritis reflects disruption of some proinflammatory action of sympathetic fibers or an interaction of sympathetic efferents and peptidergic afferents.

Presumably arthritis reflects an immu-

nological response, with the severity of the inflammation being attributable to the density of joint innervation and, more specifically, to the release of SP into the joint by peripheral afferent fibers. Conceivably the increase in SP levels in nerves that innervate an inflamed joint is secondary to the immunological mechanisms operating in the joint. The SP would exacerbate the inflammation, and thus may not have the reparative role proposed by Lembeck and Gamse (*16*).

The mechanism by which SP acts is unknown. It is significant that local application of SP produces many of the tissue changes of acute inflammation, including vasodilation, increased vascular permeability (*17*), pavementing of leukocytes in venules, stimulation of phagocytosis by polymorphonuclear leukocytes, and mast cell degranulation (*18*). Thus, SP may directly increase the inflammatory response in arthritic joints. It follows that attempts to diminish SP levels in these joints may prove effective in reducing the inflammation and tissue destruction.

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Transmission of HTLV-III Infection from Human Plasma to Chimpanzees: An Animal Model for AIDS

Abstract. *Two of three chimpanzees given plasma from patients with acquired immune deficiency syndrome (AIDS) or pre-AIDS showed serum antibodies to type III human T-cell leukemia virus (HTLV-III) 10 to 12 weeks after transfusion. One animal also developed lymphadenopathy, transient depression of the ratio of T4 to T8 lymphocytes, and impaired blastogenic responses. No opportunistic infections occurred. Adenopathy persisted for 32 weeks, and antibody to HTLV-III persisted for at least 48 weeks. This transmission of HTLV-III by lymphocyte-poor plasma confirms the potential risk of such plasma or plasma derivatives to recipients. The susceptibility of the chimpanzee to HTLV-III infection and the ability to simulate the human lymphadenopathy syndrome in this animal makes it a valuable model for further study of AIDS.*

Originally identified in male homosexuals and abusers of intravenous drugs (*1*), the acquired immune deficiency syndrome (AIDS) has more recently been recognized as a potential consequence of blood transfusion (*2*). Our investigation, designed to determine whether there was a transmissible agent in human blood capable of inducing AIDS and to establish an animal model in which the pathogenesis, treatment, and prevention of AIDS could be studied, began before the virologic investigations (*3–5*) that linked human AIDS to a type C retrovirus. Retroviruses designated human T-cell leukemia virus type III (HTLV-III) (*3*) and lymphadenopathy-associated virus (LAV) (*5*) have been reproducibly recovered from patients with AIDS and the AIDS-related syndrome. Whether or not HTLV-III and LAV are identical viruses remains to be determined. Common to both these agents is tropism for the T4 lymphocyte, a cell whose depletion is the focal point of the chain of immunologic and clinical events that comprises AIDS (*1*).

In experiments to maximize the potential for AIDS transmission to chimpanzees, each of three study animals (CH132, CH114, and CH133) was sequentially infused with plasma from three different patients selected to represent the spectrum of AIDS-related disorders (specifically, the lymphadenopathy syndrome, Kaposi's sarcoma, and life-threatening opportunistic infection) as defined by the Centers for Disease Control (*6*). One animal served as a control and received 3 units of normal donor plasma. To enhance further the potential for transmission of an agent with a low titer of antibody and to simulate the human transfusion experience, inocula were given in large volume (50 to 150 ml) to each chimpanzee. Plasmas were ABO-compatible, and no adverse reactions were associated with this interspecies transfusion program. The chimpanzees were housed individually in an isolation hut at the Southwest Foundation for Biomedical Research (SFBR) in San Antonio, Texas. Their clinical status was monitored by biweekly physical examination, and their immunologic status was assessed by biweekly determination of absolute lymphocyte counts, the number of T3, T4, and T8 subsets, B cells, natural killer cell activity, lymphocyte mitogen responsiveness, interleukin-2 activity, and reactions in mixed lymphocyte culture by means of established techniques (*7*).

Antibodies to HTLV-III were determined in a solid-phase ELISA (enzyme-linked immunosorbent assay) with the H9 HTLV-III clone (*3*) as described (*8*). Assays were performed in duplicate, and