Mediators of substance P-induced inflammation in the rat knee joint

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Abstract

Substance P (SP) injected into the synovial cavity of the rat knee resulted in an inflammatory response as measured by plasma protein extravasation into the joint capsule. This response was dose dependant over the range of ~4 μM to ~200 μM. Part of this inflammatory response was mediated via mast cells as pre-treatment of the animals with a mast cell degranulator (compound 48/80) resulted in a 66% reduction of the response. A direct effect of SP on the vascular receptors may also contribute to the inflammatory response as pre-treatment with the substance P antagonist (SPA) D-Pro⁴ D-Trp⁷⁹⁺¹⁰ SP₄₋₁₁ also reduces the inflammatory response. Intra-articular injections of the H₁ blocker diphenhydramine or the H₂ blocker cimetidine significantly blocked the SP-induced inflammatory response. The 5-hydroxytryptamine (5-HT) antagonist methysergide proved to be even more potent in blocking the SP-induced inflammatory response. No synergistic inhibition was observed with combinations of the different antagonists. Intra-articular injections of 5-HT elicited a much more pronounced inflammatory response than that produced by a 10-fold higher concentration of histamine.

The results suggest that SP produces increased vascular permeability partly via direct actions on the blood vessels and partly via mast cells. The inflammatory response occurring via mast cells appears to be mediated by histamine and to a greater extent by 5-HT.

Introduction

It has recently been demonstrated that the early phase of acute inflammation in the rat knee joint induced by intra-articular injection of 2% carrageenan has a significant neurogenic component as it can be inhibited by either denervation of the joint or by prior intra-articular injection of 1% capsaicin suspension [1]. It is likely that this component is mediated by release of substance P (SP) normally localised in sensory C-fibres [2], as the potent substance P antagonist (SPA) D-Pro⁴ D-Trp⁷⁹⁺¹⁰ SP₄₋₁₁ substantially inhibited the inflammatory response [1]. Antidromic electrical stimulation of articular C-fibres has been shown to produce plasma extravasation into the synovial cavity of the cat knee [3]. This neurogenically induced plasma extravasation is completely inhibited by prior intra-articular administration of the SPA D-Pro⁴ D-Trp⁷⁹⁺¹⁰ SP₄₋₁₁ [3] which strongly suggests that SP is the mediator of the response. Additional evidence implicating substance P is that electrical stimulation of the nerve supplying the cat knee joint causes release of SP from articular nerve fibres [4].

In a chronic model of arthritis such as adjuvant-induced arthritis, infusion of SP into the rat knee resulted in more pronounced inflammation and destructive changes of bone and cartilage than those whose joints had been infused with the sub-
stance P antagonist D-Pro²Trp⁷.⁹-⁹-SP [5]. It has also been found that neonatal capsaicin treatment reduced the inflammatory response of adjuvant induced arthritis in the rat [6]. Substance P has also been detected in the synovial fluid aspiration from inflamed joints in a patient with rheumatoid arthritis [7].

More recently, direct intra-articular injections of SP into rat knees have been shown to elicit marked inflammatory responses [1, 8]. This can be inhibited by prior intra-articular administration of D-Pro⁴-D-Trp⁷.⁹.¹⁰SP₄⁻¹₁ [1] or by capsaicin [8]. Thus, SP may have a role in inflammatory processes affecting joints, but so far there have been no studies performed to examine the inflammatory actions of SP on joint tissue. The purpose of the present investigation was to examine the potency of SP in eliciting an inflammatory response in the rat knee joint, and establish the extent to which the effects of SP are mediated by histamine and 5-hydroxytryptamine (5-HT).

**Materials and methods**

Experiments were performed on male Wistar rats (~300 g) deeply anaesthetised by intraperitoneal injection of urethane (1.13 g/kg) and diazepam (2.5 mg/kg). Evans blue (75 mg/kg) was injected into the external jugular vein. The experimental procedure consisted of injection of 0.2 ml of one of the following pro-inflammatory agents: substance P (Cambridge Research), histamine (Sigma), and 5-hydroxytryptamine (5-HT) (Sigma), or the mast cell degranulator, compound 48/80 (Sigma) into the synovial cavity of one knee, the other being injected with 0.2 ml of 0.9% saline to provide an internal control. These were left in the joint for four hours and anaesthesia maintained, after which the animals were injected with Euthatal and exsanguinated.

The anterior and posterior portions of the knee joint capsule on both sides were dissected free from each rat. The amount of tissue obtained from each animal was small, necessitating pooling of samples from five rats. These samples were weighed and Evans blue extracted using a modified dye extraction technique [9], details of which have been reported previously [1]. The amount of dye recovered was calculated by comparing the absorbance of the fluid obtained at 620 nm (LKB Ultraspec II) with that of a standard curve prepared with known concentrations of Evans blue solutions. As Evans