British Journal of Pharmacology (1996) 118, 2107-2114

Characterization of tachykinin receptors mediating plasma extravasation and vasodilatation in normal and acutely inflamed knee joints of the rat

[1]F.Y. Lam & M.C.S. Wong

Department of Pharmacology, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong

1 Inflammatory actions of tachykinins in normal rat knee joints were compared with those of animals with acutely inflamed joints induced by intra-articular injection of 2% carrageenan. Plasma protein extravasation in rat knee joints, measured by protein micro-turbidimetry, was induced by intra-articular perfusion of selective tachykinin receptor agonists. Changes in joint blood flow, measured by laser Doppler perfusion imaging, were produced by topical applications of selective tachykinin receptor agonists to the joint capsule.

2 Carrageenan-injected rat knee joints showed significantly higher ($P<0.001$) basal plasma extravasation (56 ± 4 $\mu g\ ml^{-1}$, $n=5$) than normal rat knee joints (10 ± 4 $\mu g\ ml^{-1}$, $n=6$). Intra-articular perfusion of the selective neurokinin$_1$ (NK_1) receptor agonist [Sar^9, Met(O$_2$)^{11}]-substance P (0.8 nmol min^{-1}) for 60 min elevated the basal plasma extravasation to 90 ± 17 $\mu g\ ml^{-1}$ ($n=6$, $P<0.001$) in normal joints, and to 150 ± 14 $\mu g\ ml^{-1}$ ($n=5$, $P<0.001$) in inflamed joints. Perfusion of the selective NK_1 receptor antagonist N^2-[(4**R**)-4-hydroxy-1-(1-methyl-1H-indol-3-yl)carbonyl-L-prolyl]-*N*-methyl-*N*-phenylmethyl-3-(2-naphthyl)-L-alaninamide (FK888; 0.8 nmol min^{-1}) for 20 min followed by co-perfusion with the NK_1 receptor agonist (0.8 nmol min^{-1}) produced complete inhibition of the NK_1 receptor agonist-induced plasma extravasation in the two groups of animals (for both groups; $n=3$, $P<0.001$).

3 Intra-articular perfusion of the selective NK_2 receptor agonist [Nle^{10}]-neurokinin A_{4-10} (0.8 nmol min^{-1}) and the selective NK_3 receptor agonist [MePhe^7]-neurokinin B (0.8 nmol min^{1}) produced no increase in plasma extravasation in normal or in inflamed rat knee joints ($n=4$ and 11, $P>0.05$).

4 Topical bolus applications of the NK_1 receptor agonist [Sar^9, Met(O$_2$)^{11}]-substance P onto normal joint capsules produced dose-dependent vasodilatation expressed as a voltage increase from control level. The maximum increase in blood flow was 2.05 ± 0.21 V from a basal voltage of 3.42 ± 0.07 V ($n=13$, $P<0.001$). To a much lesser extent, administration of the NK_2 receptor agonist [Nle^{10}]-neurokinin A_{4-10} also produced dose-dependent vasodilatation with a maximum increase of 0.46 ± 0.08 V from a basal level of 3.38 ± 0.1 V ($n=7$, $P<0.01$). Animals with acutely inflamed joints showed enhanced vasodilator responses to the NK_1 and NK_2 receptor agonists (for both: P vs non-inflamed joints <0.001). Thus, the NK_1 and NK_2 receptor agonists produced maximum increases of 2.56 ± 0.19 V (basal level = 5.84 ± 0.07 V; $n=7$, $P<0.001$) and 1.97 ± 0.26 V (basal level = 6.31 ± 0.23 V; $n=11$, $P<0.001$), respectively. The NK_3 receptor agonist [MePhe^7]-neurokinin B produced no change in blood flow in normal or in inflamed rat knee joints ($n=7$ and 5, $P>0.05$).

5 Bolus administration of the NK_1 receptor antagonist FK888 (10 pmol) alone followed 5 min later by another dose of 10 pmol FK888 (*i.e.* total dose of 2×10 pmol) applied together with the NK_1 receptor selective agonist [Sar^9, Met(O$_2$)^{11}]-substance P produced partial, but significant inhibition of the NK_1 receptor agonist-induced vasodilatation in both normal (maximum response reduced by $51.9\pm5.4\%$; $n=6$, $P<0.001$) and inflamed rat knee joints (maximum response reduced by $49.3\pm6.1\%$; $n=5$, $P<0.001$). The NK_2 receptor agonist [Nle^{10}]-neurokinin A_{4-10}-induced vasodilator responses in inflamed joints were not affected by this treatment ($n=6$, $P>0.05$). However, with two higher doses of FK888 (both 1 nmol), the NK_1 and the NK_2 receptor agonist-induced vasodilator responses were abolished in the two groups of animals ($n=6-8$, $P<0.005$).

6 Administration of two doses of the selective NK_2 receptor antagonist (S)-*N*-methyl-*N*-[4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)-butyl]benzamide (SR48968; both 10 pmol) produced no change in NK_1 receptor agonist-induced vasodilatation in normal ($n=11$, $P>0.05$) or in inflamed joints ($n=4$, $P>0.05$). However, the vasodilator responses to the NK_1 receptor agonist [Sar^9, Met(O$_2$)^{11}]-substance P in inflamed joints were abolished by two bolus doses of SR48968 (1 nmol), with the exception of the vasodilator response to the highest dose of the NK_1 receptor agonist (100 pmol) tested, where a small but significant ($P<0.001$) vasodilator response (0.58 ± 0.04 V; basal level = 5.37 ± 0.12 V; $n=6$) was still present. In contrast, the NK_2 receptor agonist [Nle^{10}]-neurokinin A_{4-10}-induced vasodilator responses were abolished by the two low doses of SR489868 (10 pmol) in normal ($n=6$, $P<0.005$) and in inflamed joints ($n=8$, $P<0.001$), except that to a high dose of the NK_2 receptor agonist (100 pmol), where a small but significant ($P<0.001$) vasodilator response (0.45 ± 0.22 V; basal level = 5.52 ± 0.05 V; $n=8$) still remained in the inflamed joint. This residual vasodilator response in the inflamed joint was also resistant to higher doses of SR48968 (two doses at 1 nmol) (0.53 ± 0.11 V; basal level = 5.57 ± 0.12 V; $n=7$, $P<0.001$), but was abolished by prior co-administration of the NK_2 receptor antagonist SR48968 and the NK_1 receptor antagonist FK888 both at 1 nmol ($n=4$, $P>0.05$).

[1]Author for correspondence.

7 The present findings indicate that NK_1 receptors are important in mediating tachykinin-induced plasma extravasation and vasodilatation in normal and acutely-inflamed rat knee joints. In normal joints, NK_2 receptor-mediated vasodilatation is of minor importance, but it has greater significance in inflamed joints. The data do not support a role for NK_3 receptors in mediating tachykinin-induced vasodilatation or plasma extravasation. It is speculated that suppression of NK_1 and NK_2 receptor-mediated responses might be useful in attenuating inflammatory responses in the rat knee joint where tachykinin release is implicated.

Keywords: Tachykinins; vasodilatation; joint inflammation; plasma extravasation; SR48968; FK888; [Sar^9, Met$(O_2)^{11}$]-substance P; [Nle^{10}]-neurokinin A_{4-10}; [MePhe^7]-neurokinin B

Introduction

Involvement of the nervous system in inflammation has long been recognised and Lewis (1936) first suggested that cutaneous wheal and flare responses occurred by the release of substances from the peripheral terminals of nociceptive afferents. The term 'neurogenic inflammation' has been used to describe the finding that antidromic stimulation of cutaneous nerves leads to vasodilatation and increased vascular permeability in the area innervated by the stimulated nerve (Jancso 1967; Lembeck & Holzer, 1979). In the last ten years, substantial evidence has accumulated to indicate that neurogenic inflammation occurs in the joint as well as in the skin. Thus, antidromic nerve stimulation at C-fibre strength in the joint (Ferrell & Russell, 1986; Ferrell & Cant, 1987), as in the skin (Jancso *et al.*, 1967), could elicit symptoms typical of inflammation, namely, plasma extravasation and vasodilatation. The neuropeptides substance P and neurokinin A contained in primary sensory neurones have been implicated, along with the potent vasodilator calcitonin gene-related peptide (CGRP), as important mediators of neurogenic inflammation in the joint. Thus, various experimental models of monoarthritis in the rat knee joint have shown significant increases of substance P- and neurokinin A-like immunoreactivity in knee joints pretreated with inflammatory agents compared to those found in control knee joints (Bileviciute *et al.*, 1993). Substance P and neurokinin A have also been shown to evoke potent dilatation of articular blood vessels in the rat knee joint (Lam & Ferrell, 1993a). However, of the two neuropeptides, substance P was the only one effective in increasing permeability of articular blood vessels in the rat knee joint (Lam & Ferrell, 1991b). The latter finding suggests that substance P may be more important than neurokinin A as a mediator of neurogenic inflammation in the rat knee joint. Other studies (Ferrell & Cant, 1987; Yaksh *et al.*, 1988; Lam & Ferrell 1989a,b; 1991a) have also suggested a role for substance P in the inflammatory process in the joint.

Substance P is a member of the tachykinin family consisting of a group of structurally related peptides including neurokinin A and neurokinin B. The tachykinins are known to interact with three major receptor types; the neurokinin$_1$ (NK_1), NK_2 and NK_3 receptors, at which substance P, neurokinin A, and neurokinin B are the preferential endogenous agonists, respectively, although all three receptors are activated by all three peptides (Burcher *et al.*, 1991; Mussap *et al.*, 1993). Plasma extravasation in rat skin (Andrew *et al.*, 1989) and in rat knee joint (Lam & Ferrell, 1991b) has been shown to be mediated predominantly by the NK_1 receptor subtype, though one study with naturally-occurring tachykinins indicated that multiple tachykinin receptor types could be involved in mediating tachykinin-induced vasodilatation in the rat knee joint (Lam & Ferrell, 1993a). However, naturally-occurring tachykinins are inherently unselective and at high concentrations are able to interact with all tachykinin receptors (Burcher *et al.*, 1991; Mussap *et al.*, 1993). The development of selective tachykinin receptor agonists provides the ability of stimulating one tachykinin receptor type only (Regoli *et al.*, 1988). Furthermore, selective tachykinin receptor antagonists now exist, and are proving useful tools in receptor characterization (Watling, 1992; Maggi *et al.*, 1993). Two such antagonists are N^2-[(4**R**)-4-hydroxy-1-(1-methyl-1H-indol-3-yl)carbonyl-L-prolyl]-*N*-methyl-*N*-phenylmethyl-3-(2-naphthyl)-L-alaninamide (FK888; Fujii *et al.*, 1992b) and (*S*)-*N*-methyl-*N*-*N*-[4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)-butyl]-benzamide (SR48968; Emonds-Alt *et al.*, 1992) which are selective for NK_1 and NK_2 receptors, respectively.

In a model of acute inflammation in the joint induced by intra-articular injection of 2% carrageenan, plasma extravasation (Scott *et al.*, 1992) and vasodilatation (Lam & Ferrell, 1993b) produced by substance P were enhanced compared to responses obtained in the normal joint. Preliminary findings with the selective NK_1 receptor antagonist, FK888, indicated that substance P-induced vasodilatation in the two groups of animals was mediated through the activation of NK_1 receptors (Lam *et al.*, 1993). It is likely that NK_1 receptors also play a role in mediating the increase in plasma extravasation evoked by substance P in normal joints (Lam *et al.*, 1993), but their significance in mediating substance P-induced plasma extravasation in inflamed joints is unknown. At present, the mechanisms contributing to the hypersensitivity (increased vasodilatation and plasma extravasation) of inflamed joints to substance P actions remain obscure. To shed further light on this, in the present study we investigated the tachykinin receptor types mediating plasma extravasation and vasodilatation in normal and acutely-inflamed rat knee joint using selective tachykinin receptor agonists and antagonists.

Methods

Experiments were performed on male Sprague-Dawley rats (300–370 g) deeply anaesthetized with urethane (1.7 g kg^{-1}, i.p.). In some rats acute inflammation was induced by prior (24 h) intra-articular injection of 0.2 ml (2%) λ-carrageenan into the synovial cavity of the knee (Lowther & Gillard, 1976).

Assessment of plasma protein extravasation

Assessment of plasma protein extravasation was as described previously (Scott *et al.*, 1991). Briefly, for each animal, two needles (21 gauge) were inserted into one of the knee joints. The tip of the inflow needle was placed in the posterior region of the synovial cavity and the outflow needle positioned in the anterior region of the cavity. A peristaltic pump (Gilson minipuls) was used for continuous perfusion of fluid into the synovial cavity at a constant rate of 50 μl min^{-1}. The effluent was collected by an automated fraction collector (Gilson FC 203B) into sample tubes over 4 min periods. NaCl (0.9%) was continuously perfused for 90 min to achieve a steady-state before collection began. To serve as a control, 5 sample tubes (20 min) of the aspirate were collected during the NaCl perfusion, after which the perfusate was changed to one containing drug solutions. Collection was then continued for a further 60 min which, according to our previous studies (Scott *et al.*, 1992), should be ample time to allow for maximum plasma extravasation to occur. In experiments involving antagonist administration, the antagonist was perfused into the

joint 20 min before co-perfusion with the agonist. This procedure has been confirmed by two independent studies to be adequate for antagonists to exert their inhibitory effects (Scott *et al.*, 1992; Hirayama *et al.*, 1993).

A micro-turbidimetric method (Scott *et al.*, 1991) was used to measure the protein content of each sample with a Hitachi (UV-1201) spectrophotometer at 277 nm. The amount of protein recovered was calculated by comparing the absorbance of test samples with that of the calibration curve obtained with standard rat albumin samples of known concentrations.

Assessment of changes in articular blood flow

The method of laser Doppler perfusion imaging (LDI) described by Lam and Ferrell (1993b) was used to measure the relative change in rat knee joint blood flow. The skin over the knee joint of the anaesthetized rat was removed to expose the anteromedial aspect of the joint capsule. To prevent tissue dehydration, 100 μl NaCl (0.9%) was added to the exposed surface every 5 min throughout the experiment except during drug administration. A laser Doppler perfusion imager (Lisca Development AB, Sweden), placed 12 cm above the joint, directs a helium-neon laser (633 nm) to the tissue and scans the surface of the object in a rectangular pattern of 6 x 7 cm in approximately 1 min. A colour-coded perfusion image can subsequently be generated and displayed on the monitor. The actual voltage values at each point in the image are stored on disc and can be utilised for calculation of the mean voltage within a given area, enabling the determination of voltage difference of the same selected area on the LDI image before and after experimental manipulation.

Drugs were administered as a bolus applied to the surface of the joint in a volume of 100 μl. Dose-response curves were constructed with 15 min intervals in between each bolus application to allow for recovery and to avoid tachyphylaxis to the tachykinins. In experiments where an antagonist was included, the joints were pretreated with the antagonist 5 min before the control measurement. This was followed by co-administration of the antagonist with the test agent. Possible changes in systemic blood pressure induced by drugs applied on the joint surface were monitored via a cannula inserted into the carotid artery connected to a pressure transducer (Gould, P231D) and the transducer output was displaced on a pen recorder (Graphtec Miniwriter, WTR751).

Drugs

The following drugs were used: [Sar^9, Met(O$_2$)^{11}]-substance P, [MePhe^7]-neurokinin B, and λ-carrageenan dissolved in 0.9% NaCl; [Nle^{10}]-neurokinin A$_{4-10}$ dissolved in ammonium hydroxide (0.01%); FK888 and SR48968 dissolved in ethanol (15%). The percentage of solvent refers to that of the stock solution, which was 10 μM. Subsequent dilutions of the stock solution were made in 0.9% NaCl. λ-Carrageenan was purchased from Sigma Chemical Co. (U.S.A.). All other drugs were purchased from Cambridge Research Biochemicals (U.K.) except FK888 and SR48968 which were gifts from Fujisawa Pharmaceutical Co. Ltd. (Japan) and Sanofi Recherche (France), respectively.

Statistical analysis

The results obtained from protein micro-turbidimetry are expressed as means (in μg ml^{-1}) $\pm$ s.e.mean of the amount of plasma protein extravasated. Those from LDI are represented as the mean voltage difference (*i.e.* test minus control) $\pm$ s.e.mean of the same selected scanned area in each animal. Mean values were compared by unpaired Student's *t* test, and differences between curves were analysed by repeated measures two-factor analysis of variance (ANOVA), followed where appropriate by comparisons of means by Planned Contrasts (SuperANOVA, Abacus Concepts, U.S.A.). The latter procedure is very efficient for comparing a limited subset of possible contrasts. This is useful for testing hypotheses about data that are more specific than the hypothesis automatically tested for each term in the ANOVA model (Gagnon *et al.*, 1989). P values <0.05 were considered statistically significant.

Results

Effects of tachykinin receptor agonists and antagonist on plasma protein extravasation in normal joints

In the normal rat knee joint, the basal plasma protein extravasation measured after 90 min perfusion with 0.9% NaCl was 10 ± 4 μg ml^{-1} ($n=6$). Switching the perfusate to one containing the selective NK$_1$ receptor agonist [Sar^9, Met(O$_2$)^{11}]-substance P (0.8 nmol min^{-1}) promptly increased plasma extravasation. The maximum response (90 ± 20 μg ml^{-1}; $n=6$) was achieved at 8 min and despite the continuous presence of the drug, it returned to the basal level 32 min after commencement of the drug perfusion. Administration of the NK$_2$ receptor agonist [Nle^{10}]-neurokinin A$_{4-10}$ (0.8 nmol min^{-1}; $n=8$) or the NK$_3$ receptor agonist [MePhe^7]-neurokinin B (0.8 nmol min^{-1}; $n=4$) produced no significant change in basal plasma extravasation ($P>0.05$). Perfusion of the selective NK$_1$ receptor antagonist FK888 (0.8 nmol min^{-1}; $n=3$) alone did not affect the basal protein level ($P>0.05$). Co-perfusion of the antagonist with [Sar^9, Met(O$_2$)^{11}]-substance P (0.8 nmol min^{-1}; $n=3$) abolished the NK$_1$ receptor agonist-induced response ($P<0.001$). These results are illustrated in Figure 1.

Effects of tachykinin receptor agonists and antagonist on plasma protein extravasation in acutely-inflamed joints

The basal protein extravasation observed in the acutely-inflamed joint (56 ± 4 μg ml^{-1}; $n=5$) was significantly greater

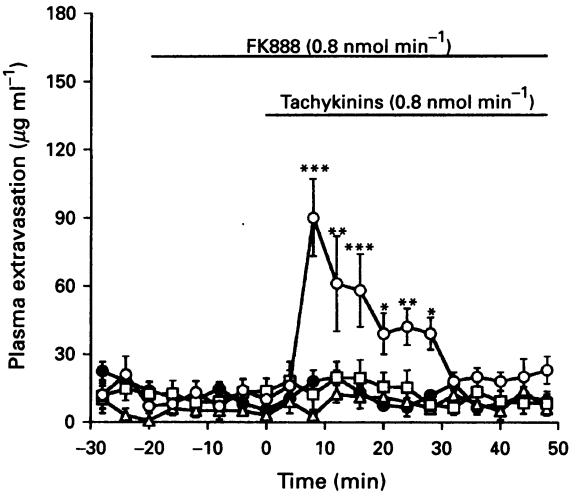

Figure 1 Plasma protein extravasation in normal rat knee joints. Effects of intra-articular perfusion of the selective NK$_1$ receptor agonist [Sar^9 Met (O$_2$)^{11}-SP (○; $n=6$), the selective NK$_2$ receptor agonist [Nle^{10}]-NKA$_{4-11}$ (□; $n=8$), the selective NK$_3$ receptor agonist [MePhe^7]-NKB (△; $n=4$), and [Sar^9 Met (O$_2$)^{11}]-SP in the presence of the selective NK$_1$ receptor antagonist FK888 (●; 0.8 nmol min^{-1}; $n=3$). The perfusion rate was 0.8 nmol min^{-1} throughout. The antagonist was administered 20 min before co-perfusion with the NK$_1$ receptor agonist into the joint. Data are shown as means $\pm$ s.e.mean (shown by vertical lines) of the amount of plasma protein extravasated in μg ml^{-1}. The curves for the NK$_1$ receptor agonist in the absence and in the presence of FK888 are not significantly different ($P>0.05$; two-factor ANOVA). However, subsequent analyses by Planned Contrasts (see Methods) showed significant differences between their means at time points indicated: $^*P<0.05$; $^{**}P<0.01$, $^{***}P<0.001$.

($P<0.001$) than that observed in the normal joint (10 ± 4 μg ml^{-1}; $n=6$). Perfusion of the selective NK_1 receptor agonist [Sar^9, Met(O$_2$)^{11}]-substance P (0.8 nmol min^{-1}) produced a peak increase in plasma extravasation (150 ± 14 μg ml^{-1}; $n=5$) 12 min following perfusion of the drug. The difference between the peak and the basal protein level (95 ± 19 μg ml^{-1}; $n=6$) was not significantly different ($P>0.05$; unpaired Student's t test) to that observed in the normal joint (80 ± 15 μg ml^{-1}; $n=5$). Nevertheless, the NK_1 receptor agonist-induced effect was more prolonged in the inflamed joint as the protein level did not return to basal value after 40 min, whilst in the normal joint, the response returned to the basal level after 32 min. Perfusion of the NK_2 receptor agonist [Nle^{10}]-neurokinin A_{4-10} (0.8 nmol min^{-1}; $n=5$) or the NK_3 receptor agonist [MePhe^7]-neurokinin B (0.8 nmol min^{-1}; $n=11$) into the inflamed joint, as in the normal joint, produced no change in the basal plasma extravasation ($P>0.05$). The basal protein level in the inflamed joint was not affected by perfusion of the NK_1 receptor antagonist FK888 (0.8 nmol min^{-1}; $n=3$, $P>0.05$), but plasma extravasation induced by the NK_1 receptor agonist [Sar^9, Met(O$_2$)^{11}]-substance P (0.8 nmol min^{-1}), as in the normal joint, was abolished in the presence of FK888 (0.8 nmol min^{-1}; $n=3$, $P<0.001$). These results are shown in Figure 2.

Effects of tachykinin receptor agonists and antagonists on blood flow in normal joints

Topical administration of the NK_1 receptor agonist [Sar^9, Met(O$_2$)^{11}]-substance P produced dose-dependent (0.1–100 pmol) increases in blood flow of the articular blood vessels. The maximum vasodilator response was 2.05 ± 0.21 V, representing a 60% increase from basal blood flow of 3.42 ± 0.07 V ($n=13$, $P<0.001$), and this was produced at 100 pmol of the agonist. A higher dose of agonist (1 nmol) produced a less pronounced increase in blood flow which was associated with a corresponding drop in systemic blood pressure and thus was not included in the figure (Figure 3a). Administration of the NK_2 receptor agonist [Nle^{10}]-neurokinin A_{4-10} produced a much smaller dose-dependent (0.1–100 pmol) increase in blood flow than the NK_1 receptor agonist (Figure 3b). At the highest dose tested (100 pmol), [Nle^{10}]-neurokinin A_{4-10} produced a maximum vasodilator response of 0.46 ± 0.08 V (basal level = 3.38 ± 0.1 V; $n=7$, $P<0.01$). The NK_3 receptor agonist [MePhe^7]-neurokinin B at all doses tested (0.1–100 pmol), produced no change in the knee joint blood flow ($n=5$, $P>0.05$) (Figure 3b).

Pretreatment of the rat knee joint with the selective NK_1 receptor antagonist FK888 (10 pmol followed 5 min later by co-administration of another 10 pmol of the antagonist with

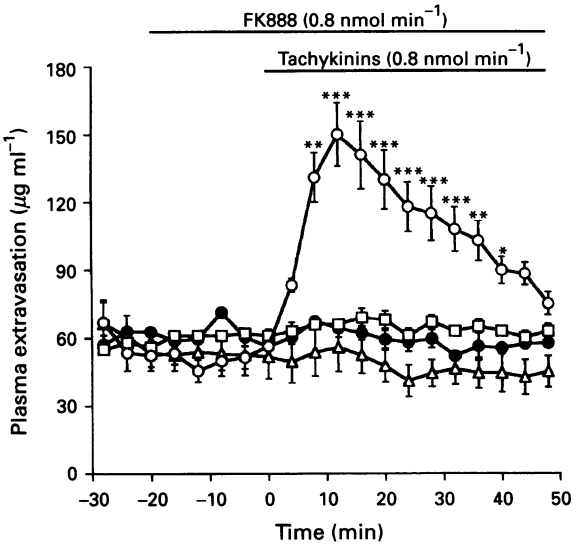

Figure 2 Plasma protein extravasation in acutely-inflamed rat knee joints. Effects of intra-articular perfusion of the selective NK_1 receptor agonist [Sar^9 Met (O$_2$)^{11}]-SP (○; $n=5$), the selective NK_2 receptor agonist [Nle^{10}]-NKA$_{4-11}$ (□; $n=5$), the selective NK_3 receptor agonist [MePhe^7]-NKB (△; $n=11$), and [Sar^9 Met (O$_2$)^{11}]-SP in the presence of the selective NK_1 receptor antagonist FK888 (●; 0.8 nmol min^{-1}; $n=3$). Acute inflammation was induced by intra-articular injection of 0.2 ml (2%) carrageenan into the synovial cavity of the joint 24 h before the experiment. The increase in plasma extravasation evoked by [Sar^9 Met (O$_2$)^{11}]-SP was significantly inhibited by FK888 ($P<0.005$; two way ANOVA). Subsequent analyses by Planned Contrasts showed significant differences between their means at time points indicated: $^*P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

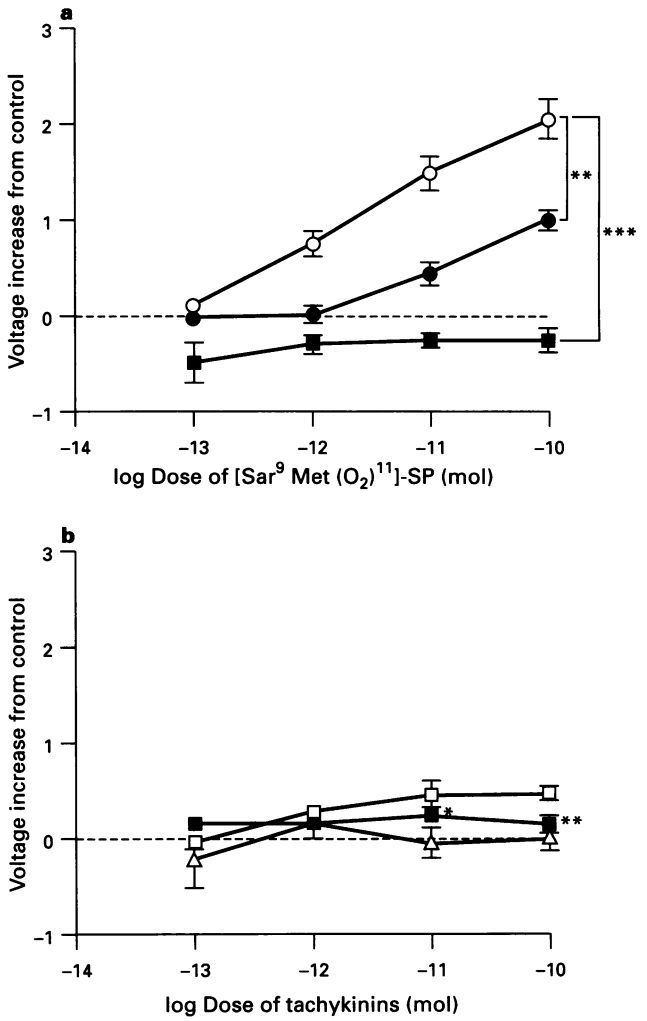

Figure 3 Articular blood flow of normal rat knee joint capsules. (a) Effects of the selective NK_1 receptor agonist [Sar^9 Met (O$_2$)^{11}]-SP alone (○; $n=13$) and in the presence of two 10 pmol doses (●, $n=6$) and two 1 nmol doses (■; $n=8$) of the selective NK_1 receptor antagonist FK888 (see Methods). (b) Effects of the selective NK_2 receptor agonist [Nle^{10}]-NKA$_{4-11}$ (□; $n=7$), the selective NK_3 receptor agonist [MePhe^7]-NKB (△; $n=7$), and [Nle^{10}]-NKA$_{4-11}$ in the presence of two doses of 1 nmol FK888 (■; $n=8$). Drugs were administered as a bolus applied to the rat knee joint surface in a volume of 0.1 ml. Data are shown as means±s.e.mean (shown by vertical lines) expressed as voltage difference of test image minus control image. Dose-response curves for the NK_1 receptor agonist were significantly inhibited by FK888 ($^{**}P<0.01$; $^{***}P<0.001$; two factor ANOVA). Dose-response curves for the NK_2 receptor agonist in the absence and in the presence of FK888 are not significantly different ($P>0.05$; two-factor ANOVA). However, subsequent comparisons of means by Planned Contrasts showed significant differences between control and 10 pmol means ($^*P<0.05$) and control and 100 pmol means ($^{**}P<0.01$).

[Sar^9, Met(O$_2$)^{11}]-substance P), resulted in a partial, but significant ($P>0.001$), inhibition of the dose-response curve of the NK$_1$ receptor agonist. The maximum increase in blood flow observed at 100 pmol of the NK$_1$ receptor agonist was significantly inhibited by 51.9±5.4% ($n=6$, $P>0.001$) in the presence of FK888. Complete inhibition of responses to all doses of the NK$_1$ receptor agonist was achieved with two 1 nmol doses of FK888 ($n=8$, $P>0.05$). At these doses, FK888 also significantly ($P<0.005$) reduced the vasodilator response to the maximum dose (100 pmol; $n=8$) of the NK$_2$ receptor agonist. These results are shown in Figure 3a and b, respectively.

As illustrated in Figure 4, pretreatment of the rat knee joint with two 10 pmol doses of the NK$_2$ receptor antagonist SR48968 i.e. 10 pmol followed 5 min later by co-administration of another 10 pmol of the antagonist with [Sar^9, Met(O$_2$)^{11}]-substance P, produced no change in the dose-response curve of the NK$_1$ receptor agonist ($n=11$, $P>0.05$). However, the same treatment of the joint with SR48968 abolished the vasodilator response to [Nle^{10}]-neurokinin A$_{4-10}$ ($n=6$, $P<0.005$). Administration of either FK888 (two 1 nmol doses; $n=10$) or SR48968 (two 1 nmol doses; $n=11$) produced no change in basal blood flow on their own ($P>0.05$).

Effects of tachykinin receptor agonists and antagonists on blood flow in acutely-inflamed joints

The basal blood flow observed in the acutely-inflamed joint (5.75±0.36 V; $n=10$) was significantly ($P>0.001$) higher than that observed in the normal joint (3.43±0.17 V; $n=12$). Administration of the NK$_1$ receptor agonist [Sar^9, Met(O$_2$)^{11}]-substance P elicited dose-dependent vasodilator responses in inflamed joints that were more pronounced than those observed in normal joints (Figure 5a). The maximum vasodilator responses produced in inflamed and normal joints were 2.56±0.19 V and 2.05±0.21 V from basal levels of 5.84±0.07 V ($n=7$) and 3.42±0.07 V ($n=13$), respectively. In the case of the NK$_2$ receptor agonist [Nle^{10}]-neurokinin A$_{4-10}$ (Figure 5b), the augmentation of the vasodilator response was even more profound, producing a peak response at 100 pmol of 1.97±0.26 V (basal level = 6.31±0.23 V; $n=11$) in the inflamed joint compared to that of 0.46±0.08 V (basal level = 3.38±0.1 V; $n=7$) in the normal joint. However, the duration of the vasodilator responses to the NK$_1$ and NK$_2$ receptor agonists was the same in normal and inflamed joints, and they lasted no more than 5 min in both cases. The NK$_3$ receptor agonist [MePhe^7]-neurokinin B (0.1–100 pmol), as in normal joints, was without effect on blood flow in the inflamed joints ($n=5$, $P>0.05$) (Figure 5b).

The NK$_1$ receptor antagonist FK888 produced the same pattern of inhibition on the NK$_1$ receptor agonist [Sar^9, Met(O$_2$)^{11}]-substance P-induced vasodilatation in inflamed and normal rat knee joints. Thus, two doses of 10 pmol FK888 produced partial inhibition of the NK$_1$ receptor agonist response ($n=5$, $P<0.001$), whereas two doses of 1 nmol FK888 abolished the NK$_1$ agonist response ($n=7$; $P<0.001$). The NK$_2$ receptor agonist (Nle^{10}]-neurokinin A$_{4-10}$-induced vasodilatation was unaffected by two doses of FK888 at 10 pmol ($n=6$, $P>0.05$), but was completely inhibited by two doses of FK888 at 1 nmol ($n=6$, $P<0.001$). These results are shown in Figure 5a and b, respectively. Unlike that in the normal joint, the basal blood flow (5.05±0.47 V, $n=9$) of the inflamed joint was significantly reduced (−0.61±0.41 V; $n=9$, $P<0.005$) by two 1 nmol doses of FK888.

As illustrated by the identical dose-response curves in Fig-

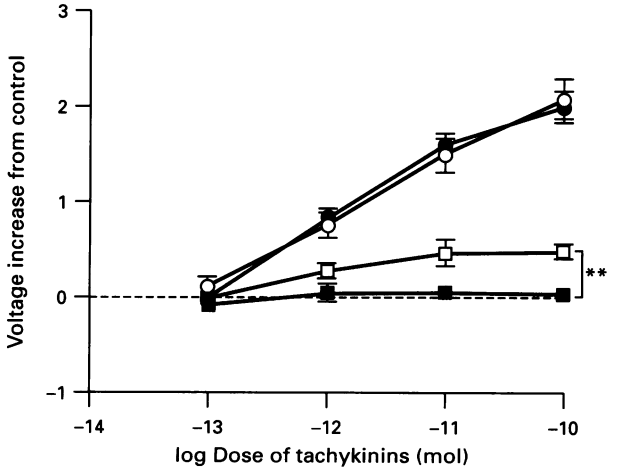

Figure 4 Articular blood flow of normal rat knee joint capsules. Effects of the selective NK$_1$ receptor agonist [Sar^9 Met (O$_2$)^{11}]-SP (○) and the selective NK$_2$ receptor agonist [Nle^{10}]-NKA$_{4-11}$ (□) in the absence ($n=13$ and 7, respectively) and in the presence of two 10 pmol doses of the selective NK$_2$ receptor antagonist SR48968 (●; $n=11$, ■; $n=7$, respectively). Dose-response curves of the NK$_2$ receptor agonist in the absence and in the presence of SR48968 are significantly different (**$P<0.005$; two-factor ANOVA).

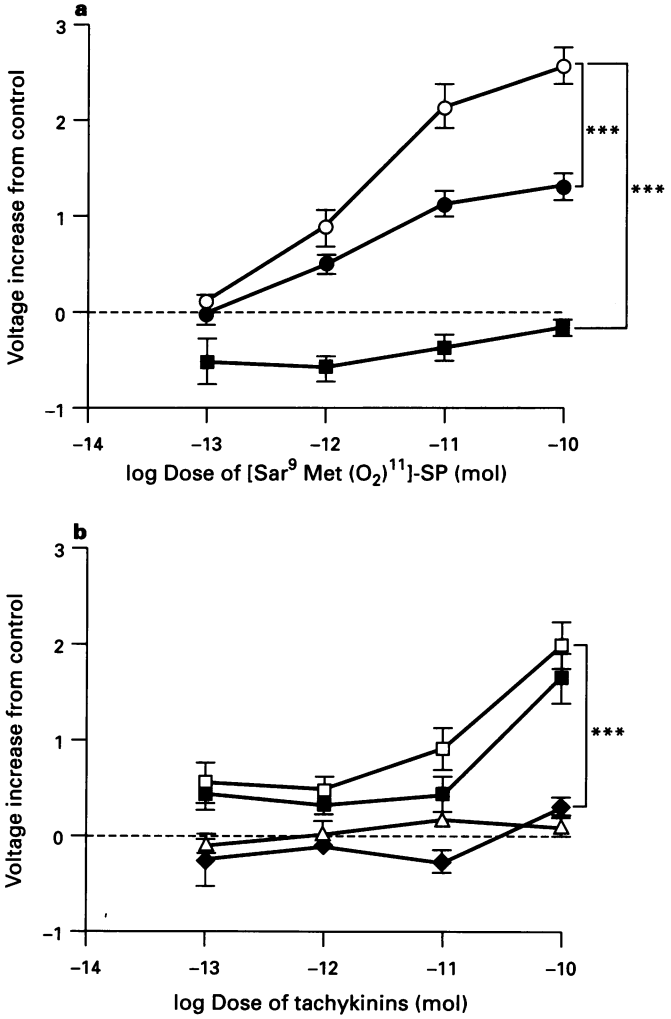

Figure 5 Articular blood flow of acutely-inflamed rat knee joint capsules. (a) Effects of the selective NK$_1$ receptor agonist [Sar^9 Met (O$_2$)^{11}]-SP alone (○; $n=7$) and in the presence of two 10 pmol doses (●; $n=5$) and two 1 nmol doses (■; $n=7$) of the selective NK$_1$ receptor antagonist FK888. (b) Effects of the selective NK$_2$ receptor agonist [Nle^{10}]-NKA$_{4-11}$ (□; $n=11$), the selective NK$_3$ receptor agonist [MePhe^7]-NKB (△, $n=5$), and [Nle^{10}]-NKA$_{4-10}$ in the presence of two 10 pmol doses (■; $n=6$) and two 1 nmol doses (◆; $n=6$) of FK888. Significant difference: ***$P>0.001$ (two factor ANOVA).

ure 6a, two doses of the selective NK_2 receptor antagonist SR48968 at 10 pmol produced no change in the NK_1 receptor agonist [Sar^9, $Met(O_2)^{11}$]-substance P-induced vasodilatation ($n=4$, $P>0.05$). However, two higher doses of SR48968 (both at 1 nmol) inhibited the NK_1 receptor agonist response except at the highest dose of the agonist (100 pmol) tested, where a small vasodilator response (0.58 ± 0.04 V; basal level = 5.37 ± 0.12 V; $n=6$, $P<0.001$) still remained. SR48968, at two 10 pmol doses and two 1 nmol doses, produced virtually identical inhibition on the NK_2 receptor agonist [Nle^{10}]-neurokinin A_{4-10}-induced response. Thus, the NK_2 receptor agonist (100 pmol) produced small vasodilator responses of 0.45 ± 0.22 V (basal level = 5.52 ± 0.05 V; $n=8$, $P<0.001$) and 0.53 ± 0.11 V (basal level = 5.57 ± 0.12 V; $n=7$, $P<0.001$) in the presence of the two multiple doses of SR48968, respectively. Whereas, vasodilator responses to the lower doses of the agonist were abolished by the two multiple doses of SR48968 ($P<0.005$ and 0.001). Co-administration of FK888 and SR48968, both two doses at 1 nmol, produced complete inhibition of the vasodilator responses to all doses of the NK_2 receptor agonist ($P>0.05$). These results are illustrated in Figure 6b. As in the normal joint, the basal blood flow of the inflamed joint was not affected by two 1 nmol doses of SR48968 ($n=9$, $P>0.05$).

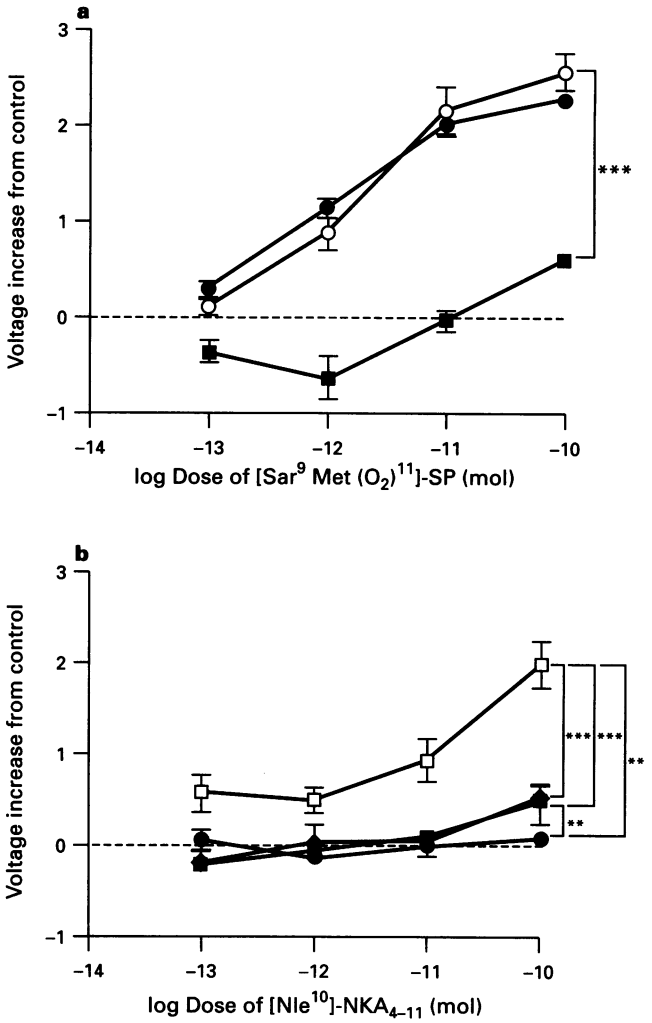

Figure 6 Articular blood flow of acutely-inflamed rat knee joint capsules. (a) Effects of the selective NK_1 receptor agonist [Sar^9 $Met(O_2)^{11}$]-SP alone (○; $n=7$) and in the presence of two 10 pmol doses (●; $n=4$) and two 1 nmol doses (■; $n=6$) of the selective NK_2 receptor antagonist SR48968. (b) Effects of the selective NK_2 receptor agonist [Nle^{10}]-NKA_{4-11} alone (□; $n=11$), in the presence of two 10 pmol doses (■, $n=8$) and two 1 nmol doses (◆; $n=7$) of SR48968, and in the presence of SR48968 plus the selective NK_1 receptor antagonist FK888 (both two 1 nmol doses; ●; $n=4$). Significant difference: $^{**}P>0.005$; $^{***}P>0.001$ (two factor ANOVA).

Discussion

The present study demonstrates that intra-articular perfusion of the selective NK_1 receptor agonist [Sar^9, $Met(O_2)^{11}$]-substance P is effective in eliciting plasma protein extravasation in normal rat knee joints, while the selective NK_2 receptor agonist [Nle^{10}]-neurokinin A_{4-10} and the NK_3 receptor agonist [$MePhe^7$]-neurokinin B are ineffective. This is also true in studies performed on acutely-inflamed rat knee joints. In blood flow studies, [Sar^9, $Met(O_2)^{11}$]-substance P produced the most pronounced vasodilatation in blood vessels of the normal rat knee joints; the vasodilator responses to [Nle^{10}]-neurokinin A_{4-10} were much smaller, and [$MePhe^7$]-neurokinin B was ineffective. In inflamed rat knee joints, the vasodilator responses to NK_1 and NK_2 receptor agonists were exacerbated, but [$MePhe^7$]-neurokinin B remained ineffective. Studies with tachykinin receptor antagonists indicate that tachykinin-induced plasma protein extravasation is mediated via the activation of NK_1 receptors, whereas, tachykinin-induced vasodilatation is likely to involve activation of both NK_1 and NK_2 receptors.

The characteristics of the NK_1 receptor agonist response in normal joints resembles those seen with substance P in previous studies (Scott *et al.*, 1991; 1992), in that the two tachykinins both produced marked yet transient plasma extravasation; thus their responses peaked at 8 min and then returned to basal levels within 32 min in both cases, despite continuous perfusion of tachykinin into the joint. The transient nature of the action of substance P in the normal joint has been suggested to serve as a useful protective mechanism for the joint (Scott *et al.*, 1992; 1994). It was postulated that after injury of a joint, substance P could initiate short-term recruitment of mediators that are useful for the healing process, whilst avoiding prolonged inflammatory actions of the tachykinin which could be detrimental to the joint. In this context, it is interesting to note that, CGRP, a non-tachykinin neuropeptide known to co-exist with substance P and neurokinin A in sensory neurones, produces sustained (>1 h) plasma protein extravasation in the rat knee joint (Karimien & Ferrell, 1994). In some respects this parallels the vasodilator effects of the two neuropeptides, with substance P producing transient dilatation of synovial blood vessels whereas CGRP produces long-lasting dilatation (Lam & Ferrell, 1993a). However, Karimien and Ferrell (1994) also showed that the β_1,β_2-adrenoceptor agonist isoprenaline produced dilatation of articular blood vessels equivalent to that of CGRP, but this was not accompanied by plasma extravasation. Thus, as with substance P (Cruwys *et al.*, 1992; Lam & Ferrell, 1991b; 1993a,b), these workers proposed that CGRP may increase vascular permeability independent of its vasodilator effect. In rat skin, it has been suggested that substance P-evoked release of proteolytic enzymes from local mast cells could lead to accelerated degradation of CGRP, thereby shortening the duration of the response to CGRP (Brain & Williams, 1989). This phenomenon has yet to be demonstrated in the rat knee joint, nevertheless, it has been shown that the duration of CGRP-induced vasodilatation in the rat knee joint can be drastically reduced by the co-administration of substance P with CGRP (Lam & Ferrell, 1993a). Thus, although continuous perfusion of CGRP can produce a persistent increase in plasma extravasation (Karimien & Ferrell, 1994), whether endogenous release of CGRP together with SP can evoke sustained plasma extravasation in the rat knee joint remains uncertain.

In the inflamed joint, both substance P (Scott *et al.*, 1992) and the NK_1 receptor agonist evoked more persistent plasma extravasation, although the sustained effect was less pronounced with the NK_1 receptor agonist than substance P. These findings indicate that in inflammatory conditions activation of the NK_1 receptors produces greater inflammatory responses than in normal physiological conditions. The exact

mechanisms of this enhanced NK_1 receptor-mediated response is unknown at present, but it is speculated that in the acute inflammatory state, NK_1 receptors on the articular blood vessels may have been up-regulated, thereby producing a more sustained response. Whether this is due to increased NK_1 receptor numbers, or increased affinity of the NK_1 receptors towards their preferential ligands, or to alterations at the level of the second messenger system remains to be elucidated. Involvement of other tachykinin receptor types in the plasma extravasation response is unlikely as both the selective NK_2 receptor agonist and NK_3 receptor agonist were without effect in normal and in inflamed joints.

Of the three selective tachykinin receptor agonists tested, the NK_1 receptor agonist was the most effective in producing vasodilatation of the articular blood vessels in the rat knee joint. The maximum vasodilatation produced by the NK_2 receptor agonist was only 27% of that produced by the NK_1 receptor agonist, and the NK_3 receptor agonist was ineffective. This order of potency is in agreement with a similar study using naturally-occurring tachykinins, where a rank order of potency of SP = NKA > NKB in eliciting vasodilator responses in the rat knee joint was demonstrated (Lam & Ferrell, 1993a). Thus, the use of selective tachykinin receptor agonists in the present study confirms the hypothesis that NK_1 receptors are of greater importance than NK_2 or NK_3 receptors in mediating vasodilator responses in the rat knee joint. Furthermore, the use of selective tachykinin receptor antagonists in the present study consolidated the view that the NK_1 receptor agonist-induced effect is mediated by its action on NK_1 receptors. Hence, in the presence of two 10 pmol doses of the selective NK_1 receptor antagonist FK888, the NK_1 receptor agonist-induced vasodilatation was partially inhibited, whilst at higher doses of the antagonist (two doses at 1 nmol), it was abolished. In addition, the vasodilator response of the NK_1 receptor agonist was unaffected by the presence of the selective NK_2 receptor antagonist SR48968 (two doses at 10 pmol), implying that NK_2 receptors are not involved. On the other hand, the same doses of SR48968 produced complete inhibition of the NK_2 receptor agonist-induced vasodilatation, indicating that it is mediated by the NK_2 receptors.

In comparison to normal joints, a small potentiation of the vasodilator response to the NK_1 receptor agonist was observed in inflamed joints. FK888 was effective in abolishing the potentiated vasodilator response, suggesting that it too was also NK_1 receptor-dependent. The involvement of NK_2 receptors is unlikely since SR48968 (two doses at 10 pmol) was not effective in blocking the NK_1 receptor agonist-induced response. Higher doses of SR48968 (two doses at 1 nmol) significantly reduced the NK_1 receptor agonist response but this could well be due to antagonism of NK_1 receptors by SR48968. In fact, SR48968 at high concentrations has been shown to exhibit significant binding to NK_1 receptors of the guinea-pig lung (Kudlacz *et al.*, 1993). SR48968 is not expected to have non-specific inhibitory effects in the rat knee joint as it has been shown that SR48968 did not affect 5-hydroxytryptamine-induced Evan's blue extravasation in the rat knee joint, even at a dose of 180 nmol per knee (Hirayama *et al.*, 1993). Thus, the enhanced vasodilator action of the NK_1 receptor agonist in inflamed joints is more likely attributed to increased participation of the NK_1 receptors in the inflamed condition. It is possible that reduced sympathetic vasoconstriction in the inflamed joint as demonstrated by Lam & Ferrell (1993b) also contributes to the enhanced vasodilatation.

The augmentation of the NK_2 receptor agonist-induced vasodilator response in the inflamed rat knee joint was even more striking than that observed with the NK_1 receptor agonist. FK888, at two 10 pmol doses, was without effect on the NK_2 receptor agonist-induced response, but it abolished the NK_2 receptor agonist response at two 1 nmol doses which indicate that NK_1 receptors might be responsible for the increased vasodilator action of the NK_2 receptor agonist in the inflamed joint. However, FK888 is much more effective in inhibiting the NK_1 receptor agonist-induced responses; it abolished the NK_1 receptor agonist-induced vasodilatation at two 1 nmol doses and even at two 10 pmol doses, it partially reduced the NK_1 receptor agonist response. No non-specific inhibitory actions of FK888 have been observed (e.g. Wang *et al.*, 1994), although it has been shown to possess a weak affinity for guinea-pig NK_2 receptors (Fujii *et al.*, 1992a). Taking these points into consideration, and in spite of FK888 being labelled as a 'selective' NK_1 receptor antagonist, it might be more appropriate to suggest that in the present preparation the reduction in the NK_2 receptor agonist response by the higher dose of FK888 may be due to block of NK_2 receptors. In addition, the present study showed that FK888 at two 1 nmol doses produced a small but significant reduction in basal blood flow of the inflamed joints, suggesting that there may be tonic release of neuropeptides responsible for the augmented basal blood flow in inflamed joints. As FK888 lowers the basal blood flow in inflamed joints, it is likely that this also contributes to its effect in reducing the NK_2 receptor agonist-induced response.

Further evidence in support of the idea that NK_2 receptors are involved in mediating the NK_2 receptor agonist effects is that SR48968 at two low doses of 10 pmol was effective in inhibiting the NK_2 receptor agonist-induced vasodilatation, albeit a small residual vasodilator response still remained with 100 pmol of the agonist. This residual vasodilator response persisted even in the presence of two 1 nmol doses of SR48968, but a mixture of FK888 and SR48968 (both two doses at 1 nmol) abolished the response. The lowering of the basal blood flow by FK888 may have helped in abolishing the vasodilator response, but it is also possible that NK_1 receptors are involved in mediating the small residual vasodilator response to the NK_2 receptor agonist.

As with plasma extravasation, [MePhe^7]-neurokinin B possesses no vasodilator action on either normal or inflamed rat knee joints. It is thus unlikely that NK_3 receptors have a role in mediating inflammatory processes in the rat knee joints.

In conclusion, the present studies confirm the hypothesis that NK_1 receptors are solely responsible for mediating tachykinin-induced plasma protein extravasation in normal and inflamed rat knee joints. On the other hand, tachykinin-induced vasodilatation could be mediated by both NK_1 and NK_2 receptors. The contribution of NK_2 receptor-mediated vasodilatation is of minor importance in normal joints, but it is of greater significance in inflamed joints as revealed by the augmented vasodilator response to the NK_2 receptor agonist in the inflamed condition. Thus, the data support the idea that tachykinins are important in the initiation of inflammatory responses in the rat knee joint, and this is primarily mediated by NK_1 receptors. Moreover, the results indicate that tachykinins could exacerbate the inflammatory process once manifested. This latter contribution to the inflammatory process is likely to involve both NK_1 and NK_2 receptor activation. In addition to the tachykinins, it is possible that CGRP constitutes another neurogenic mediator of joint inflammation as this peptide is also known to produce potent vasodilatation (Lam & Ferrell, 1993a) and plasma extravasation (Karimian & Ferrell, 1994) in the rat knee joint.

Finally, it is worth noting that the tachykinin receptor antagonists were without significant effect *per se* on basal plasma extravasation in inflamed joints. This does not necessarily imply that the tachykinins are not involved in the continuing inflammatory process as it was shown that FK888 was effective in lowering the augmented basal blood flow in inflamed joints. Furthermore, in our previous studies, we have demonstrated a neurogenic component in carrageenan-induced joint inflammation (Lam & Ferrell, 1991a) and that a substance P antagonist given before the administration of carrageenan produced substantial reduction in the inflammatory response (Lam & Ferrell, 1989b). Taken together, it is more appropriate to conclude that the tachykinin antagonists are more effective in inhibiting the neurogenic contribution to the inflammatory process when administered

before the induction of inflammation, and perhaps even better, if combined with a CGRP antagonist. In addition, since tachykinins have greater effects in inflamed joints, if further release of tachykinins occurs in these joints, this could lead to drastically exacerbated inflammatory responses in the joints. In these circumstances, long term treatment of the inflamed joints with tachykinin antagonists might be useful in limiting the progress of the disease.

This research was supported by the Research Grants Council (RGC) of Hong Kong. The authors are grateful to Fujisawa Pharmaceutical Co. Ltd. (Japan) and Sanofi Recherche (France) for the gifts of FK888 and SR48968, respectively. The authors thank Prof. Robert Jones for his helpful advice in the preparation of this manuscript and also thank Miss Amber Lo Ming Yip for her invaluable technical assistance.

References

ANDREW, P.V., HELME, R.D. & THOMAS, K.L. (1989). NK_1 receptor mediation of neurogenic plasma extravasation in rat skin. *Br. J. Pharmacol.*, **97,** 1232–1238.

BILEVICIUTE, I., LUNDBERG, T., EKBLOM, A. & THEODORSSON, E. (1993). Bilateral changes of substance P-, neurokinin A-, calcitonin gene-related peptide- and neuropeptide Y-like immunoreactivity in rat knee joint synovial fluid during acute monoarthritis. *Neurosci. Lett.*, **152,** 37–40.

BRAIN, S.D. & WILLIAMS, T.J. (1989). Interactions between the tachykinins and calcitonin gene-related peptide lead to modulation of oedema formation and blood flow in rat skin. *Br. J. Pharmacol.*, **97,** 77–82.

BURCHER, E., MUSSAP, D.J., GERAGHTY, D.P., McCLURE-SHARP, J.M. & WATKINS, D.J. (1991). Concepts in characterization of tachykinin receptors. *Ann. N.Y. Acad. Sci.*, **632,** 123–136.

CRUWYS, S.C., KIDD, B.L., MAPP, P.I., WALSH, D.A. & BLAKE, D.R. (1992). The effects of calcitonin gene-related peptide on formation of intra-articular oedema by inflammatory mediators. *Br. J. Pharmacol.*, **107,** 116–119.

EMONDS-ALT, X., VILAIN, P., GOULAOUIC, P., PROIETTO, V., VAN BROECK, D., ADVENIER, C., NALINE, E., NELIAT, G., LE FUR, G. & LE BRELIERE, J.C. (1992). A potent and selective non-peptide antagonist of the neurokinin A (NK_2) receptor. *Life Sci.*, **50,** PL-101-PL-106.

FERRELL, W.R. & CANT, R. (1987). Vasodilatation of articular blood vessels induced by antidromic electrical stimulation of joint C-fibres. In *Fine Afferent Nerve Fibres and Pain.* ed. Schmidt, R.F., Schaible, H.G. & Vahle-Hinz, C. pp. 187–192. Weinheim, F.R.G.: VCH Verlagsgesellschaft.

FERRELL, W.R. & RUSSELL, N.J.W. (1986). Extravasation in the knee induced by antidromic stimulation of articular C fibre afferents of the anaesthetised cat. *J. Physiol.*, **379,** 407–416.

FUJII, T., MURAI, M., MORIMOTO, H., HAGIWARA, D., MIYAKE, H. & MATSUO, A. (1992a). Effects of novel SP antagonist, FK888 on airway constriction and airway edema in guinea-pigs. *Neuropeptides*, **22,** 24.

FUJII, T., MURAI, M., MORIMOTO, H., MAEDA, M., YAMAOKA., HAGIWARA., MIYAKE, H., IKARI, N. & MATSUO, M. (1992b). Pharmacological profile of a high affinity dipeptide NK_1 receptor antagonist, FK888. *Br. J. Pharmacol.*, **107,** 785–789.

GAGNON, J., ROTH, J.M., FINZER, W.F., HAYCOCK, K., FELDMAN, D.S. & SIMPSON, J. (1989). Contrasts. In *Abacus Concepts, SuperANOVA*, pp. 199–205. Berkeley, CA: Abacus Concepts, Inc.

HIRAYAMA, Y., YASUMITSU, R., KAWAMURA, A. & FUJII, T. (1993). NK_1 receptors mediate tachykinin-induced plasma extravasation in the rat knee joint. *Agents Actions*, **40,** 171–175.

JANSCO, N., JANSCO-GABOR, A. & SZOLCSANYI, J. (1967). Direct evidence for neurogenic inflammation and its prevention by denervation and pretreatment with capsaicin. *Br. J. Pharmacol.*, **31,** 138–151.

KARIMIAN, M. & FERRELL, W.R. (1994). Plasma protein extravasation into the rat knee joint induced by calcitonin gene-related peptide. *Neurosci. Lett.*, **166,** 39–42.

KUDLACZ, E.M., LOGAN, D.E., SHATZER, S.A., FARRELL, A.M. & BAUGH, L.E. (1993). Tachykinin-mediated respiratory effects in conscious guinea-pigs: modulation by NK_1 and NK_2 receptor antagonists. *Eur. J. Pharmacol.*, **241,** 17–25.

LAM, F.Y. & FERRELL, W.R. (1989a). Capsaicin suppresses substance P-induced inflammation in the rat. *Neurosci. Lett.*, **105,** 155–158.

LAM, F.Y. & FERRELL, W.R. (1989b). Inhibition of carrageenan-induced inflammation in the rat knee joint by substance P antagonist. *Ann. Rheum. Dis.*, **48,** 928–932.

LAM, F.Y. & FERRELL, W.R. (1990). Mediators of substance P-induced inflammation in the rat knee joint. *Agents Actions*, **31,** 298–307.

LAM, F.Y. & FERRELL, W.R. (1991a). Neurogenic component of different models of acute inflammation in the rat knee joint. *Ann. Rheum. Dis.*, **50,** 747–751.

LAM, F.Y. & FERRELL, W.R. (1991b). Specific neurokinin receptors mediate plasma extravasation in the rat knee joint. *Br. J. Pharmacol.*, **103,** 1263–1267.

LAM, F.Y. & FERRELL, W.R. (1993a). Effects of interaction of naturally-occurring neuropeptides on blood flow in the rat knee joint. *Br. J. Pharmacol.*, **108,** 694–699.

LAM, F.Y. & FERRELL, W.R. (1993b). Acute inflammation in the rat knee joint attenuates sympathetic vasoconstriction but enhances neuropeptide-mediated vasodilatation assessed by laser Doppler perfusion imaging. *Neuroscience*, **52,** 443–449.

LAM. F.Y., FERRELL, W.R. & SCOTT, D.T. (1993). Substance P-induced inflammation in the rat knee joint is mediated by neurokinin 1 (NK_1) receptors. *Regul. Pept.*, **46,** 198–201.

LEMBECK, F. & HOLZER, P. (1979). Substance P as a neurogenic mediator of antidromic vasodilatation and neurogenic plasma extravasation. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **310,** 175–183.

LEWIS, T. (1936). Experiments relating to cutaneous hyperalgesia and its spread through somatic nerves. *Clin. Sci.*, **2,** 373–417.

LOWTHER, D.A. & GILLARD, G.C. (1976). Carrageenin-induced arthritis. *Arthritis Rheum.*, **19,** 769–776.

MAGGI, C.A., PATACCHINI, R., ROVERO, P. & GIACHETTI, A. (1993). Tachykinin receptors and tachykinin receptor antagonists. *J. Auton. Pharmacol.*, **13,** 23–93.

MUSSAP, C.J., GERAGHTY, D.P. & BURCHER, E. (1993). Tachykinin receptors: a radioligand binding perspective. *J. Neurochem.*, **60,** 1987–2009.

REGOLI, D., DRAPEAU, G., DION, S. & COUTURE, R. (1988). New selective agonists for neurokinin receptors: pharmacological tools for receptor characterization. *Trends Pharmacol. Sci.*, **9,** 290–295.

SCOTT, D.T., LAM, F.Y. & FERRELL, W.R. (1991). Time course of substance P-induced protein extravasation in the rat knee joint measured by micro-turbidimetry. *Neurosci. Lett.*, **129,** 74–76.

SCOTT, D.T., LAM, F.Y. & FERRELL, W.R. (1992). Acute inflammation enhances substance P-induced plasma protein extravasation in the rat knee joint. *Regul. Pept.*, **39,** 227–235.

SCOTT, D.T., LAM, F.Y. & FERRELL, W.R. (1994). Acute joint inflammation-mechanisms and mediators. *Gen. Pharmacol.*, **25,** 1285–1296.

WANG, Z-Y., TUNG, S.R., STRICHARTZ, G.R. & HÅKANSON, R. (1994). Investigation of the specificity of FK888 as a tachykinin NK_1 receptor antagonist. *Br. J. Pharmacol.*, **111,** 1342–1346.

WATLING, K.J. (1992). Nonpeptide antagonists herald new era in tachykinin research. *Trends Pharmacol. Sci.*, **13,** 66–69.

YAKSH, T.L., BAILLY, J., RODDY, D.R. & HARTY, G.J. (1988). Peripheral release of substance P from primary afferents. In *Proceedings of Vth World Congress on Pain.* ed. Dubner, R., Gebhart, G.F. & Bond, M.R. pp. 51–55. Amsterdam: Elsevier.

(Received March 28, 1996
Revised May 22, 1996
Accepted May 23, 1996)