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Unique gradual and sustained vasodilator response to substance P in the rabbit knee joint

Francis F.Y. Lam *, Amber L.M. Yip

Department of Pharmacology, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, People's Republic of China

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Abstract

The effects of substance P on blood flow, plasma extravasation, and knee joint sizes in the rabbit were investigated. Topical bolus application of substance P (1 nmol) onto the exposed rabbit knee joint capsule increased its blood flow from 15 min onwards and reached a peak of 46% at 90 min compared to saline administration. However, administration by the same route and the same dose of the NK_1 receptor agonist [Sar^9, Met $(O_2)^{11}$]substance P produced no change on the knee joint blood flow compared to the saline control. The NK_1 receptor antagonist N^2-[(4*R*)-4-hydroxy-1-(1-methyl-1*H*-indol-3-yl)carbonyl-L-prolyl]-*N*-methyl-*N*-phenylmethyl-3-(2-naphthyl)-L-alaninamide (FK888) and the NK_2 receptor antagonist (*S*)-*N*-methyl-*N*-[4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)-butyl] benzamide (SR48968), at 2×1 nmol and 2×10 nmol, had no effect on the substance P-induced response, which however was reduced by pyrilamine, cimetidine, and flurbiprofen (all at 2×10 nmol). N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-nitro-D-arginine methyl ester (D-NAME), both at 2×100 nmol, did not significantly affect the substance P-induced response. Unilateral intra-articular administration of substance P (1 nmol) into synovial cavities of the rabbit knee joint increased basal blood flow of the ipsilateral joint at 4 h post-injection, and bilateral increase of basal blood flow was observed at 24 h. Plasma extravasation was significantly higher in the substance P-injected knee compared to the contralateral saline-injected knee at 4 h after intra-articular administrations, but not at 24 h. Knee joint sizes were not affected at both time points. The present study is the first to demonstrate that substance P possesses a gradual and persistent vasorelaxant action in the rabbit knee joint. This novel action of substance P is not mediated by NK_1 or NK_2 receptors, but involves histamine and prostaglandins. The degree of plasma extravasation elicited by substance P in the rabbit knee joint is small and short-lived, and with no concurrent oedema of the joint. These results suggest that substance P can evoke acute inflammatory responses in the rabbit knee joint, but on its own, it is unlikely to cause chronic joint inflammation in this species.

Keywords: Substance P; Vasodilatation; Plasma extravasation; Knee joint, Rabbit

1. Introduction

Peripheral branches of sensory nerves are known to have dual ''sensory-efferent'' function such that activation of these neurones will increase afferent nerve discharge centrally as well as causing release of neuropeptides locally (see Maggi and Meli, 1986). One of the neuropeptides contained in sensory neurones is substance P (Walsh et al., 1992; Grönblad et al., 1988; Konttinen et al., 1990), and this acts as a neurotransmitter for nociceptive pathways when it is released at central sites (Schaible et al., 1990; Neugebauer et al., 1995). On the other hand, substance P has been shown to be very widespread in the periphery (Maggi, 1995), and it is transported to the peripheral sensory neurones four times as much as that transported centrally (Keen et al., 1982). These findings indicate that it may have more important role(s) in the periphery, one of which is to mediate neurogenic inflammation; a phenomenon that describes antidromic stimulation of nociceptive neurones leads to vasodilatation and increased vascular permeability in the area innervated by the stimulated nerve (Jancso et al., 1967; Lembeck and Holzer, 1979).

Substance P is implicated as a mediator of neurogenic inflammation because it has been shown that electrical stimulation of nerves supplying the cat knee joint can evoke the release of this peptide from articular nerve fibres (Yaksh et al., 1988) and produce plasma extravasation into the synovial cavity (Ferrell and Russell, 1986). This neuro-

* Corresponding author. Tel.: +852-2609-6790; fax: +852-2603-5139.
E-mail address: francislam@cuhk.edu.hk (F.F.Y. Lam).

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genically induced plasma extravasation can be abolished by prior intra-articular administration of a substance P antagonist (Ferrell and Russell, 1986). In addition, it has been shown that exogenously administered substance P in rat knee joints can cause dilation of the joint blood vessels (Lam and Ferrell, 1993a,b) and increase their permeability (Scott et al., 1991, 1992). Moreover, in various experimental models of monoarthritis, rat knee joints pretreated with inflammatory agents were shown to have higher substance P-like immunoreactivity compared to those found in control knee joints (Bileviciute et al., 1993).

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 produce biological responses via interactions with three major receptor types: the tachykinin NK_1, NK_2, and NK_3 receptors. Substance P displays the highest affinity for the NK_1 receptors, but it can also act on the other receptor types (Burcher et al., 1991; Mussap et al., 1993). Tachykinin-induced plasma extravasation in rat skin (Andrew et al., 1989) and in rat knee joint (Lam and Ferrell, 1991; Lam and Wong, 1996; Ferrell et al., 1997) have been shown to be mediated predominantly by the NK_1 receptor subtype. On the other hand, both NK_1 and NK_2 receptors (especially in inflamed joints) could be involved in mediating tachykinin-induced vasorelaxation in the rat knee joint (Lam and Ferrell, 1993a; Lam and Wong, 1996).

The accumulated evidence suggests that substance P plays a part in joint inflammation. These evidences were obtained mostly in rats where substance P effects are attributed to the activation of predominantly NK_1 receptors. However, NK_1 receptors located in rats are not identical to those found in rabbits and human (Patacchini and Maggi, 1995), and thus, it is uncertain if these findings can be extrapolated to other species. The present study examines this question by investigating the actions of substance P on blood vessels of the rabbit knee joint.

2. Materials and methods

2.1. Induction of anaesthesia

Experiments were performed on adult New Zealand white rabbits (2.5–3.5 kg) bred at the Chinese University of Hong Kong. The animals were anaesthetised initially by intravenous injection of sodium pentobarbitone (40 mg/kg) via the marginal ear vein. After canulating the trachea, the animal was allowed to breathe spontaneously, or via an artificial respirator, when necessary. Another cannula was inserted into the ear vein and a slow continuous infusion of sodium pentobarbitone (0.6 mg/min) was administered throughout the remainder of the experiment. Deep anaesthesia was maintained throughout as judged by the absence of a flexor withdrawal reflex response to a pinch applied to the forelimb. All experiments were conducted in accordance with the Animal Research Ethics Committee, The Chinese University of Hong Kong.

2.2. 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 knee joint blood flow. Rabbits were anaesthetised as described above, and then the skin over the knee joint was removed to expose the anteromedial aspect of the joint capsule. A laser Doppler perfusion imager (Lisca Development, 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 6×7 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 0.1 ml. In experiments involving the use of the NK_1 receptor antagonist (N^2-[(4*R*)-4-hydroxy-1-(1-methyl-1*H*-indol-3-yl)carbonyl-L-prolyl]-*N*-methyl-*N*-phenylmethyl-3-(2-naphthyl)-L-alaninamide (FK888); Fujii et al., 1992) or the NK_2 receptor antagonist (*S*)-*N*-methyl-*N*-[4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)-butyl] benzamide (SR48968); Emonds-Alt et al., 1992), the joints were pretreated with a dose of the antagonist for 10 min prior to the control measurement. This was followed by co-administration of another dose of the antagonist together with substance P. For experiments involving pretreatment with histamine receptor antagonists or flurbiprofen, the interval between the two doses of antagonists was 15 min. For pretreatment with the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) or its enantiomer N^G-nitro-D-arginine methyl ester (D-NAME), the interval was 30 min.

2.3. Assessment of plasma extravasation

The method of Evans blue extravasation was employed to assess plasma extravasation responses. Rabbits were anaesthetised with 40 mg/kg sodium pentobarbitone via the marginal ear vein, followed by administration of 50 mg/kg Evans blue via the same route. Substance P (1 ml) was injected into synovial cavities of one knee and the same volume of saline was injected into the contralateral knee to provide an internal control. To provide controls for saline injections, a separate group of animals received 1 ml of saline in one knee and the other knee was left untreated. After 4 or 24 h, an overdose of anaesthetic was given and

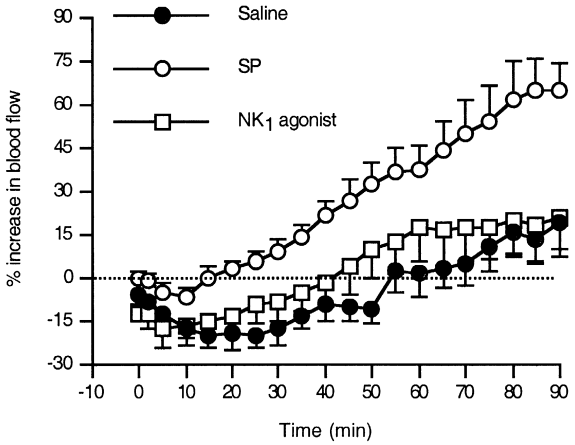

Fig. 1. Effects of substance P (SP) and the selective NK_1 receptor agonist [Sar^9, Met $(O_2)^{11}$]substance P on rabbit knee joint blood flow. Topical bolus application of substance P (1 nmol) onto the rabbit knee joint surface increased blood flow from 15 min onwards and peaked at 90 min with 46% increase compared to saline administration ($n = 7$ for each, $P < 0.001$; two-factor ANOVA). Application of [Sar^9, Met $(O_2)^{11}$]substance P (1 nmol) produced no change in the knee joint blood flow compared to the saline control ($n = 6$, $P > 0.05$ two-factor ANOVA). Data are shown as means ± S.E.M. (shown by vertical lines) of percentage change of blood flow from basal values that are normalised to zero.

the animals were exsanguinated.

The anterior and posterior portions of the knee joint capsule on both sides were dissected free from each animals. Tissue from each knee was pooled and weighed and Evans blue extracted as described by Lam and Ferrell (1989). This entailed cutting the capsules into smaller pieces and mixing them with acetone and 1% aqueous solution of sodium sulphate (in a ratio of 7:3) in a drug bottle. The bottle was capped and placed in an electrical shaker for 24 h at room temperature with continuous shaking. Each preparation was then centrifuged for 10 min at 200 rpm and the supernatant was separated. The amount of dye extracted was calculated by comparing the absorbance of the fluid obtained at 620nm (Pharmacia Biotech, Biochrom 4060) with that of a standard curve prepared with known concentrations of Evans blue solution. As Evans blue binds to plasma protein normally restricted to the vascular compartment, its presence in the capsule provides an index of altered vascular permeability.

2.4. Assessment of changes in knee joint sizes

At 4 or 24 h after intra-articular administration of substance P or saline, knee joint sizes of each animal were measured by placing a micrometer across the medial aspect of the knee joint.

2.5. Drug formulation

The following drugs were used: substance P, sodium pentobarbitone, L-NAME, D-NAME and pyrilamine dissolved in distilled water; flurbiprofen dissolved in absolute alcohol; FK888 and SR48968 dissolved in ethanol (15%); cimetidine dissolved in dimethyl sulphoxide (DMSO; 50%). The percentage of the solvent refers to that of the stock solution, which is 10 μM. Subsequent dilutions of the stock solution were made in 0.9% NaCl. All drugs were purchased from Sigma except FK888 and SR48968, which were gifts from Fujisawa Pharmaceutical (Japan) and Sanofi Recherche (France), respectively.

2.6. Statistical analysis

Results obtained from blood flow studies are expressed as means ± S.E.M. of percentage change of blood flow from basal values that are normalised to zero. Those from plasma extravasation experiments are expressed as means (in μg/g tissue) ± S.E.M. of the amount of Evans blue extravasated. Knee joint sizes are expressed as means ± S.E.M. of sizes measured in millimeters. Mean values were compared by paired or unpaired Student's *t*-test as appropriate. 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, USA). The latter procedure is very efficient for comparing a limited subset of possible contrasts. This is useful for testing hypothesis 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.

3. Results

3.1. Topical administration of neuropeptides

The mean basal blood flow determined in 58 rabbit knee joints was 3.25 ± 0.16 V. Single topical application of substance P (1 nmol) onto the exposed rabbit knee joint

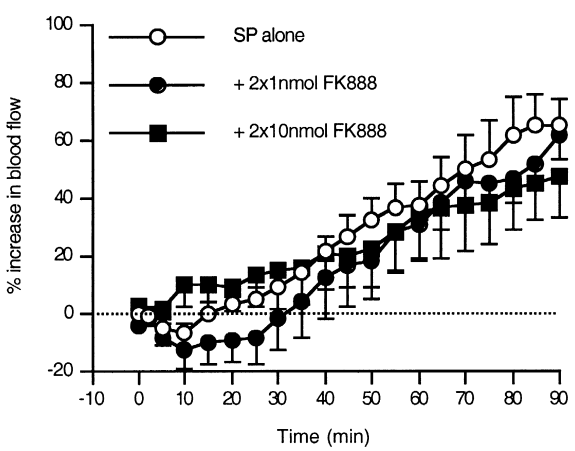

Fig. 2. Effects of the NK_1 receptor antagonist, FK888, on substance P-induced vasodilatation. Administrations of two 1 nmol ($n = 7$) or two 10 nmol ($n = 5$) FK888 (see Materials and methods) had no effect on the substance P-induced response ($n = 7$; $P > 0.05$ for both; two-factor ANOVA).

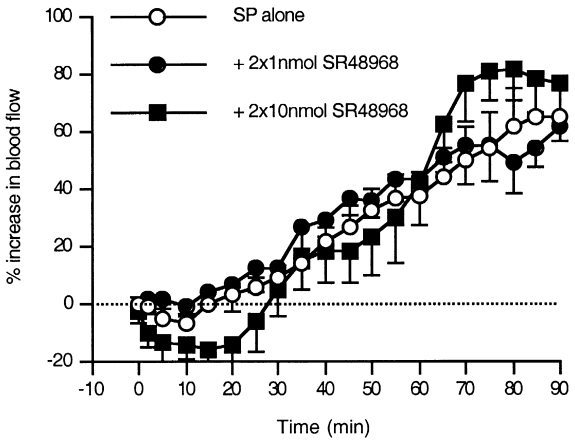

Fig. 3. Effects of the NK_2 receptor antagonist, SR48968, on substance P-induced vasodilatation. Administrations of two 1 nmol ($n = 5$) or two 10 nmol ($n = 4$) had no effect on the substance P-induced response ($n = 7$; $P > 0.05$ for both; two-factor ANOVA).

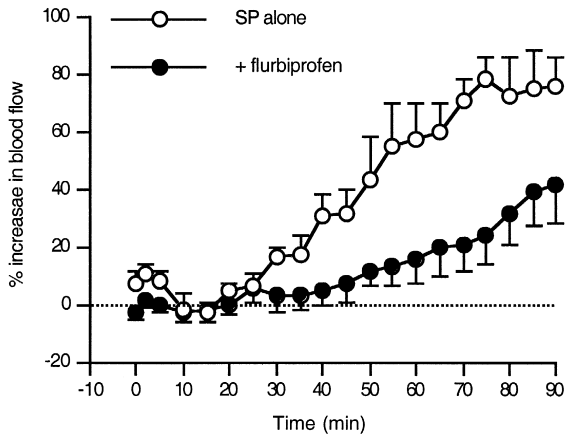

Fig. 5. Effects of the cyclo-oxygenase inhibitor flurbiprofen on substance P-induced vasodilatation. Administrations of two 10 nmol flurbiprofen produced significant inhibition on the substance P-induced response ($n = 7$ for both; $P < 0.05$; two-factor ANOVA).

capsule produced a gradually developing vasodilator response that is significant from 15 min onwards and peak at 90 min with 46% increase in blood flow compared to saline administration ($n = 7$ for each; $P < 0.001$). The same treatment with the selective NK_1 receptor agonist [Sar^9, Met $(O_2)^{11}$]substance P produced no change in the knee joint blood flow compared to the saline control ($n = 6$; $P > 0.05$). These results are shown in Fig. 1.

3.2. Topically-applied antagonists on substance P

Topical applications of two 1 nmol and two 10 nmol doses of either the selective NK_1 receptor antagonist FK888 ($n = 7$ and 5, respectively) (Fig. 2), or the selective NK_2 receptor antagonist SR48968 ($n = 5$ and 4, respectively) (Fig. 3), produced no change on the substance P-induced vasodilator response ($P > 0.05$ for all). On the other hand, applications of two 10 nmol doses of the selective H_1 receptor antagonist pyrilamine produced significant inhibition on the substance P-induced vasodilator response ($n = 5$; $P < 0.05$). Treatment with two 10 nmol doses of the selective H_2 receptor antagonist cimetidine had no effect on substance P until at 60 min and onwards where there were small reductions on the vasodilator responses ($n = 7$; $P < 0.05$–0.001) (Fig. 4).

Applications of two 10 nmol doses of the cyclo-oxygenase inhibitor flurbiprofen caused substantial inhibition on the substance P-induced vasodilator response ($n =$

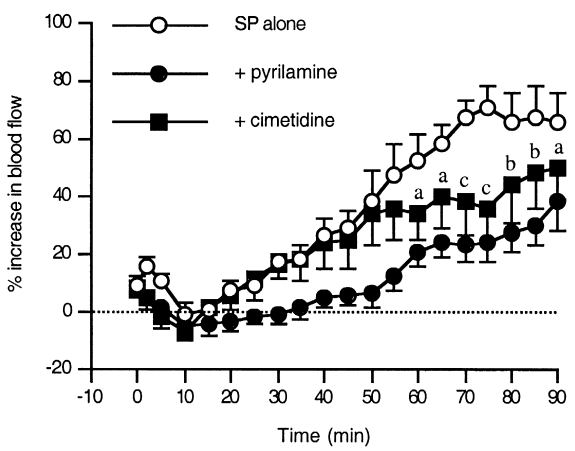

Fig. 4. Effects of the H_1 receptor antagonist, pyrilamine, and the H_2 receptor antagonist, cimetidine, on substance P-induced vasodilatation. Administrations of two 10 nmol pyrilamine produced significant inhibition on the substance P-induced response ($n = 5$ and $n = 7$, respectively; $P < 0.05$, two-factor ANOVA). Applications of two 10 nmol cimetidine ($n = 7$) had no effect on the substance P-induced response ($P > 0.05$; two-factor ANOVA). However, subsequent comparisons of means by planned contrasts showed significant difference between their means from 60 min onwards: $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$.

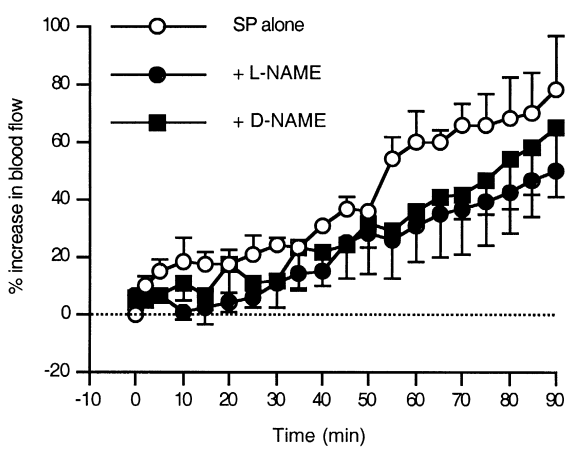

Fig. 6. Effects of the nitric oxide synthase inhibitor L-NAME, and its enantiomer D-NAME, on substance P-induced vasodilatation. Administrations of two 100 nmol L-NAME produced significant inhibition on the substance P-induced response ($n = 4$ for both; $P < 0.01$; two-factor ANOVA), whereas, two 100 nmol D-NAME had no effect ($n = 4$; $P > 0.05$; two-factor ANOVA). However, the curves of substance P in the presence of L-NAME and D-NAME are not significantly different ($P > 0.05$; two-factor ANOVA).

7; $P < 0.05$) (Fig. 5). A small but significant suppression ($n = 4$; $P < 0.01$) of the substance P-induced vasodilator response was also observed in joints treated with two 100 nmol doses of the nitric oxide synthase inhibitor L-NAME, but not in joints treated with two 100 nmol doses of its enantiomer D-NAME ($n = 4$; $P > 0.05$) (Fig. 6). However, the curves of substance P with L-NAME and substance P with D-NAME were not significantly different ($P > 0.05$) (Fig. 6).

Basal blood flow of the rabbit knee joint was not significantly affected by pretreatment of the above antagonists ($P > 0.05$ for all), except for L-NAME and pyrilamine which caused $40.07 \pm 3.83\%$ ($n = 5$; $P < 0.001$) and $24.62 \pm 7.38\%$ ($n = 4$; $P < 0.01$) reductions on the basal blood flow, respectively.

3.3. Intra-articular administration of substance P

The effects of unilateral intra-articular administrations of substance P (1 nmol) on basal blood flow, Evans blue extravasation, and knee joint sizes in the rabbit are shown in Fig. 7. Basal blood flow of substance P-injected knees were significantly higher than their respective contralateral saline-injected knees at 4 and 24 h post-injections ($n = 5$; $P < 0.05$ for all). For the latter time point, basal blood flow of the saline-injected knees in the substance P-treated animals were significantly higher than those of the saline-injected knees in the control animals that did not receive substance P ($P < 0.001$) (Fig. 7A). Substance P-injected knees produced greater Evans blue extravasation than their contralateral saline-injected knees at 4 h post-injection

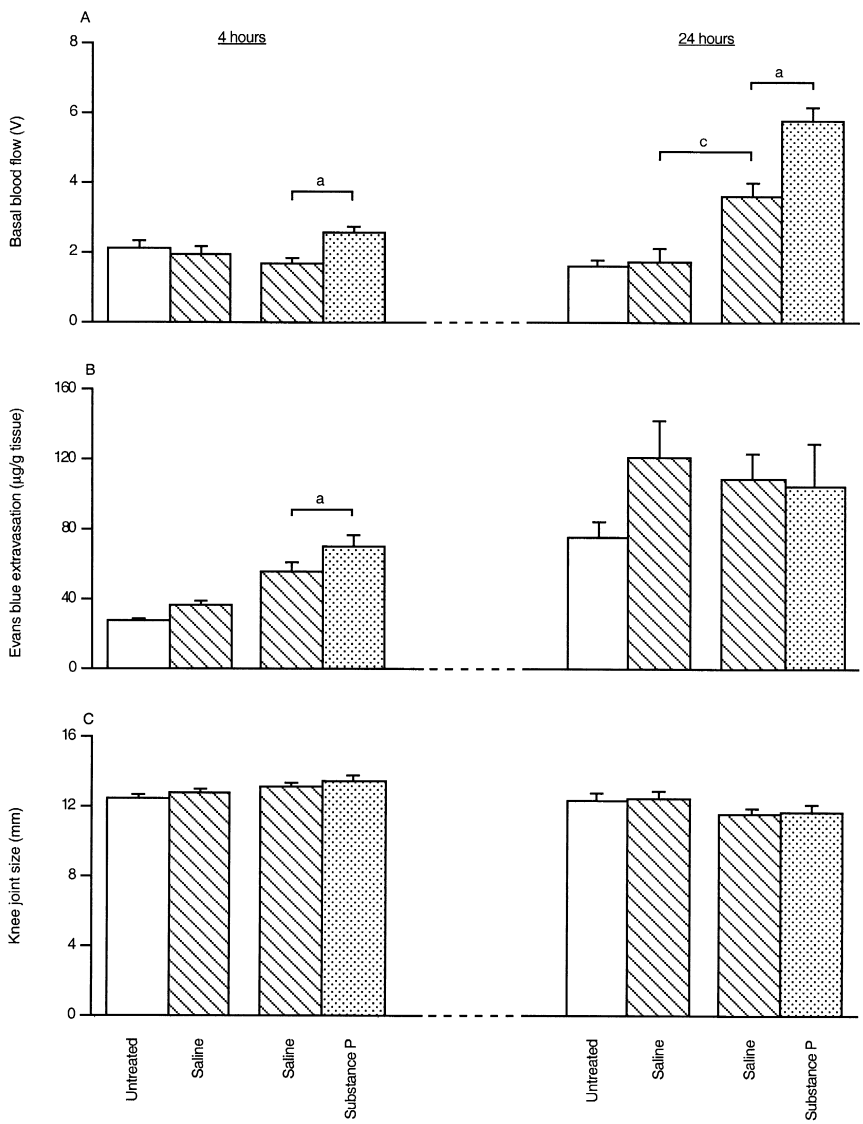

Fig. 7. Effects of intra-articular administrations of substance P (1 nmol) on (A) basal blood flow, (B) Evans blue extravasation, and (C) knee joint sizes in the rabbit. Basal blood flow of substance P-injected knees were significantly higher than their respective contralateral saline-injected knees at 4 and 24 h post-injections: $^{a}P < 0.05$ for both (Student's paired *t*-test). The amount of Evans blue extravasated in substance P-injected knees were greater than those of their contralateral saline-injected knees at 4 h post-injection: $^{a}P < 0.05$ (Student's paired *t*-test), but not at 24 h post-injection. Knee joint sizes were not significantly different in all treatment groups: $P > 0.05$ (Student's paired and unpaired *t*-test). $n = 5$ for all.

($n = 5$; $P < 0.05$), but not at 24 h ($n = 5$; $P > 0.05$) (Fig. 7B). When comparing knee joints that received the same treatments, Evans blue extravasation was always higher for the knee joints at 24 h than those at 4 h ($n = 4–5$; $P > 0.01–0.05$), except for the knee joints that received substance P which produced similar degree of Evans blue extravasation at both time points ($n = 5$; $P > 0.05$) (Fig. 7B). No significant change in knee joint sizes was detected in all treatment groups at the two time points. ($n = 5$; $P < 0.05$ for all) (Fig. 7C).

4. Discussion

The present study demonstrates that substance P has a unique vasodilator action in the rabbit knee joint that is slow in onset and long-lasting. Hitherto, substance P has not been shown to possess a vasodilator action of this pattern. Besides, the NK_1 receptor agonist [Sar^9, $Met(O_2)^{11}$]substance P lacks vasodilator action on the present preparation. The NK_1 and NK_2 receptor antagonists are ineffective in blocking the substance P-induced vasodilator response, which is attenuated by histamine antagonists and flurbiprofen. These findings suggest that the unique vasodilator action of substance P in the rabbit is not mediated by NK_1 or NK_2 receptors but involves the participation of histamine and prostaglandins. In addition, the degree of plasma extravasation elicited by substance P is small and short-lived, and there is no concurrent oedema of the joint. Taken together, these data suggest that substance P can evoke acute inflammatory responses in the rabbit knee joint, but it is unlikely to result in chronic inflammation of the joint when acting alone.

Substance P-induced vasodilatation has previously been shown to be fast in onset and short in duration. For example, in in vivo studies performed in rat knee joints (Lam and Ferrell, 1993a) and in rat skin (Ralevic et al., 1995), substance P produced immediate vasodilator responses that lasted no more than 10 min. Intravenously applied substance P also caused fast and transient vasodilator responses (less than 3 min) in various splanchnic vascular beds of the dog (Prokopiw and McDonald, 1994). Similarly, in isolated preparations of guinea pig (Hoover and Hossler, 1993) and human (Franco-Cereceda and Rudehill, 1989) coronary blood vessels, substance P-induced vasodilator responses were found to subside within 3 to 10 min. This fast and transient pattern of the substance P-induced vasodilator response suggests that it is appropriate for immediate and short-term regulation of blood flow.

In contrast to previous findings, the present study showed that topically applied substance P produced a unique vasodilator action on the rabbit knee joint that is slow in onset and persistent. Furthermore, when substance P was administered via the intra-articular route, a vasodilator response can be detected even at 24 h after administration of the drug. Clearly, the pattern of the vasorelaxant effect of substance P is not the same on knee joints of all species.

A simple speculation for the delayed vasodilator response to substance P is that the agonist requires some time to reach its site of action when applied topically onto the joint surface. However, this theory is opposed by the finding that substance P also lacked vasodilator action when continuously infused close intra-arterially into the rabbit knee joint (unpublished observation). Furthermore, our previous study showed that topically applied calcitonin gene-related peptide produced immediate vasodilatation in the rabbit knee joint, and yet, this neuropeptide is almost three times the size of substance P (Yip and Lam, 1995).

Another possibility for the delayed vasodilator response to substance P would be that the concurrent plasma extravasation effect of substance P had somehow affected the detection of blood flow changes in the rabbit knee joint. On the other hand, the finding that substance P elicited immediate vasodilator response alongside with plasma extravasation in the rat knee joint is in discordance with this view (Lam and Ferrell, 1990, 1993a). Moreover, the present study showed that in the rabbit knee joint, vasodilatation can be observed in parallel with plasma extravasation effect after intra-articular administration of substance P. Hence, it is unlikely that the plasma extravasation effect of substance P can influence blood flow measurement by the laser Doppler imager.

Interestingly, the present study showed that unilateral injection of substance P produced significant bilateral and sustained increase in blood flow at 24 h. This is in line with symmetrical joint involvement typically seen in rheumatoid arthritis patients, and which has been proposed to be due to the sensory innervation of these joints (Levine et al., 1984). Nonetheless, substance P had no effect on knee joint sizes of the rabbit at 4 and 24 h after intra-articular administrations of the drug. This implies that the hyperaemic action of substance P (together with its small plasma extravasation effect) does not provoke oedema in the joint. Therefore, it is suspected that substance P cannot elicit chronic inflammation in the rabbit knee joint, unless when the joint is already inflamed, then substance P may promote the accumulation of other inflammatory mediators and augment their actions in the affected joint, and thereby, it may perpetuate the inflammatory conditions.

Substance P-induced vasorelaxation has been attributed to the activation of NK_1 receptors in the endothelium of arterial vessels (Lam and Wong, 1996; Saito et al., 1990). Therefore, a selective NK_1 receptor agonist, [Sar^9 $Met(O_2)^{11}$]substance P, was tested in the present study. Surprisingly, this NK_1 agonist did not affect blood flow of the rabbit knee joint in spite of the dose of the agonist used was 10 times that required to elicit maximum vasodilatation in the rat knee joint (Lam and Wong, 1996). Two tachykinin receptor antagonists were used to further clarify the involvement of tachykinin receptors in mediating the substance P-induced response: FK888, a peptide-based

selective NK_1 receptor antagonist (Fujii et al., 1992), and SR48968, the first selective nonpeptide NK_2 receptor antagonist synthesized (Emond-Alts et al., 1992). The results showed that applied topically, neither FK888 nor SR48968 had an effect on the substance P-induced vasodilator response. Thus, the present study does not support a role for NK_1 or NK_2 receptors in mediating the substance P-induced response.

Taking into consideration that: (1) FK888 and SR48968 applied by the same protocol and at a dose 10 times lower than that of the maximum dosage used in the present study substantially inhibited tachykinin-induced vasodilatation in the rat knee joint (Lam and Wong, 1996); (2) the affinity of FK888 for rat NK_1 receptors is 320 times less than that for the human NK_1 receptors (Aramori et al., 1994), and that tachykinin receptors are known to be similar in man and rabbit (Coge and Regoli, 1994); and (3) FK888 and SR48968 have been shown to possess potent antagonist activities on rabbit vascular tissues with pA_2 values of 9.07 and 9.6, respectively (Coge and Regoli, 1994), the lack of inhibitory action of these antagonists in the present study cannot be ascribed to the method of drug administration or to the dosages used. Nevertheless, Khoshbaten and Ferrell (1993) reported that a substance P antagonist [D-Pro^4 D-$Trp^{7,9,10}$]substance P-(4–11) can substantially reduce neurogenic vasodilatation in the rabbit knee joint which implies tachykinin receptor involvement in the vasodilator response. However, 500 μg of the antagonist was used in their study, whereas in another study in the rat knee joint, 10 μg of the same antagonist was found to be adequate in blocking the substance P-induced response (Lam and Ferrell, 1989). At 500 μg, it is possible that the antagonist is producing vasoconstriction independent of tachykinin receptor inhibition (Cox et al., 1988), which accounts for the discrepancy of their result to the present findings.

Although the vasorelaxant effect of substance P in the rabbit knee joint does not require activation of tachykinin receptors, it should not be interpreted as a lack of involvement of the vascular endothelium in the response. In the present study, L-NAME and D-NAME were used to investigate the requirement of the endothelium-derived vasorelaxant factor (EDRF; nitric oxide) in the substance P-induced response. L-NAME is capable of blocking nitric oxide synthesis by inhibiting nitric oxide synthase; its enantiomer D-NAME is much less effective for this action (Rees et al., 1990). Accordingly, the present study showed that L-NAME produced a small but significant inhibition on the substance P-induced vasodilator response, but D-NAME did not. However, it should not be overlooked that L-NAME produced marked vasoconstriction on its own, and this may have, to some extent, counteracted the vasodilator response to substance P. Furthermore, statistical analysis of the curves of substance P in the presence of L-NAME and D-NAME showed that they are not significantly different from each other. Therefore, the small suppression of substance P-induced response by L-NAME is unlikely to be representative of nitric oxide involvement. On the other hand, endothelium-derived vasodilator prostaglandins may be the alternative candidates for mediating the substance P-induced vasodilator response. This is because flurbiprofen, an inhibitor of cyclo-oxygenase enzymes that catalyse the production of prostaglandins, produced substantial inhibition of the substance P-induced vasodilator response in the present study. Other possible sources for local production of prostaglandins may include the pericytes and smooth muscle cells within the microvasculature, as well as interstitial cells (e.g. fibroblasts, mast cells) and various cells in the vascular compartment (e.g. leukocytes, platelets) (see Gerritsen, 1996; Lotz et al., 1987).

One of the factors that has been attributed to the development of joint inflammation elicited by substance P in the rat knee joint is the release of histamine from mast cells by substance P (Lam and Ferrell, 1990). The presence of mast cells in the rabbit knee joint is uncertain. Nevertheless, substance P and compound 48/80 have been reported to cause histamine release from mast cells in the rabbit lung (Nemmar et al., 1999a,b). More importantly, the present study showed that histamine H_1 and H_2 receptor antagonists were effective in reducing the substance P-induced vasodilator response, thus, providing indirect evidence that mast cells are present in the rabbit knee joint. The release of histamine from mast cells by substance P has been suggested to be mediated via a receptor-independent mechanism that involves direct interaction with G proteins (Mousli et al., 1990). This is in line with the present findings that showed substance P-induced vasodilatation in the rabbit knee joint does not require the activation of tachykinin receptors, but involved the release of histamine from mast cells.

In summary, the present study reports a novel, gradual, and persistent vasodilator action of substance P on blood vessels of the rabbit knee joint. The mechanism of this vasodilator action is also unique because it does not require the activation of tachykinin NK_1 or NK_2 receptors, and involves the participation of histamine and prostaglandins. Substance P also elicited a small degree of plasma extravasation in the rabbit knee joint, but it did not cause oedema of the joint. Taken together, these results suggest that substance P can evoke acute inflammatory responses in the rabbit knee joint, but on its own, it would not yield chronic inflammation.

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