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Effects of interactions of naturally-occurring neuropeptides on blood flow in the rat knee joint

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1 Changes of blood flow in the rat knee joint, measured by laser Doppler flowmetry, were produced by topical application of naturally-occurring neuropeptides to the joint capsule.

2 Substance P (SP), neurokinin A (NKA), and neurokinin B (NKB) all produced dose-dependent transient vasodilatation of the rat knee joint microvasculature. NKB showed significantly smaller vasodilator responses compared to SP and NKA which were similar in their potencies.

3 Calcitonin gene-related peptide (CGRP) produced dose-dependent vasodilatation which was more pronounced than that produced by the neurokinins. The rank order of potency was: CGRP>SP = NKA>NKB. The vasodilator effect of CGRP was also more prolonged and this extended phase was abolished by co-administration of SP.

4 Cross-tachyphylaxis was not observed with the different neurokinins, but SP and NKA showed novel antagonistic effects on NKB-induced vasodilatation.

5 Co-administration of 1 nmol of the specific NK_1 receptor antagonist, CP-96345, with 1 nmol of each of the neurokinins produced significant inhibition of the vasodilator response to SP but did not affect vasodilator responses to NKA and NKB. Co-administration of CP-96345 with the neurokinins plus superfusion of the rat knee joint with a solution containing 0.1 mM CP-96345 further reduced the vasodilator responses to SP but again the vasodilator responses to NKA and NKB were not significanly altered.

6 The results suggest that multiple neurokinin receptor types may be present in the rat knee joint which could mediate the vasodilator responses of the different neurokinins. Co-release of neuropeptides from sensory nerve endings in the rat knee joint may have inter-regulatory actions on their individual responses on the microvasculature.

Keywords: Neuropeptides; vasodilatation; neurokinins; substance P; neurokinin A; neurokinin B; calcitonin gene-related peptide; joint inflammation; CP-96345

Introduction

Substance P (SP) is a neuropeptide localized in central and peripheral terminals of primary sensory neurones (Hokfelt *et al.*, 1975) and has been considered as the putative transmitter (Otsuka & Konishi, 1976) involved in the mediation of pain (Randic & Miletic, 1977). When administered into the synovial cavity of rat knee, SP produced marked plasma extravasation (Lam & Ferrell, 1989a,b) and when applied on exposed rat knee joint blood vessels, SP produced transient but pronounced vasodilatation (Grice *et al.*, 1990). Antidromic electrical stimulation of articular C-fibres has been shown to produce plasma extravasation into the synovial cavity of the cat knee (Ferrell & Russell, 1986). Prior intra-articular administration of the substance P antagonist, D-Pro^4, D-Trp^{7,9,10} SP $_{(4-11)}$ totally inhibited this response (Ferrell & Russell, 1986). Thus, the available evidence suggests that apart from a neurotransmitter role in conveying nociceptive information from the periphery to the central nervous system, SP may also have an important role in mediating neurogenic inflammation.

In recent years a number of investigators have suggested the possibility of multiple receptor types for tachykinins (Falconeri-Erspamer *et al.*, 1980; Lee *et al.*, 1982). Within a relatively short period since then, two new mammalian tachykinins sharing similar structures to SP, neurokinin A (NKA) and neurokinin B (NBK) were isolated, purified and sequenced (Kangawa *et al.*, 1983; Kimura *et al.*, 1983). The discovery of these tachykinins, or more appropriately called neurokinins since they are synthesized and stored in nervous structures and act as neurotransmitters, permitted the identification of neurokinin receptor types according to their respective endogenous ligands. At present, three distinct neurokinin receptor types in various mammalian tissues have been confirmed (Beaujouan *et al.*, 1984; Buck *et al.*, 1984; Regoli *et al.*, 1985; 1987a,b); the neurokinin-1 (NK_1), neurokinin-2 (NK_2), and neurokinin-3 (NK_3) receptors, at which SP, NKA, and NKB are the preferential endogenous agonists, respectively. Plasma extravasation in rat skin (Andrews *et al.*, 1989) and in the rat knee joint (Lam & Ferrell, 1991) has been shown to be mediated by the NK_1 receptor subtype.

Sensory neurones commonly contain more than one type of neuropeptide, with SP and calcitonin gene-related (CGRP) often co-localized in many types of nociceptive afferent fibres (Fischer *et al.*, 1985). CGRP has been shown to be a potent vasodilator in several species including man (Brain *et al.*, 1985). In the rabbit, it can potentiate oedema induced by other mediators of increased microvascular permeability such as platelet-activating factor (PAF), C5a des Arg, N-formyl-methionyl-leucyl-phenylalanine (FMLP) and leukotriene B_4 (LTB_4) (Brain & Williams, 1985). In rat skin, CGRP can also potentiate SP-induced oedema (Brain & Williams, 1985). Thus, the effect of neuropeptides on microvascular tone could have an important influence on the final manifestations of inflammatory processes. The present study investigates the effects of naturally-occurring neuropeptides on blood flow in the rat knee joint. Possible interactions of these neuropeptides were also studied.

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Methods

Experiments were performed on male Wistar rats (300–400 g) deeply anaesthetized by intraperitoneal injection of a mixture of urethane (470 mg kg^{-1}) and diazepam (1 mg kg^{-1}). Blood pressure was monitored continuously via a cannula inserted into the carotid artery. Relative changes in blood flow were monitored by laser Doppler flowmetry (LDF) using a single channel flowmeter (Moor Instruments MBF3) with an near infra-red laser (720 nm) and recorded with a time constant of 3 s. LDF is based on the principle that coherent light scattered by moving erythrocytes experiences a frequency (Doppler) shift that is proportional to the velocity of erythrocytes flowing through the volume of tissue illuminated by the laser radiation (Nilsson *et al.*, 1980a,b). Although this technique provides a continuous and non-invasive signal related to blood flow, it cannot measure absolute flow but can be usefully employed to assess relative changes in blood flow.

The anterior region of the knee was exposed and a fibre-optic probe containing two fibres (each of diameter 100 μm; one fibre emitting laser radiation whilst the other collects the backscattered photons) was placed just above the surface which was continuously superfused with physiological saline at a rate of 0.1 ml min^{-1} by a peristaltic pump (Watson-Marlow 101U). The tip of the probe was placed 1–2 mm central to the medial edge of the patellar ligament (Figure 1), over an area of joint capsule which we have confirmed histologically to consist only of synovial tissues. It has been shown that a laser Doppler flowmeter using a near infra-red laser source samples a larger volume of tissue than an instrument using a shorter wavelength laser (Obeid *et al.*, 1990). In preliminary experiments we have shown that the instrument used in the present study is able to sample blood flow in deeper synovial vessels as vasodilatation is still detected with flowmeter probe placed over the exposed surface of the capsule when the synovial cavity is perfused with CGRP.

Drugs were administered as a bolus applied to the surface in a volume of 0.1 ml. Data are presented as maximum change in the laser flux signal from the preceding basal level. The basel reading may vary in different animals depending on probe position, thus necessitating expression of the vasodilator response as a percentage change from the control level which was normalised to 0%. Analysis of Variance (ANOVA) was used to analyse differences between dose-response curves. Differences were considered significant if the P values were 5% or less. Values in the histograms represent means ± s.e.mean. An unpaired one-tailed t test was used to analyse the difference between means.

Human α-CGRP, SP, and CP-96345 ((2S,3S)-cis-2-(diphenylmethyl)-N-((2-methoxyphenyl)methyl)-1-azabicyclo[2.2.2]oct-an-3-amine) were kindly donated by Celltech Ltd (Berkshire), ICI Pharmaceuticals, and Pfizer Ltd, respectively. All other drugs were purchased from Cambridge Research Biochemicals Ltd. Neurokinin B was dissolved in 10% ammonium hydroxide and subsequent dilutions were made in physiological saline. In all other cases physiological saline was used as the solvent.

Results

Vasodilatation of rat knee joint blood vessels induced by naturally-occurring neuropeptides

The four neuropeptides SP, NKA, NKB, and CGRP all produced an increase in the basal laser flux signal, indicating that they were all effective in eliciting vasodilatation of rat knee joint blood vessels. With the exception of NKB, the neuropeptides produced a drop in blood pressure when applied to the knee joint at doses above 1 nmol. Thus, higher doses were not used as the hypotension they induce would tend to offset their vasodilator effects. With the doses used in the present study, blood pressure was found to remain constant throughout the experiment as long as the animal remained deeply anaesthetized. The vasodilator effects of these neuropeptides were tachyphylactic as was observed in our previous study on specific neurokinin receptor agonists (Grice *et al.*, 1990). Thus, dose-response curves to the neurokinins were achieved by allowing 15 min to elapse between each drug application to avoid tachyphylaxis. Figure 2 shows that all the neurokinins were effective in producing dose-dependent vasodilatation in the rat knee joint. The maximum vasodilator responses achieved at 1 nmol were 55.8 ± 3.4%, 68.5 ± 10.2%, and 47.6 ± 5.6% increase from basal perfusion levels for SP, NKA and NKB, respectively. Statistical ana-

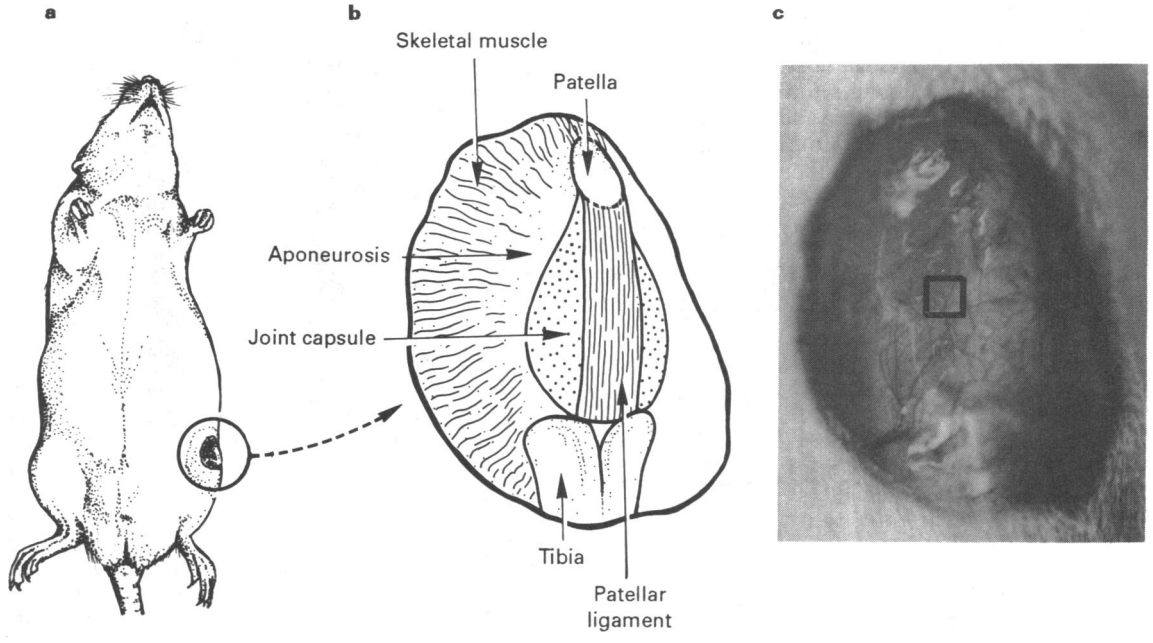

Figure 1 Photographic illustration of an exposed rat knee prepared for assessment of knee joint perfusion by a laser Doppler flowmeter (LDF). (a) Drawing of a rat indicating the exposed medial aspect of the knee joint which is photographically illustrated in (c). The principal anatomical features are shown diagrammatically in (b). The black rectangle in (c) outlines areas which were suitable for LDF probe placement.

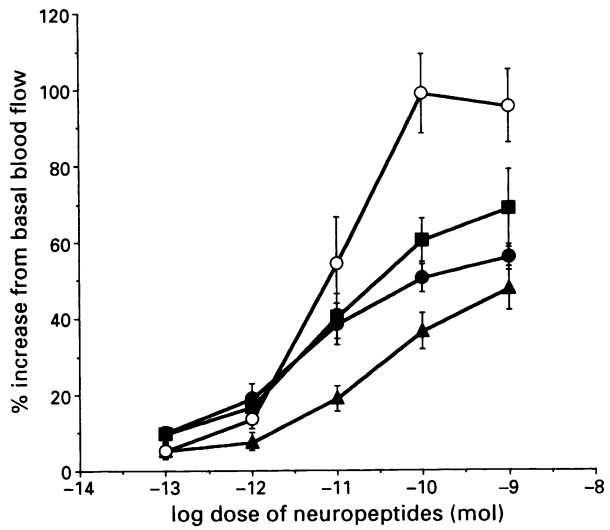

Figure 2 Effect of calcitonin gene-related peptide (○, $n = 7$), substance P (●, $n = 7$), neurokinin A (■, $n = 7$), and neurokinin B (▲, $n = 7$) on articular blood flow of the rat knee joint capsule. Drugs were administered as a bolus applied to the rat knee joint surface in a volume of 0.1 ml. Data are shown as means ± s.e.mean (shown by vertical bars) expressed as a percentage change from control (normalised to 0%).

lysis showed no difference between the SP and NKA dose-response curves ($P = 0.394$), whereas the dose-response curve to NKB differed significantly from that of SP ($P = 0.005$) and NKA ($P = 0.003$) indicating the lesser potency of this agent.

Application of CGRP to the rat knee joint produced substantially greater vasodilator responses than those induced by the neurokinins. CGRP produced a maximum vasodilatation of 95.3 ± 9.5% at 1 nmol and its dose-response curve was significantly different from those of SP ($P = 0.013$) and NKB ($P = 0.0005$), but not significantly different from that of NKA ($P = 0.157$). However, there were significant differences when their responses at the two highest concentrations were compared ($P = 0.034$ at 0.1 nmol and $P = 0.040$ at 1 nmol). The characteristic vasodilator response to CGRP was also unique as it took longer to peak (92 ± 9.2 s; $n = 7$) and often remained visible more than 30 min after administration, whereas the neurokinins showed a transient vasodilator effect with a peak at 40 ± 5.35 s ($n = 7$) and returned to the control level in 111.43 ± 8.07 s ($n = 7$) after administration. The durations of the vasodilator responses to SP and CGRP are shown in Figure 3.

Effects of different combinations of neurokinins

Possible interactions of the neurokinins on rat knee joint blood flow were studied by investigating the effects of the different agents after an initial dose of another neurokinin. As illustrated in Figure 4, the vasodilator effect of 1 nmol SP (60.5 ± 7.0%) was not significantly different 5 min after pretreatment with 1 nmol NKA (51.7 ± 7.9%) or NKB (57.6 ± 7.4%). Similarly, the vasodilator effect of NKA (49.4 ± 6.8%) at the same concentration, was essentially the same after pretreatment with SP (56.5 ± 6.3%) or NKB (47.1 ± 2.5%). However, the vasodilator effect of 1 nmol NKB (48.5 ± 4.2%) was substantially reduced after pretreatment with SP (6.1 ± 2.9%; $P < 0.001$) or NKA (5.5 ± 1.9%; $P < 0.001$).

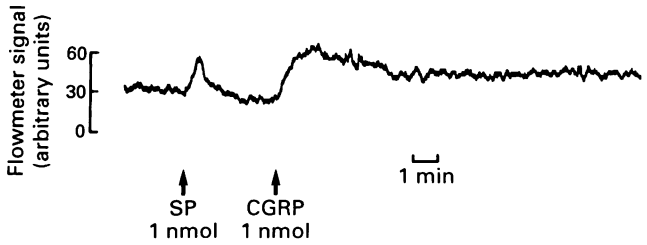

Figure 3 Trace showing the effect on the laser Doppler flux signal of topical application (0.1 ml) of substance P (SP) and calcitonin gene-related peptide (CGRP) to the rat knee joint capsule.

Effects of a specific NK_1 receptor antagonist on neurokinin-induced vasodilatation

The effects of a non-peptide specific NK_1 antagonist, CP-96345 (Snider *et al.*, 1991), on the vasodilator responses to the different neurokinins are shown in Figure 5. Co-administration of 1 nmol CP-96345 with 1 nmol of the neurokinins resulted in a reduction of the vasodilator response to SP, but the responses to NKA and NKB were not affected. Co-administration of 1 nmol CP-96345 and 1 nmol neurokinins plus superfusion of the rat knee joint with a solution containing 0.1 mM of CP-96345 produced a further reduction of the vasodilator response to SP but the vasodilator responses to NKA and NKB were again unaffected when compared statistically to the control response. Control experiments with CP-96345 alone showed no alteration of the basal blood flow in the presence of the antagonist.

Effects of SP on CGRP-induced vasodilatation

Application of 1 nmol CGRP to the rat knee joint produced a long lasting vasodilator response with 51.1 ± 10.6% of the maximum response still persisting 30 min after drug adminis-

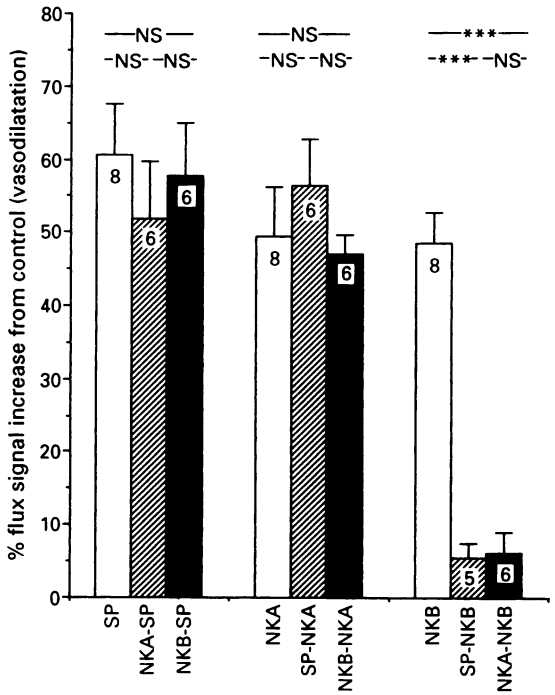

Figure 4 Effects of substance P (SP), neurokinin A (NKA), and neurokinin B (NKB) alone, and after pretreatment with either substance P (SP-), neurokinin A (NKA-), or neurokinin B (NKB-) on the laser Doppler signal obtained from the rat knee joint. All drugs were adminstered as a bolus of 1 nmol applied to the rat knee joint surface in a volume of 0.1 ml. In experiments where two applications of neurokinins were applied, a 5 min interval was allowed between the applications. Data are shown as means ± s.e.mean (vertical bars). NS = no significant difference; *** = $P < 0.001$.

tration. However, when the same dose of CGRP was co-administered with 1 nmol SP, the duration of the vasodilator response was substantially reduced. Only 11.6 ± 5.6% of the maximum response was observed 5 min after co-administration. The vasodilator response was no longer visible after 30 min. These results are shown in Figure 6.

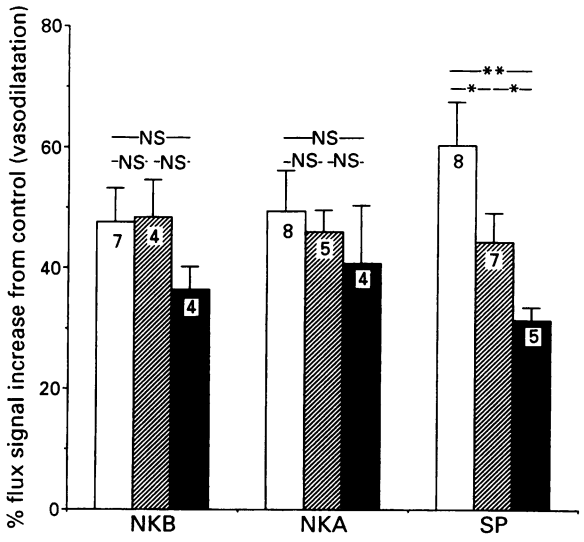

Figure 5 Effects of the specific NK_1 receptor antagonist, CP-96345, on vasodilator responses to the neurokinins NKB, NKA, and substance P (SP). The open columns illustrate control response to 1 nmol doses of the neurokinins; cross-hatched columns illustate co-administration of 1 nmol CP-96345 with 1 nmol neurokinins applied directly onto the rat knee joint in a volume of 0.1 ml; solid columns illustrate co-administration of the antagonist with neurokinins plus superfusion of the rat knee joint with 0.1 mM CP-96345. Data are shown as means ± s.e.mean (vertical bars). NS = no significant difference; * = $P<0.05$; ** = $P<0.01$.

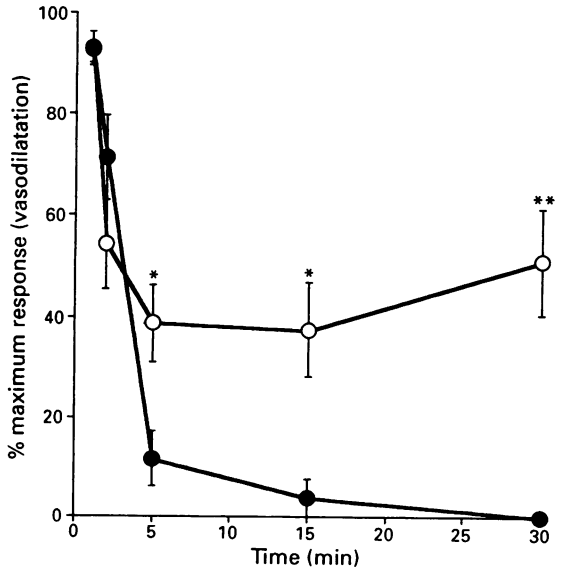

Figure 6 Effects of substance P (SP) on the time course of calcitonin gene-related peptide (CGRP)-induced vasodilatation in the rat knee joint. The open symbols (○; $n = 14$) represent the time course of the vasodilator response to 1 nmol CGRP applied as a bolus onto the rat knee joint surface in a 0.1 ml volume. The closed symbols (●; $n = 10$) illustrate the time course of the vasodilator response with co-administration of 1 nmol CGRP and 1 nmol SP to the rat knee joint. Data are shown as percentage of the maximum response (means ± s.e.mean, vertical bars). *$P<0.05$; **$P<0.01$.

Discussion

Laser-Doppler flowmetry is a convenient and simple way to measure continuously changes in microvascular blood flow. The technique does not measure blood flow in units of velocity but rather measures relative blood flow changes. It has mainly been employed for the measurement of blood flow changes in skin (Nilsson *et al.*, 1980a,b; Andrews & Helme, 1989; Hornyak *et al.*, 1990). The present study extends this method for the measurements of microvascular blood flow changes in the rat knee joint.

The results show that naturally-occurring neuropeptides can significantly influence rat knee joint microvascular tone. All the neuropeptides tested in the present study were effective vasodilators. Andrews & Helme (1989) have shown that the neurokinins were equipotent in producing vasodilator responses in rat skin and the duration of these responses was no more than 5 min. Our results confirmed the transient nature of neurokinin-induced vasodilatation but showed NKB to be less potent than SP and NKA which were similar in their responses in the rat knee joint. Although plasma extravasation in the rat knee joint has been shown to be mediated predominately by the NK_1 receptor subtype (Lam & Ferrell, 1991), the present study shows that the specific NK_1 receptor antagonist, CP-96345, selectively inhibited SP-induced vasodilatation whilst leaving the vasodilator responses to NKA and NKB unaltered. This suggests that unlike plasma extravasation, blood flow changes in the rat knee joint could be mediated by multiple neurokinin receptor types.

It has been shown that CP-96345 has higher affinity for NK_1 receptors found in man, guinea-pig and rabbit, than for NK_1 receptors found in rat, mouse, and chicken (Gitter *et al.*, 1991). The fact that CP-96345 produced incomplete inhibition of the SP response even at high concentrations (0.1 mM) in the present studies may reflect this low affinity characteristic of CP-96345 in rats. Alternatively, the incomplete inhibition by CP-96345 could suggest only partial involvement of NK_1 receptors in mediating the vasodilator response to SP. The use of higher concentrations of CP-96345 to achieve total blockade of the SP responses was prevented due to possible non-specific effects of CP-96345 at higher concentrations. Recently, a new non-peptide NK_1 receptor antagonist (RP-67580) has been developed and this has been shown to possess higher affinity than CP-96345 in rats and mice (Fardin *et al.*, 1992; Boyce *et al.*, 1992). Further experiments with RP-67580 may provide evidence to support or discard the possibilities of total or partial involvement of NK_1 receptors in mediating the SP vasodilator response.

Our experiments on combination effects of the different neurokinins provided further evidence for the involvement of multiple receptor types. The results show that the vasodilator response to SP was not affected by pretreatment with NKA, or NKB. Neither was the vasodilator effect produced by NKA affected by pretreatment with SP, or NKB. Had the vasodilator effects of the different neurokinins been mediated by the same receptor type, cross-tachyphylaxis would have been observed as was found to be the case in our previous study between SP and the specific NK_1 receptor agonist, [Sar^9, $Met(O_2)^{11}$]-substance P (Grice *et al.*, 1990). There is speculation that the equipotency of these neurokinins in producing vasodilatation in rat skin might indicate the existence of a fourth neurokinin receptor subtype (Andrews & Helme, 1989), but there is limited evidence in support of this view. Furthermore, the neurokinins do not appear to be equipotent in the rat knee joint. It seems more reasonable at this stage to assume that there are multiple neurokinin receptor types mediating the vasodilator responses of neurokinins in the rat knee joint.

An interesting finding was that although NKB did not show cross-tachyphylaxis on the vasodilator responses to SP or NKA, pretreatment of the rat knee joint with SP or NKA

resulted in potent inhibition of the vasodilator response to NKB. As this inhibition of the NKB response is unlikely to have resulted from cross-tachyphylaxis, this suggests that SP and NKA could have novel antagonistic effects on the NK_3 receptor subtype. Furthermore, it is interesting to note that the inhibitory effect was observed 5 min after application of SP or NKA when their vasodilator effects had subsided. In future studies it would be worthwhile to repeat these experiments with longer time intervals to determine the duration of the inhibition. The significance of this novel antagonistic action, and whether the antagonism is specific to joint blood vessels or occurs in other microvascular beds, remains to be elucidated.

The vasodilator effect of CGRP on the rat knee was found to be more potent and longer in duration than those produced by the neurokinins. The long lasting effect of CGRP was also observed in rabbit (Brain & Williams, 1985), rat (Brain & Williams, 1989), and human skin (Brain *et al.*, 1985). CGRP has also been shown to be a more potent vasodilator than SP or NKA in the human (Franco-Cereceda & Rudehill, 1989) and pig (Franco-Cereceda *et al.*, 1987) precontracted coronary artery. In the rat skin, it has been shown that co-administration of SP with CGRP abolished the prolonged vasodilator effect of CGRP (Brain & Williams, 1989). This was attributed to the SP-induced release of proteases from skin mast cells which degrade CGRP and thus terminated its vasodilator activity. Our results are in support of this view as co-administration of SP with CGRP also inhibited the prolonged vasodilator effects of CGRP in the rat knee joint. Nerves containing SP and CGRP have been identified in the rat synovium (Hukkanen *et al.*, 1991). These findings may suggest that SP has a regulatory role on the vasodilator responses of CGRP, especially as the two neuropeptides may be co-released from the same nerve endings in the rat synovium.

In conclusion, the present study has shown that blood flow measurements can be made in rat knee joint by laser Doppler flowmetry. All four naturally-occuring neuropeptides tested were effective vasodilators on the rat knee joint and showed a rank order of potency of CGRP > SP = NKA > NKB. The lack of cross-tachyphylaxis by the different neurokinins and more importantly, the selective inhibition of CP-96345 on SP-induced vasodilatation suggest different neurokinin receptor types may be involved in mediating vasodilator responses to neurokinins. SP may be a possible regulator of the vasodilator actions of CGRP in the rat knee joint. The present study also indicates a possible novel antagonistic activity of SP and NKA on NKB-induced vasodilatation.

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References

ANDREWS, P.V. & HELME, R.D. (1989). Tachykinin-induced vasodilatation in rat skin measured with a laser-Doppler flowmeter: evidence for receptor-mediated effects. *Regul. Pept.*, **25**, 267–275.

ANDREWS, 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.

BEAUJOUAN, J.L., TORRENS, Y., VIGER, A. & GLOWINSKI, J. (1984). A new type of tachykinin binding site in the rat brain characterised by specific binding of a labelled eledoisin derivative. *Mol. Pharmacol.*, **26**, 248–254.

BOYCE, S., RUPNIAK, N.M.J., YOUNG, L., RYCROFT, W., BRISTOW, L., IVERSON, S.D. & HILL, R.G. (1992). Antinociceptive effects of racemic RP-67580, but not racemic CP-96345, on acetic acid-induced writhing and formalin paw in mice. *Neuropeptides*, **22**, 10.

BRAIN, S.D. & WILLIAMS, T.J. (1985). Inflammatory oedema induced by synergism between calcitonin gene-related peptide (CGRP) and mediators of increased vascular permeability. *Br. J. Pharmacol.*, **86**, 655–660.

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

BRAIN, S.D., WILLIAMS, T.J., TIPPINS, J.R., MORRIS, H.R. & MACINTYRE, I. (1985). Calcitonin gene-related peptide is a potent vasodilator. *Nature*, **313**, 54–56.

BUCK, S.H., BURCHER, E., SCHULTZ, C.W., LOVENBERG, W. & O'DONAHUE, T.L. (1984). Novel pharmacology of substance K-binding sites: a third type of tachykinin receptor. *Science*, **226**, 987–989.

FALCONERI-ERSPAMER, G., ERSPAMER, V. & PICCINELLI, D. (1980). Parallel bioassay of physalaemin and kassinin, a tachykinin dodecapeptide from the skin of the African frog *Kassina senegalensis*. *Naunyn-Schmeideb. Arch. Pharmacol.*, **311**, 61–65.

FARDIN, V., FLAMAND, O., FOUCAULT, F., JOLLY, A., BOCK, M.D. & GARRETT, C. (1992). RP 67580, a non-peptide substance P antagonist: comparison with other antagonists and affinities in different animal species. *Neuropeptides*, **22**, 22.

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

FISCHER, J., FORSSMAN, W.G., HOKFELT, T., LUNDBERG, T.M., REINECKE, M., TSCHOPP, F.A. & WIESENFELD-HALLIN, Z. (1985). Immunoreactive calcitonin gene-related peptide and substance P: co-existence in sensory neurones and behavioural interactions after intrathecal administration in the rat. *J. Physiol.*, **362**, 29P.

FRANCO-CERECEDA, A. & RUDEHILL, A. (1989). Capsaicin-induced vasodilatation of human coronary arteries in vitro is mediated by calcitonin gene-related peptide rather than substance P or neurokinin A. *Acta Physiol. Scand.*, **136**, 575–580.

FRANCO-CERECEDA, A., RUDEHILL, A. & LUNDBERG, J.M. (1987). Calcitonin gene-related peptide but not substance P mimics capsaicin-induced coronary vasodilatation in pig. *Eur. J. Pharmacol.*, **142**, 235–243.

GITTER, B.D., WATERS, D.C., BRUNS, R.F., MASON, N.R., NIXON, J.A. & HOWBERT, J.J. (1991). Species differences in affinities of non-peptide antagonists for substance P receptors. *Eur. J. Pharmacol.*, **197**, 237–238.

GRICE, C.A., LAM, F.Y. & FERRELL, W.R. (1990). Response of knee joint blood vessels to neurokinin receptor agonists in anaesthetised rats. *J. Physiol.*, **429**, 75P.

HOKFELT, T., KELLERTH, J.O., NILSSON, G. & PERNOW, B. (1975). Experimental immunohistochemical studies on the localization and distribution of substance P in the cat primary sensory neurones. *Brain Res.*, **100**, 235–252.

HORNYAK, M.E., NAVER, H.K., RYDENHAG, B. & WALLIN, B.G. (1990). Sympathetic activity influences the vascular axon reflex in the skin. *Acta Physiol. Scand.*, **139**, 77–84.

HUKKANEN, M., GRÖNBLAD, M., REES, R., KOTTINEN, Y.T., GIBSON, S.J., HIETANEN, J., POLAK, J.M. & BREWERTON, N.A. (1991). Regional distribution of mast cells and peptide containing nerves in normal and adjuvant arthritis rat synovium. *J. Rheumatol.*, **18**, 177–183.

KANGAWA, K., MINAMINO, N., FUKUDA, A. & MATSUO, H. (1983). Neuromedin K: A novel mammalian tachykinin identified in porcine spinal cord. *Biochem. Biophys. Res. Commun.*, **114**, 533–540.

KIMURA, S., OKADA, M., SUGITA, Y., KANAZAWA, I. & MUNEKATO, E. (1983). Novel neuropeptides, neurokinin α and β, isolated from porcine spinal cord. *Proc. Jpn. Acad. (B)*, **59**, 101–104.

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

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

LEE, C.M., IVERSEN, L.L., HANLEY, M.R. & SANDBERG, B.E.B. (1982). Possible existence of multiple receptors for substance P. *Naunyn-Schmiedeb. Arch. Pharmacol.*, **318**, 281–287.

NILSSON, G.E., TENLAND, T. & OBERG, P.A. (1980a). A new instrument for continuous measurement of tissue blood flow by light beating spectroscopy. *IEEE Trans. Biomed. Eng. BME.*, **27**, 12–19.

NILSSON, G.E., TENLAND, T. & OBERG, P.A. (1980b). Evaluation of a laser Doppler flowmeter for measurement of tissue blood flow. *IEEE Trans. Biomed. Eng. BME.*, **27**, 597–604.

OBEID, A.N., DOUGHERTY, G. & PETTINGER, S. (1990). *In vivo* comparison of a twin wavelength laser Doppler flowmeter using He-Ne and laser diode sources. *J. Med. Eng. Tech.*, **14**, 102–110.

OTSUKA, M. & KONISHI, S. (1976). Substance P and excitatory transmitter of primary sensory neurones. *Cold Spring Harbor Symp. Quant. Biol.*, **40**, 135–143.

RANDIC, M. & MILETIC, V. (1977). Effect of substance P in cat dorsal horn neurones activated by noxious stimuli. *Brain Res.*, **128**, 164–169.

REGOLI, D., DRAPEAU, G., DION, S. & D'ORLEANS-JUSTE, P. (1987a). Mini review. Pharmacological receptors for substance P and neurokinins. *Life Sci.*, **40**, 107–117.

REGOLI, D., DRAPEAU, G., DION, S. & D'ORLEANS-JUSTE, P. (1987b). Receptors for neurokinins in peripheral organs. In *Substance P and Neurokinins*, Montreal '86. ed. Henry, J.L., Couture, R., Cuello, A.C., Pelletier, G., Quirion, R. & Regoli, D. pp. 99–107. New York: Springer-Verlag.

REGOLI, D., MIZRALI, J., D'ORLEANS-JUSTE, P., DION, S., DRAPEAU, G. & ESCHER, E. (1985). Substance P antagonists showing some selectivity for different-receptor types. *Eur. J. Pharmacol.*, **109**, 121–125.

SNIDER, R.M., CONSTANTINE, J.W., LOWE, J.A., LONGO, K.P., LEBEL, W.S., WOODY, H.A., DROZDA, S.E., DESAI, M.C., VINICK, F.J., SPENCER, R.W. & HESS, H.-J. (1991). A potent nonpeptide antagonist of the substance P (NK_1) receptor. *Science*, **251**, 435–437.

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