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Modulation of synovial blood flow by the calcitonin gene-related peptide (CGRP) receptor antagonist, $CGRP_{(8-37)}$

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1 The effect of the calcitonin gene-related peptide (CGRP) receptor antagonist, $CGRP_{(8-37)}$ on blood flow in the knee joint of the anaesthetized rat was investigated.

2 Synovial blood flow in both exposed and intact, skin-covered knees was measured by laser Doppler perfusion imaging.

3 Topical application of $CGRP_{(8-37)}$ caused a dose-dependent fall in synovial blood flow in the exposed knee joint of the rat. At low (1.5 nmol) doses of $CGRP_{(8-37)}$ there was no significant effect on synovial blood flow. In rats treated with 7.5 nmol $CGRP_{(8-37)}$ there was a fall in synovial blood flow (maximum effect at 10 min: $-28.8 \pm 4.6\%$; $n=7$), which returned to resting levels within 30 min. The highest dose (15 nmol) of antagonist used in this study caused a marked (maximum at 10 min: $-35.6 \pm 9.3\%$; $n=8$), and prolonged (up to 30 min) fall in blood flow.

4 Ten days after surgical denervation, $CGRP_{(8-37)}$ (15 nmol, topical) had no significant effect on blood flow in the rat exposed knee joint (change in flux at 10 min: $-5.1 \pm 3.6\%$; $n=4$). This suggests that $CGRP_{(8-37)}$ acts selectively to antagonize the actions of a neurally derived product, probably CGRP, on the rat synovial vasculature.

5 In skin-covered knee joints, intra-articular injection of $CGRP_{(8-37)}$ (15 nmol; bolus) elicited a significant fall in synovial blood flow (maximum effect at 10 min: $-15.5 \pm 5.8\%$; $n=6$).

6 CGRP (0.01, 0.1 or 1.0 nmol; topical) caused a dose-dependent increase in exposed knee joint blood flow, which was attenuated by co-administration of 1.5 nmol $CGRP_{(8-37)}$. For example, 1 nmol CGRP elicited a peak increase in flux at 10 min of $94.7 \pm 31.8\%$ ($n=8$) and $28.8 \pm 8.9\%$ ($n=7$) in the absence and presence of $CGRP_{(8-37)}$, respectively. The vasodilator responses induced by acetylcholine (ACh) (10 nmol, topical; $n=4-5$) or sodium nitroprusside (SNP) (10 nmol, topical; $n=4-5$) were unaltered in the presence of $CGRP_{(8-37)}$ (1.5 nmol, topical).

7 Thus, the CGRP receptor antagonist $CGRP_{(8-37)}$ elicits vasoconstriction in the rat synovium. This suggests that the endogenous, basal release of CGRP may play a physiological role in the regulation of blood flow in the rat knee joint.

Keywords: Calcitonin gene-related peptide (CGRP); calcitonin gene-related peptide receptor antagonist ($CGRP_{8-37}$); rat synovial blood flow

Introduction

The 37 amino acid peptide, calcitonin gene-related peptide (CGRP) is a potent vasodilator in many species, including man (Brain *et al*., 1985; Poyner, 1992). In addition, CGRP potentiates oedema induced by other mediators of increased microvascular permeability such as, platelet-activating factor, leukotriene B_4 and substance P (Brain & Williams, 1985). Unmyelinated, afferent neurones which innervate synovial joints are thought to be the primary source of neuropeptides such as CGRP and substance P (Grondblad *et al*., 1988; Mapp *et al*., 1990). In the rat knee joint, exogenous application of CGRP causes vasodilatation (Cambridge & Brain, 1992; Lam & Ferrell, 1993a) and an increase in microvascular permeability (Karimian & Ferrell, 1994). These findings suggest that CGRP may play an important role in the regulation of microvascular tone, particularly in inflammatory processes.

At present there are 2 known CGRP receptors, namely, $CGRP_1$ and $CGRP_2$ (Dennis *et al*., 1989; 1990; Mimeault *et al*., 1991; Tomlinson & Poyner, 1996). A number of C-terminal fragments of CGRP have been shown to be antagonists at these receptors, the most well characterized of which is $CGRP_{(8-37)}$ (Chiba *et al*., 1989; Mimeault *et al*., 1991). $CGRP_{(8-37)}$ acts preferentially at $CGRP_1$ receptors and is a reversible antagonist of CGRP-induced vasodilatation in many isolated tissues including, rat mesentery (Han *et al*., 1990), pig coronary artery (Franco-Cereceda, 1991) and guinea-pig atrium (Maggi *et al*., 1991). In addition, $CGRP_{(8-37)}$ is a selective and competitive antagonist *in vivo* (Donoso *et al*., 1990; Gardiner *et al*., 1990; Hughes & Brain, 1991). A vasoconstrictor effect of $CGRP_{(8-37)}$ has been demonstrated in rabbit skin (Hughes & Brain, 1994) and rat mesentery (Han *et al*., 1990), kidney, hindquarters (Gardiner *et al*., 1990) and *vasa nervosum* (Zochodne & Ho, 1991; 1992). This suggests that a 'tonic' release of CGRP may contribute to the physiological regulation of blood flow, although this remains a controversial area (see Maggi, 1995).

The aim of this study was to investigate, by use of the CGRP receptor antagonist $CGRP_{(8-37)}$, whether endogenous CGRP plays a physiological role in the regulation of blood flow in the rat knee joint.

The findings of this study have been published in preliminary form (McMurdo *et al*., 1996).

Methods

Surgical procedure

Male Wistar rats (250–400 g) were anaesthetized with urethane (2.5 g kg^{-1}, i.p.). The trachea was cannulated to facilitate respiration and body temperature was maintained at 37°C by means of a homeothermic blanket (Harvard, Edenbridge, Kent). The right carotid artery was cannulated and connected to a pressure transducer (Elcomatic EM.751) for

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the measurement of systemic blood pressure, from which mean arterial blood pressure (MAP) was derived and recorded on a mutitrace-4 pen recorder (Lectromed UK Ltd., Hertfordshire).

The rats were placed on their backs with the knee in the rest position. An ellipse of skin was removed to expose the medial aspect of the knee joint and blood flow was measured by laser Doppler perfusion imaging (LDI; Lisca Developments AB, Linkoping, Sweden), as previously described (Lam & Ferrell, 1993b). Briefly, a low power (1 mW) laser beam (633 nm) scans the exposed medial aspect of the rat knee joint. The backscattered Doppler-shifted photons are collected in the photodetector and are processed to generate a 2 dimensional image of knee joint perfusion. Perfusion values in these images are encoded by changes in the laser Doppler signal (voltage; V) and although these can be represented with colours or a greyscale (see Figure 1) all calculations use actual V values at each pixel. Warmed saline was regularly applied to the exposed tissue to prevent desiccation. Drugs were administered topically as 50 or 100 μl bolus applications.

A separate groups of 4 rats were subjected to halothane anaesthesia and the medial aspect of the knee joint was surgically denervated by sectioning of the saphenous nerve proximal to the knee joint. The animals were allowed to recover and 10 days later were included in the aforementioned protocol. This is a sufficient amount of time for nerve degeneration to occur distally as demonstrated by transmission electron microscopy. The distal segment of the nerve following section of the saphenous nerve proximally shows gross degeneration with invasion of macrophages, lack of myelin sheaths and no visible evidence of unmyelinated fibres. This technique has been more fully described in a previous publication from our laboratory (Ferrell *et al*., 1997).

A separate series of experiments was performed in intact, skin-covered knee joints, with the same imaging system, but by employing a near infra-red laser (830 nm) to scan the depilated knee. Although with lower sensitivity, recent work in our laboratory (Lockhart & Ferrell, 1996) has demonstrated that changes in synovial perfusion can be measured transcutaneously in intact knee joints by a near infra-red laser. The greater tissue penetration conferred by this laser enables perfusion imaging of the vascular bed without the removal of overlying skin. In these experiments drugs were given as intra-articular (50 μl, bolus) injections.

Experimental design

After skin removal animals were allowed to stabilize for 40–60 min before a control scan was taken. Rats were given either the CGRP antagonist, $CGRP_{(8-37)}$ (1.5, 7.5 or 15 nmol, topical) or vehicle (0.9% w/v saline, topical) and knee joint perfusion was measured at 5, 10, 20 and 30 min after application of drug ($n=4-8$). Two, individual rat knees were scanned in each experiment by lying the rats side-by-side. Thus, for each experiment there was a control (0.9% w/v saline) knee and a test ($CGRP_{(8-37)}$) knee. In a separate series of experiments, $CGRP_{(8-37)}$ (1.5 nmol, topical) or vehicle (0.9% w/v saline, topical) was administered and immediately afterwards CGRP (0.01, 0.1 or 1.0 nmol) was applied, in a cumulative fashion (15 min between doses), to the surface of the knee joint. Scans were conducted 10 min after each CGRP application ($n=4-8$). In addition, another group of rats were given $CGRP_{(8-37)}$ (1.5 nmol, topical) or vehicle (0.9% w/v saline, topical) and acetylcholine (ACh; 10 nmol, $n=4-5$) or sodium nitroprusside (SNP; 10 nmol, $n=4-5$) was administered immediately afterwards.

In transcutaneous imaging experiments, where knee joints were not exposed, the rats again were allowed to stabilize for 40–60 min before a control scan was taken. The CGRP antagonist, $CGRP_{(8-37)}$ (15 nmol) or vehicle (0.9% w/v saline) was administered as an intra-articular injection ($n=3-6$). Blood flow was measured at 5, 10, 20 and 30 min intervals after application of the antagonist or vehicle.

All results are expressed as the % change in flux from control scan values.

Materials

Urethane, ACh, SNP and $CGRP_{(8-37)}$ were obtained from Sigma Chemical Co. (Poole, Dorset). CGRP was purchased from Bachem (Saffron Walden, Essex). The peptides were dissolved in 0.9% w/v saline and stored as frozen ($-20^{\circ}C$) aliquots until use.

Statistical comparisons

All values in the figures and text are expressed as mean $\pm$ s.e. mean of n observations. Statistical evaluation of the data was by Student's t test for paired determinations or by 2 way ANOVA. A P value <0.05 was considered significant.

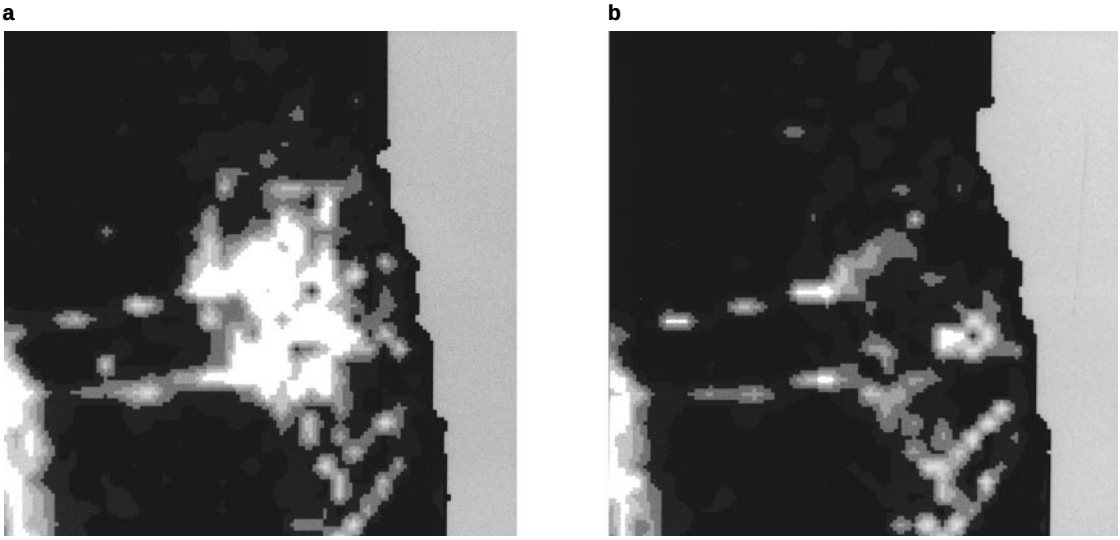

Figure 1 Laser doppler perfusion images of the medial aspect of the exposed knee joint of the rat. Representative, greyscale perfusion images of a rat knee 10 min after topical application of (a) vehicle (0.9% w/v saline) or (b) the CGRP receptor antagonist, $CGRP_{(8-37)}$ (15 nmol). Perfusion was encoded by means of a greyscale with lowest perfusion represented as black through to highest perfusion in white.

Results

$CGRP_{(8-37)}$ induced a fall in rat synovial blood flow

The mean basal value for exposed knee joint blood flow was 5.82 ± 0.53 V ($n=25$). There were no significant differences in basal flux values between any of the experimental groups ($P>0.05$). The CGRP receptor antagonist, $CGRP_{(8-37)}$ had a dose-dependent effect on rat knee joint blood flow. In rats treated with 7.5 and 15 nmol $CGRP_{(8-37)}$ there was a marked reduction in synovial blood flow (maximum at 10 min: $-28.8 \pm 4.6\%$ and $-35.6 \pm 9.3\%$, respectively; $n=7-8$, Figures 1 and 2). Blood flow had returned to resting levels within 30 min in rats treated with 7.5 nmol $CGRP_{(8-37)}$ ($n=7$; Figure 3). In contrast, the vasoconstriction induced by 15 nmol $CGRP_{(8-37)}$ was maintained for up to 30 min ($n=8$; Figure 3). A low dose (1.5 nmol) of $CGRP_{(8-37)}$ or vehicle (0.9% w/v saline, topical) had no significant effect on rat synovial blood flow throughout the duration of these experiments ($n=6$, Figures 2 and 3). Systemic blood pressure was unaffected by any of the above treatments (data not shown).

To confirm that the actions of $CGRP_{(8-37)}$ were not simply the consequence of a non-specific vasoconstrictor action, the knee joints in a group of rats were surgically denervated. The mean basal joint blood flow of denervated animals (2.65 ± 0.7 V; $n=4$) was lower than that of rats which did not undergo surgical denervation (6.35 ± 0.53 V; $n=6$), although this did not quite attain significance ($P=0.058$), $CGRP_{(8-37)}$ (15 nmol, topical) now had no significant effect on synovial blood flow in rats which were denervated 10 days before the experiment (change in flux at 10 min: $-5.1 \pm 3.6\%$, $n=4$).

To determine whether these actions of $CGRP_{(8-37)}$ were simply a consequence of removing overlying skin, experiments were performed in skin covered, intact knee joints. In this part of the study, a near infra-red laser was used to measure joint blood flow. Basal flux was 1.57 ± 0.07 V ($n=17$) when measured transcutaneously. This value was lower because the skin presents a significant optical barrier which substantially attenuates the laser Doppler signal. In these experiments, $CGRP_{(8-37)}$ (15 nmol, intra-articular) induced a significant reduction in synovial blood flow (peak fall at 10 min: $-15.5 \pm 5.8\%$; Figure 4), which was maintained for up to 30 min ($n=6$). Vehicle (0.9% w/v saline, intra-articular) had no significant effect on flux ($n=3$; Figure 4).

$CGRP_{(8-37)}$ attenuated CGRP-induced vasodilatation

In this series of experiments we wanted to determine whether $CGRP_{(8-37)}$ would attenuate the actions of the exogenously

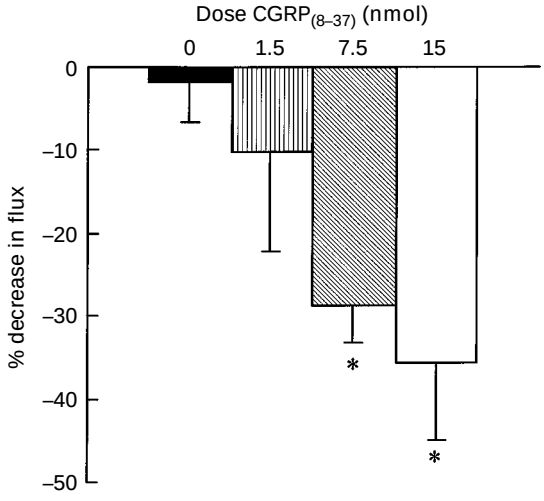

Figure 2 $CGRP_{(8-37)}$ had a dose-dependent vasoconstrictor action on synovial blood flow in the exposed knee joint of the rat. For example, 10 min post-application, $CGRP_{(8-37)}$ at a dose of 7.5 nmol (diagonally hatched column, $n=7$) or 15 nmol (open column, $n=8$) elicited substantial reductions in flux of $-28.8 \pm 4.5\%$ and $-35.6 \pm 9.3\%$, respectively. While, 1.5 nmol $CGRP_{(8-37)}$ (vertically hatched column, $n=4$) or vehicle (0.9% w/v saline; solid column, $n=6$) had no effect on synovial blood flow at 10 min post-administration. Data are expressed as mean $\pm$ s.e.mean of n observations. $^*P<0.05$ when compared to vehicle control.

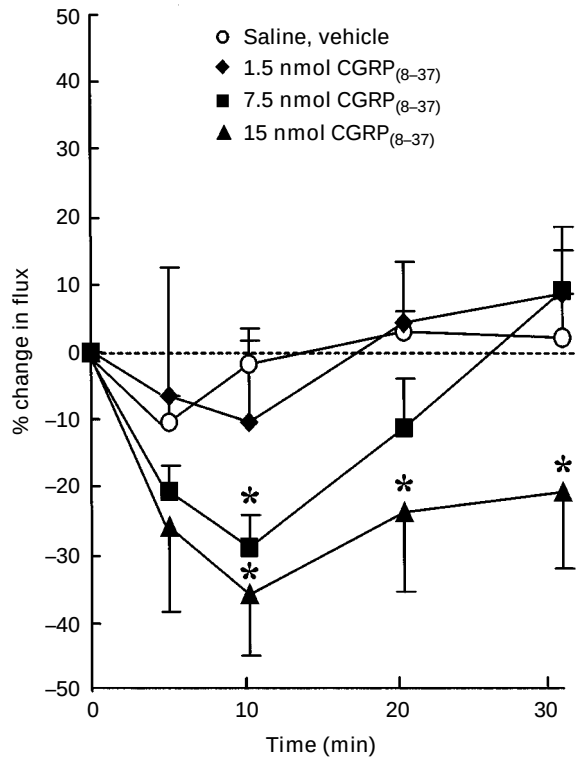

Figure 3 Time course of the effect of 1.5 nmol ($n=4$), 7.5 nmol ($n=7$) or 15 nmol ($n=8$) $CGRP_{(8-37)}$ on blood flow in the exposed knee joint of the rat. Note the significant vasoconstriction at 10 min caused by 7.5 and 15 nmol $CGRP_{(8-37)}$ and also the sustained duration of action of the highest dose used. $CGRP_{(8-37)}$ (1.5 nmol) or vehicle (0.9% w/v saline; $n=6$) had no significant effect on flux over the time course of the experiment. Data are expressed as mean of n observations; vertical lines show s.e.mean. $^*P<0.05$ when compared to vehicle control.

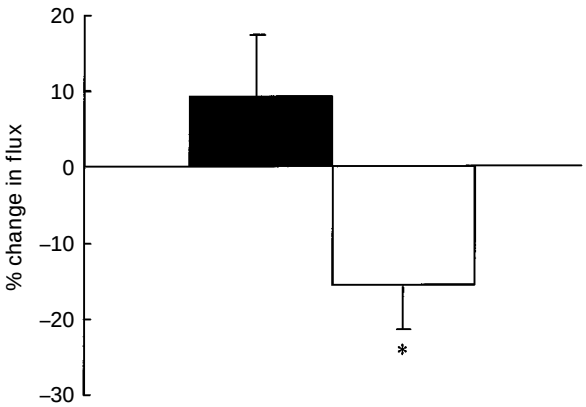

Figure 4 $CGRP_{(8-37)}$ elicited a significant reduction in synovial blood flow in the skin-covered knee joint of the rat. In these transcutaneous experiments a near infra-red laser was used to measure synovial blood flow. For example, 10 min post-application. $CGRP_{(8-37)}$ (15 nmol intra-articular; open column, $n=6$) reduced flux by $15.5 \pm 5.8\%$. While, vehicle (0.9% w/v saline intra-articular; solid column, $n=3$) had no significant effect. Data are expressed as mean $\pm$ s.e. mean of n observations. $^*P<0.05$ when compared to vehicle control.

applied agonist, CGRP. As mentioned previously, 1.5 nmol $CGRP_{(8-37)}$ has no significant vasoconstrictor effect in the joint capsule and was thus chosen for co-administration with the agonist, CGRP.

Mean, resting flux in the exposed knee joint was 3.72 ± 0.43 V ($n=15$). Again, there were no significant differences in basal flux values between the control and the experimental group ($P>0.05$). CGRP (0.01, 0.1 or 1.0 nmol) was applied in a cumulative manner to the surface of the knee. Lam & Ferrell (1993a) previously demonstrated that CGRP-induced vasodilatation in the rat knee reaches a peak within 2 min and can be maintained for up to 30 min. Here, measurements were taken at 10 min and 15 min was permitted to elapse between subsequent applications of the peptide. CGRP (0.01, 0.1 or 1.0 nmol) caused a dose-dependent increase in synovial blood flow which, was significantly attenuated by co-administration of 1.5 nmol $CGRP_{(8-37)}$ ($n=7-8$; Figure 5). For example, 1 nmol CGRP elicited a peak increase in flux at 10 min of $94.7 \pm 31.8\%$ and $28.8 \pm 8.9\%$ in the absence and presence of $CGRP_{(8-37)}$, respectively ($n=7-8$, Figure 5). CGRP (0.01, 0.1 or 1.0 nmol) had no effect on MAP, either in the absence or presence of the CGRP receptor antagonist (data not shown).

To rule out further a non-specific vasoconstrictor action of $CGRP_{(8-37)}$ we investigated whether this peptide would antagonize the actions of other (non-CGRP) exogenously applied vasodilators. Acetylcholine (ACh, 10 nmol, topical; $n=4-5$) and sodium nitroprusside (SNP, 10 nmol, topical; $n=4-5$) elicited peak increases in blood flow of $35.2 \pm 8.9\%$ and $54.0 \pm 16.9\%$, respectively. In the presence of the CGRP receptor antagonist, $CGRP_{(8-37)}$ (1.5 nmol, topical), the ACh and SNP responses were unaltered ($37.1 \pm 8.2\%$ and $43.6 \pm 11.7\%$), respectively.

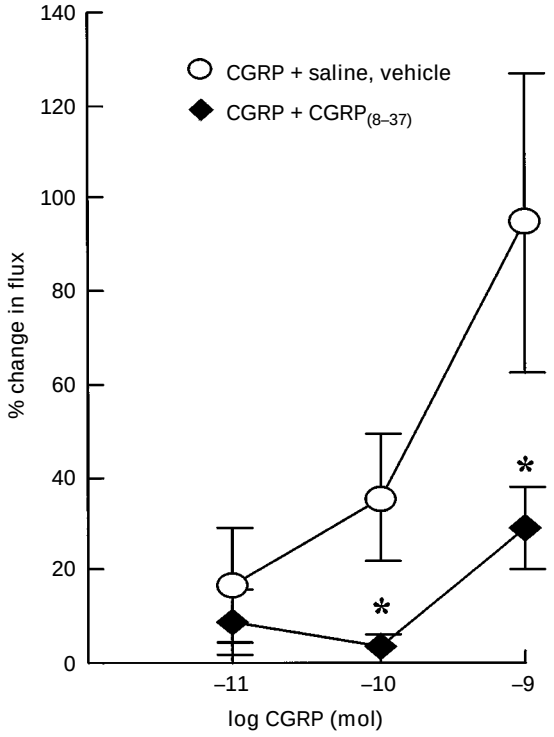

Figure 5 In the exposed knee joint of the rat, CGRP (0.01, 0.1 and 1.0 nmol, topical; $n=8$) caused a dose-dependent increase in synovial blood flow (peak at 10 min) which, was significantly attenuated by co-administration of 1.5 nmol (topical) of the CGRP receptor antagonist, $CGRP_{(8-37)}$ ($n=7$). Data are expressed as mean of n observations; vertical lines show s.e. mean. $^*P<0.05$ when compared to vehicle control.

Discussion

Here, we demonstrated that the CGRP receptor antagonist, $CGRP_{(8-37)}$ has a dose-dependent, vasoconstrictor action on synovial blood flow in the knee joint of the rat. That is, at low (1.5 nmol) doses of $CGRP_{(8-37)}$ there was no effect. At intermediate (7.5 nmol) doses there was a marked but, short-lasting effect. While, at high (15 nmol) doses of antagonist there was a pronounced and sustained vasoconstriction. This suggests that there may be a 'tonic' release of the endogenous, vasodilator CGRP which counteracts vasoconstrictor influences acting on the synovial circulation.

There is evidence both for, and against, the tonic release of neuropeptides, such as CGRP and substance P. For instance, Yonehara and colleagues (1992) demonstrated that intra-arterial administration of spantide (a substance P receptor antagonist) elicited a substantial reduction in cutaneous blood flow in the anaesthetized rat, suggesting that there may be an endogenous, basal release of the vasodilator, substance P in the skin. Further evidence supporting the hypothesis that there may be tonic release of sensory neuropeptides which regulate basal blood flow comes from experiments in the isolated perfused mesentery of the rat. In this study, infusion of $CGRP_{(8-37)}$ caused a significant increase in resting perfusion pressure, presumably as a result of inhibition of the actions of endogenous CGRP (Han *et al*., 1990). In addition, recent work from our laboratory demonstrated that, inhibition of the effects of substance P, by co-administration of FK888 and SR48968 to antagonize neurokinin$_1$ (NK_1) and NK_2 receptors, respectively, causes a significant reduction in basal synovial blood flow (Ferrell *et al*., 1997). This would suggest that there is a continuous, endogenous release of tachykinins in the rat knee joint and is in accord with the present study which points to a similar role for CGRP.

Further work is necessary to demonstrate definitively that there is a 'tonic' release of sensory neuropeptides. However, our results support the concept of CGRP acting to maintain basal microvascular tone. This conclusion is further strengthened by the absence of a vasoconstrictor response to $CGRP_{(8-37)}$ following surgical denervation, suggesting that this action of the peptide is mediated via antagonism of CGRP which is released from nerve endings. Moreover, joint basal blood flow had a tendency to be lower in denervated rats (2.65 ± 0.7 V). This may indicate that by resection of the saphenous nerve we had removed the vasodilator influences of 'tonically' released neuropeptides such as substance P or CGRP. It may be argued that the CGRP receptor antagonist is less potent as a vasoconstrictor in denervated rats as there is a lower basal blood flow. We believe that this is not the case. For example, in a normal animal which had a lower basal blood flow (2.57 V) than the mean (6.35 ± 1.15 V), $CGRP_{(8-37)}$ still caused a 22% reduction in synovial perfusion. In addition, in a separate study we have demonstrated that, topical application of noradrenaline (10^{-8} mol) can reduce joint blood flow by as much as 78% in rats with basal flows of as little as 2.53 V. Thus, we do not feel that an inability to vasoconstrict further is a viable explanation for the absence of a $CGRP_{(8-37)}$ response in rat denervated joints. Until more CGRP receptor antagonists become available we cannot totally rule out a non-selective vasoconstrictor action of $CGRP_{(8-37)}$, but this seems unlikely, as the CGRP antagonist does not affect other vasodilator agents such as ACh, SNP (this study), substance P, histamine, and bradykinin (see Maggi, 1995). It is also interesting to note that an anti-CGRP monoclonal antibody (C4.19; 3 mg per rat, intravenous, bolus) causes a significant rise in blood pressure in anaesthetized rats (Tan *et al*., 1995). These authors suggested, therefore, that circulating CGRP may play a role in the maintenance of vascular tone under resting conditions *in vivo* (Tan *et al*., 1995).

In contrast, $CGRP_{(8-37)}$ had no effect on resting skin blood flow in experiments conducted by Delay-Goyet and co-workers (1992). Moreover, tachykinin receptor antagonists had no

effect on a variety of cardiovascular parameters, such as heart rate or blood pressure in either the anaesthetized rat or guinea-pig (see Maggi, 1995). The results of these experiments, amongst others, suggest that sensory neuropeptides are not tonically released in all tissues.

Synovial joints are innervated by both myelinated and unmyelinated afferent nerves (Samuel, 1952). The large, myelinated fibres serve mainly a proprioceptive role (Ferrell, 1992). The unmyelinated and finely myelinated axons with unmyelinated terminals, which are not associated with specialized receptive structures, are termed free nerve endings. Fibres with these endings provide the majority of afferent innervation and are the main source of neuropeptides in the joint (Langford & Schmidt, 1983; Grondblad *et al*., 1988; Mapp *et al*., 1990). Many of these substance P- and CGRP-containing nerves are found in perivascular areas, with some extending through the synovium almost as far as the synovial surface (Mapp *et al*., 1990). Here, we suggest that CGRP may be released from nerve endings in the rat knee joint without the evocation of an axon reflex. In our experiments, the knee was held in a non-noxious position and the animals were normotensive. It would seem, therefore, that there would be no cause for primary afferent activation. Skin removal may be considered as an obvious, noxious stimulus which would activate CGRP-containing neurones. Yet, in this study, $CGRP_{(8-37)}$ caused vasoconstriction even when the skin covering the joint was intact, and synovial blood flow was measured transcutaneously with the laser Doppler perfusion imager. This finding is in accord with previous observations that, in the absence of inflammation, most articular nociceptive afferents show little spontaneous activity, even with the joint capsule exposed (Schaible & Schmidt, 1983). Endogenously synthesized CGRP is continually transported down the axon in a peripheral direction. Such on-going synthesis and transport is not consistent with the notion that sensory neuropeptides are released only in response to noxious events. It is more indicative of a 'micro-release' of this mediator (see Maggi, 1995). Primary afferent nerves are now thought of as having a dual 'sensory-efferent' function (Szolcsanyi, 1984; 1990; see Maggi, 1995). Previous work from our laboratory demonstrates that primary afferents are not spontaneously active under the present experimental conditions (unpublished data). Thus, it would seem that these nerves can function in an efferent capacity and may be able to release neuropeptides in the absence of a painful/noxious stimulus.

Another interesting aspect of this work was the finding that, $CGRP_{(8-37)}$ (1.5 nmol, topical) reduced (by approximately 60%) the peak vasodilator response to exogenous CGRP (1 nmol, topical). However, this was the dose of $CGRP_{(8-37)}$ which did not appear to antagonize the actions of endogenous CGRP. That is, there was no $CGRP_{(8-37)}$-induced vasoconstriction at this dose. The reason for this is unclear. It may be that, endogenous CGRP is released from sensory fibres into a 'protected' receptor site and is thereby less susceptible to antagonism, than the 'unprotected' exogenous CGRP. Alternatively, the localized, endogenous concentration of CGRP may be greater than 1 nmol (the dose that we applied exogenously). Plasma concentrations of CGRP are in the range of 10–100 pM, but concentrations at the receptor are likely to be much higher (Zaida *et al*., 1985). Thus, 1.5 nmol $CGRP_{(8-37)}$ may not be a large enough dose of antagonist to inhibit this high (>1 nmol ?), basal concentration of endogenous CGRP. Gardiner and co-workers (1990) have demonstrated that $CGRP_{(8-37)}$ is a competitive, reversible antagonist of CGRP in the rat *in vivo*. However, in our study we could not determine whether $CGRP_{(8-37)}$ was acting as a competitive or non-competitive antagonist in the rat knee joint. Although the dose-response curve to CGRP was shifted to the right, it was not clear what effect $CGRP_{(8-37)}$ had on the maximum vasodilator response. This is because higher doses (e.g. 10 nmol) of CGRP could not be studied in this model as, they caused a fall in systemic blood pressure, which in turn, reduced synovial blood flow ($n=2$, data not shown).

The synovium of the rat has a similar distribution of neuropeptide-containing nerves to that of man (Hukkanen *et al*, 1991; 1992). Thus, it is a good model for studying the involvement of agents such as CGRP in the regulation of synovial blood flow. The results presented here suggest that CGRP may play an important, physiological role in modulating vascular tone in the knee joint. It will be interesting to investigate the role of CGRP in regulating blood flow in pathophysiological situations. For instance, in inflammatory joint disease; a condition where alterations in neuropeptide levels may be a critical factor in disease progression.

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