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Spatial heterogeneity of the effects of calcitonin gene-related peptide (CGRP) on the microvasculature of ligaments in the rabbit knee joint

[1,*]W.R. Ferrell, J.J. McDougall & R.C. Bray

McCaig Centre for Joint Injury and Arthritis Research, University of Calgary, Alberta, T2N 4N1, Canada and *Institute of Biomedical & Life Sciences, West Medical Building, University of Glasgow, G12 8QQ, Scotland

1 Experiments were performed in anaesthetized rabbits to examine the effects of calcitonin gene-related peptide (CGRP) and the CGRP antagonist $CGRP_{8-37}$ on blood flow to the medial collateral ligament of the knee joint.

2 Topical application of CGRP (10^{-13} to 10^{-9} mol) to the exposed external surface of eight knee joints resulted in dose-dependent dilatation of vessels in both the ligament and the joint capsule. The magnitude of this response varied significantly in different regions of the medial collateral ligament, with the 10^{-9} mol dose of CGRP giving the maximum response ($101.5 \pm 25.3\%$ increase) at the femoral insertion site of the medial collateral ligament and lowest ($23.1 \pm 8.8\%$) at the tibial insertion site.

3 Topical application of $CGRP_{8-37}$ (0.1, 1 and 10 nmol) produced dose-dependent constriction of vessels in the ligament and the joint capsule in five knees, with a trend towards the greatest effect occurring at the femoral insertion site ($45.8 \pm 8.1\%$ reduction in blood flow). With the 10 nmol dose, the vasoconstrictor response at the femoral insertion site differed significantly ($P<0.05$) from the responses obtained at the tibial insertion and joint capsule sites.

4 Topical application of $CGRP_{8-37}$ (0.1, 1 and 10 nmol) to four chronically denervated knees produced substantially smaller vasoconstrictor responses at all sites. At the femoral insertion site, where 10 nmol $CGRP_{8-37}$ normally produces a $45.8 \pm 8.1\%$ reduction in blood flow ($n=8$), ten days following denervation this response was reduced to $6.5 \pm 6.1\%$, this difference being significant ($P=0.01$).

5 Adrenaline was applied topically to augment blood vessel tone, in order to establish how effectively co-administration of CGRP would offset this increase in tone. Adrenaline (10^{-10} mol) produced vasoconstriction at all sites ($n=6$). In the capsule this vasoconstriction was virtually abolished when CGRP (10^{-9} mol) was co-administered with adrenaline but in the ligament vasodilatation occurred at all sites. This vasodilatation was significantly greater at the femoral insertion site compared to the tibial insertion and mid ligament sites ($P<0.05$ for both) and the capsule ($P<0.01$).

6 Topical application of substance P (10^{-10} or 10^{-9} mol) failed to elicit dilatation of ligament blood vessels.

7 These results suggest that endogenous CGRP may play an important role in regulating blood flow to different structures in and around the knee joint.

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

Introduction

Ligaments of the larger diarthroses are crucial static stabilizing structures. Injury to these commonly results in temporary or permanent joint instability and the resultant abnormal stresses placed on the articular cartilage causes it to deteriorate and osteoarthritis ensues (Marshall & Olsson 1971; Pond & Nuki 1973; Jacobsen, 1977). Ligaments are known to have an extensive vascular network (Bray *et al*., 1990), with the majority of vessels found in the epiligament layer (Bray *et al*., 1990; Chowdhury *et al*., 1991) covering the surface of the dense collagenous bundles which constitute the bulk of the ligament (Bray *et al*., 1996b). These epiligamentous vessels supply the underlying relatively hypoaemic mass of the ligament and are, therefore, likely to play an important role in maintaining physiological ligament integrity. It has recently been demonstrated that deterioration of the mechanical properties of the medial collateral ligament in injured rabbit knees are closely correlated with substantial increases in ligament perfusion (Doschak *et al*., 1994). This is possibly related to the rate of formation or reabsorption of interstitial fluid in the substance of the ligament, since changes in the water content of a ligament alters its mechanical properties (Chimich *et al*., 1992). These observations suggest an important relationship between ligament perfusion and mechanical adaptive responses, but at present little is known about the factors that regulate ligament blood flow and govern the mechanical properties of ligaments. The purpose of this investigation was to examine whether epiligamentous blood vessels in the medial collateral ligament of the rabbit knee joint are influenced by sensory neuropeptides, in particular calcitonin gene-related peptide (CGRP) which is known to be a potent vasodilator in many tissues and in many species, including man (Brain *et al*., 1985) and which could play an important a role in regulating basal ligament blood flow. Unmyelinated afferent neurones innervate virtually all tissues and show immunoreactivity for a variety of neuropeptides, the most widely investigated being substance P (SP) and CGRP (for review see Maggi, 1995). Rat and human synovium also contains fibres immunoreactive for SP and CGRP (Gröndblad *et al*., 1988; Mapp *et al*., 1990) and it has also been shown recently that ligaments of the rabbit, rat and human knee joint all contain such fibres (Gröndblad *et al*., 1991; McDougall *et*

[1]Author for correspondence at: Institute of Biomedical & Life Sciences, West Medical Building, University of Glasgow G122 8QQ.

al., 1997). The function of these nerve fibres is uncertain, as is whether there might be regional variations in the effects of CGRP within the tissues of a synovial joint. In the rat knee, exogenous application of CGRP causes capsular vasodilatation (Cambridge & Brain, 1992; Lam & Ferrell, 1993b) and an increase in microvascular permeability (Karimian & Ferrell, 1994). Exogenous CGRP has also been found to potentiate the increase in microvascular permeability induced by inflammatory mediators (Cambridge & Brain, 1992; Cruwys *et al*., 1992). Recently it has been shown in the rabbit knee joint capsule that CGRP produces dose-dependent vasodilatation (Yip & Lam, 1995). These findings suggest that CGRP may play a role in inflammatory processes, but whether it has other, more subtle physiological roles is uncertain.

The finding that exogenous CGRP exerts effects on the synovial vasculature confirms the presence of CGRP receptors in this tissue, but whether this also applies to the epiligamentous vessels has not been investigated. At present there are known to be two types of CGRP receptors: $CGRP_1$ and $CGRP_2$ (Dennis *et al*., 1989; Mimeault *et al*., 1991; Tomlinson & Poyner, 1996). A number of C-terminal fragments of CGRP have been shown to have antagonistic activity at these receptors. $CGRP_{8-37}$ is the most well characterized of these (Chiba *et al*., 1989; Mimeault *et al*., 1991), acting 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) 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, 1991) and rat mesentery (Han *et al*., 1990) kidney, hindquarters (Gardiner *et al*., 1990) *vasa nervosum* (Zochodne & Ho, 1991; 1993) and synovium (McMurdo *et al*., 1996). This may suggest that a 'tonic' release of CGRP contributes to the physiological regulation of blood flow, although this remains uncertain (Maggi, 1995).

The aim of this study was to compare the effect of the CGRP receptor antagonist $CGRP_{8-37}$ on blood flow to synovium and ligaments in the rabbit knee joint, thereby establishing whether endogenous CGRP modulates basal blood flow to these structures, particularly the ligaments. In addition, the effect of exogenous CGRP on these tissues was also examined. The effectiveness of CGRP in modulating tone in the rabbit synovium and ligaments was further investigated by co-administration with adrenaline to enhance basal blood vessel tone.

Methods

Twenty-one one year old female New Zealand white rabbits (4.5–5.5 kg) were sedated with acepromazine maleate (0.2 mg, i.v.) and then anaesthetized with urethane (1 g kg^{-1}, i.p.). The right carotid artery was cannulated and connected to a pressure transducer (Elcomatic EM752) for the measurement of systemic blood pressure which was monitored by a computerized recording system with CODAS software (Dataq Instruments Inc., U.S.A.). Body temperature was maintained at 37°C by means of a homeothermic blanket (American Pharmaseal Company).

The animals were placed in dorsal recumbency with the hip externally rotated and the knee in the rest position. An ellipse of skin was removed to expose the medial aspect of the knee joint and fascial tissues overlying the joint then removed to expose the superficial aspects of the joint capsule and the medial collateral ligament. Tissue perfusion was measured by use of a laser Doppler perfusion imager (Moor Instruments Ltd, Axminster), with principles similar to those previously used in a study of the rat knee joint (Lam & Ferrell, 1993a; Karimian *et al*., 1995) and which have been validated for ligament blood flow determination (Bray *et al*., 1996a). Briefly, a low power (1 mW) laser beam (633 nm) scans over the exposed medial aspect of the knee joint. The backscattered Doppler-shifted photons are collected by photodetectors in the scanner head and are processed to generate 2 dimensional images of joint tissue perfusion. These represent spatial maps of the perfused tissue and, unlike laser Doppler flowmetry (LDF) where measurements are obtained only at a single point, laser Doppler imaging (LDI) consist of hundreds of measurement points, depending on the area scanned, which yields a more accurate assessment of overall tissue perfusion. These images were processed by use of customised software (Moor Instruments Ltd, Axminster) to yield measurements in perfusion (flux) units (pu). Four sites, corresponding to identified anatomical features, were analysed in each of the images obtained (Figure 1). For each of these sites the following numbers of measurement points (mean ± s.e. mean; $n = 14$) were obtained: the femoral insertion (FI) of the medial collateral ligament (85.3 ± 5.9), the tibial insertion (TI) of the medial collateral ligament (86 ± 6.4), the middle area (MID) of the medial collateral ligament between these two points (457 ± 19.1) and the immediately adjacent external surface of the exposed joint capsule (CAP; 398.9 ± 22.3) which was used as a reference. Warmed saline was periodically superfused over these exposed tissues to prevent desiccation. When drugs were

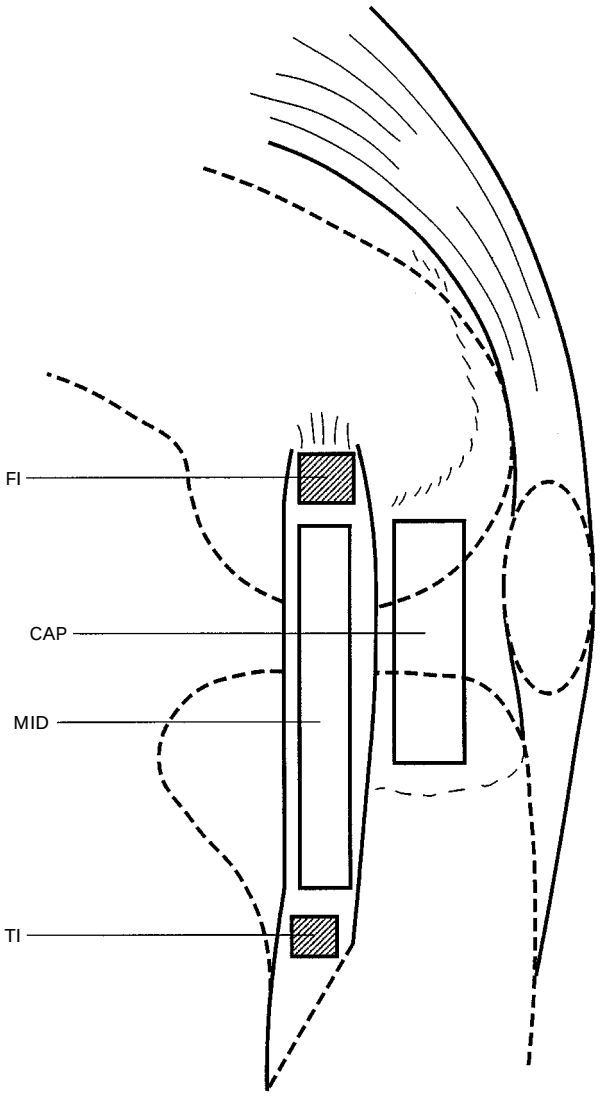

Figure 1 Diagram illustrating the measurement sites from which scans were taken. FI = femoral insertion of the medial collateral ligament; MID = mid region of medial collateral ligament; TI = tibial insertion of medial collateral ligament; CAP = external surface of joint capsule. The boxes indicate the measurement areas (see text for values).

applied to the tissues, it was important to observe the time-course of the effect of the agent, but necessary to avoid further administration of saline which would dilute the applied drug. In these circumstances, drugs were topically administered as a 100 μl bolus and the tissues then covered with cling film. This was also repeated with a single 100 μl bolus of saline administration (to give a saline time control) and as shown in the results section (Figures 3 and 5) this gave relatively stable basal blood flow values.

Experimental design

After skin removal, vehicle (0.9% w/v saline, topical) was administered for 20–50 min before administration of CGRP or the CGRP antagonist. $CGRP_{8-37}$ (0.1, 1 and 10 nmol) was administered topically and knee joint perfusion measured at 1, 5, 10 and 20 min after application ($n=5$). After each dose was administered, the tissues were repeatedly washed with warm saline until basal flux values returned to control values. In separate experiments, CGRP (10^{-13} to 10^{-9} mol topical) was applied, in a cumulative fashion (10 min between doses), to the surface of the capsule. Scans were obtained 1, 5 and 10 min after each CGRP application ($n=8$). In initial experiments, substance P (SP) was administered topically as single doses which were followed for 20 min after each application.

Topical application was used in all these experiments as it was found that administration of either SP or CGRP by close intra-arterial injection resulted in hypotension. Across the dose ranges used in the present study this did not occur with topical application of CGRP (see Table 1). Similarly, topical SP application did not result in hypotension.

All results are expressed as the % change in flux from control scan values, obtained immediately before any intervention. Actual flux values are given in Figures 2 and 4, but only for the femoral insertion site.

In four animals, under deep general anaesthesia (halothane 2%, 98% O_2) the saphenous nerve, which innervates the medial aspect of the rabbit knee joint, was transected and a 5 mm length was resected. The animals were then allowed to recover for 10 days, which is a sufficient period of time for neural degeneration to become well established (Ferrell *et al*., 1997), before being taken to the terminal experiment.

Materials

Urethane and $CGRP_{8-37}$ were obtained from Sigma Chemical Co. (U.S.A.). Atrovet (acepromazine maleate) was acquired through Ayerst Laboratories (Montreal, Canada) and adrenaline hydrochloride by Epiclor (Calgary, Canada). CGRP was purchased from Rose Scientific LTD (Edmonton, Canada) and SP was supplied by Research Biochemicals International (Natick, MA, U.S.A.). The peptides were dissolved in 0.9% w/v saline and stored in a freezer ($-30^{\circ}C$) until use.

Statistical comparisons

All values in the figures and text are expressed as mean ± s.e. mean of n observations. Statistical evaluation of the data was by Student's t test for paired determinations or by 1 or 2 way ANOVA. A P value <0.05 was considered significant.

Results

Response to CGRP

Administration of CGRP, across a 10^{-13} to 10^{-9} mol dose range, produced progressive vasodilatation (Figure 2). This was observed at all four sites, although there were marked differences between these. The femoral insertion site gave the greatest response to CGRP at all doses (Figure 3a) with a maximum response just exceeding 100% increase in perfusion ($P=0.0001$, 1-way ANOVA). Interestingly, the tibial insertion site yielded the smallest response to CGRP (Figure 3c) which did not show clear dose-dependency ($P=0.15$, 1-way ANOVA). The mid ligament region response was somewhat greater ($P=0.0012$, 1-way ANOVA) but still less than the femoral insertion site (Figure 3b). Dose-dependent vasodilatation to CGRP also occurred in the joint capsule (Figure 3d; $P=0.0025$, 1-way ANOVA). The response to CGRP at the femoral insertion site was significantly ($P=0.0001$; 2-way ANOVA) greater than at the tibial insertion site, as it was compared to the mid ligament site and the capsule ($P<0.01$, $P<0.005$ respectively; 2-way ANOVA). Significant differences were also found comparing the tibial insertion site and the capsule ($P<0.01$) and the mid ligament site with the tibial insertion site ($P<0.02$), but not

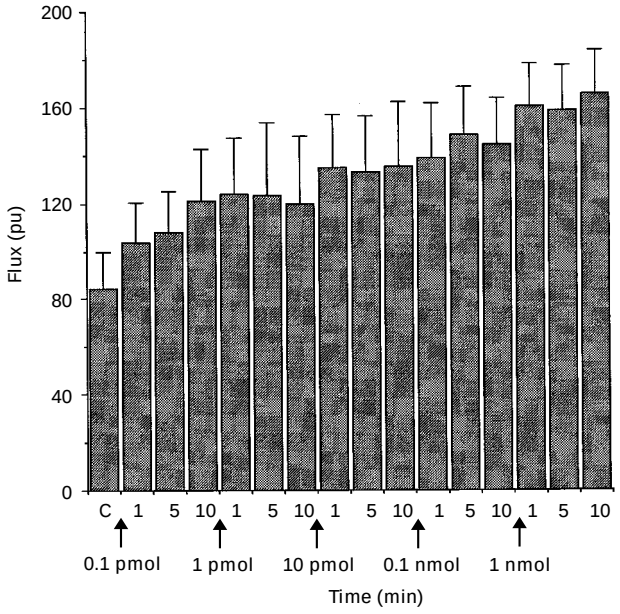

Figure 2 Perfusion values obtained from the femoral insertion site in response to cumulative doses of CGRP at 1, 5 and 10 min following each dose. C: control. Means ± s.e.mean are shown; $n=8$.

Table 1 Mean arterial blood pressure (MABP) values during administration of $CGRP_{8-37}$ and CGRP

		$CGRP_{8-37}$ (nmol)				
	Control	*0.1*	*1*	*10*		
MABP (mmHg)	84.3 ± 3	84.1 ± 3.3	82.8 ± 4.6	82.3 ± 4.7		
		CGRP (log dose, mol)				
	Control	*–13*	*–12*	*–11*	*–10*	*–9*
MABP (mmHg)	83.3 ± 4.7	83.8 ± 5.1	83.2 ± 5.6	83.6 ± 5.6	85.1 ± 5.2	86.1 ± 5.2

Data shown are means ± s.e.mean.

the mid ligament site and capsule ($P=0.82$; all comparisons by 2-way ANOVA).

Response to $CGRP_{8-37}$

Administration of $CGRP_{8-37}$ resulted in dose-dependent vasoconstriction. Both the magnitude and duration of the effect of this antagonist were dose-related and could be reversed by repeated topical application of saline (Figure 4). Overall there was a significant dose-related effect ($P=0.0001$; 1-way ANOVA). However, for individual sites the small number of doses used combined with variability of response between doses resulted in lack of significance for individual sites. Comparison of the effect of the antagonist on the different regions suggested that the greatest degree of vasoconstriction occurred at the femoral insertion region (Figure 5a). However, 2-way ANOVA showed no significant difference between the femoral insertion site and the mid ligament region (Figure 5b; $P=0.17$) or the tibial insertion site (Figure 5c; $P=0.11$), but there was a significant difference between femoral insertion and joint capsule (Figure 5d; $P=0.0075$). Comparison of the response at the femoral insertion site with the other sites for only the 10 nmol dose did reveal a significant difference in relation to the tibial insertion site ($P=0.04$) and the joint capsule site ($P=0.012$) but not the mid ligament site ($P=0.24$; one-tailed t test). Comparisons of the responses at sites other than femoral insertion failed to reveal a significant difference ($P>0.2$). For all four sites the response to the antagonist differed significantly from the saline time control ($P<0.01$ for both femoral insertion and joint capsule sites; $P<0.05$ for tibial insertion and mid ligament sites; 2-way ANOVA). When the effect of $CGRP_{8-37}$ was tested after surgical interruption of the saphenous nerve, the vasoconstrictor response was substantially attenuated (Figure 6). For the femoral insertion site which normally showed the largest reduction in blood flow to administration of $CGRP_{8-37}$, after surgical denervation of the knee this response was substantially and significantly reduced ($P=0.01$; 2-way ANOVA; $n=4$).

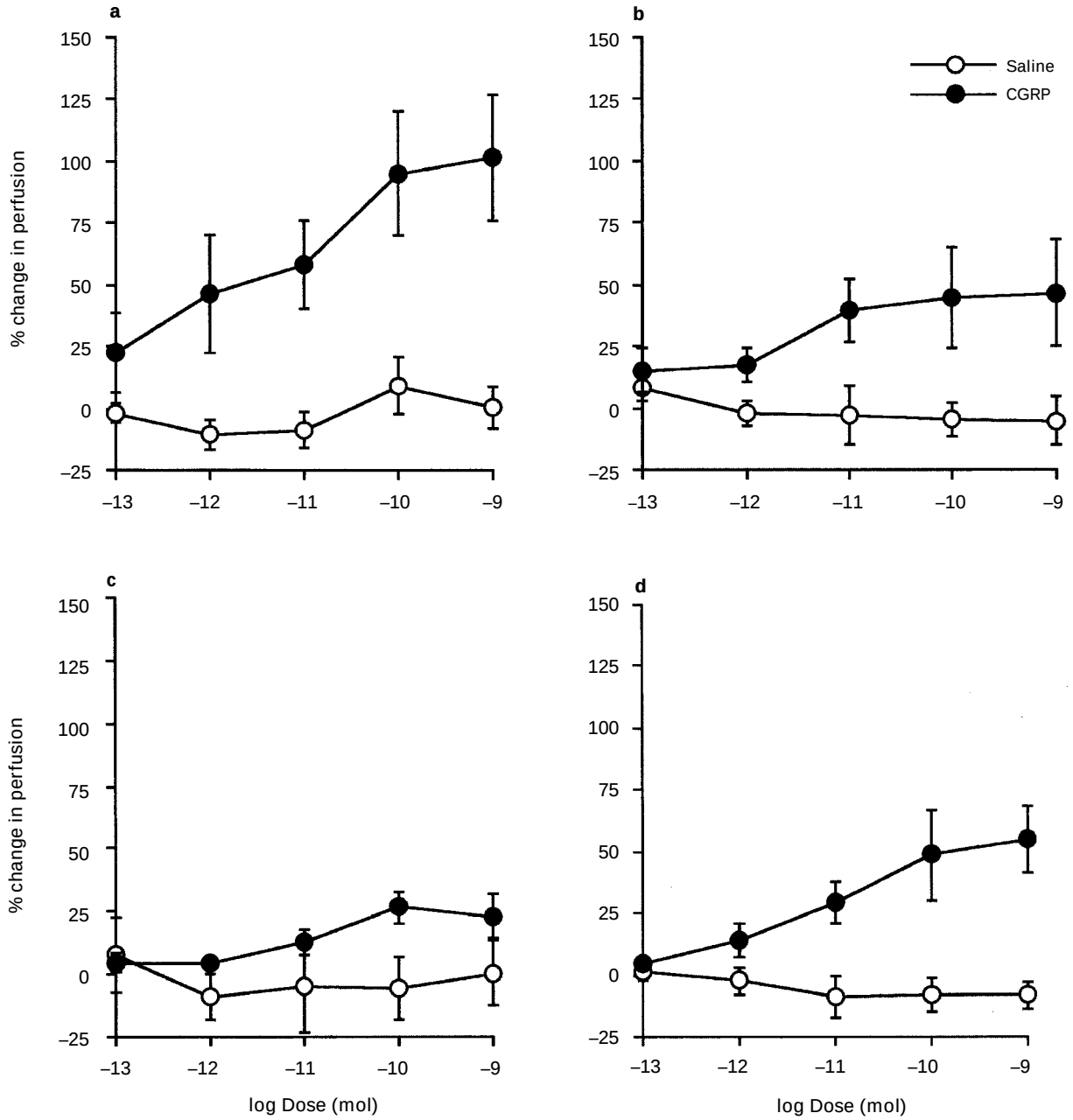

Figure 3 Response to cumulative doses of CGRP at the medial collateral ligament femoral insertion site (a), the mid region of the ligament (b), the tibial insertion site (c) and the exposed external surface of the capsule (d). The corresponding saline time controls are also shown. In each case the responses differed significantly from the saline time control ($P<0.0005$; 2-way ANOVA). Means are shown with vertical lines indicating s.e.mean; $n=8$ at each site.

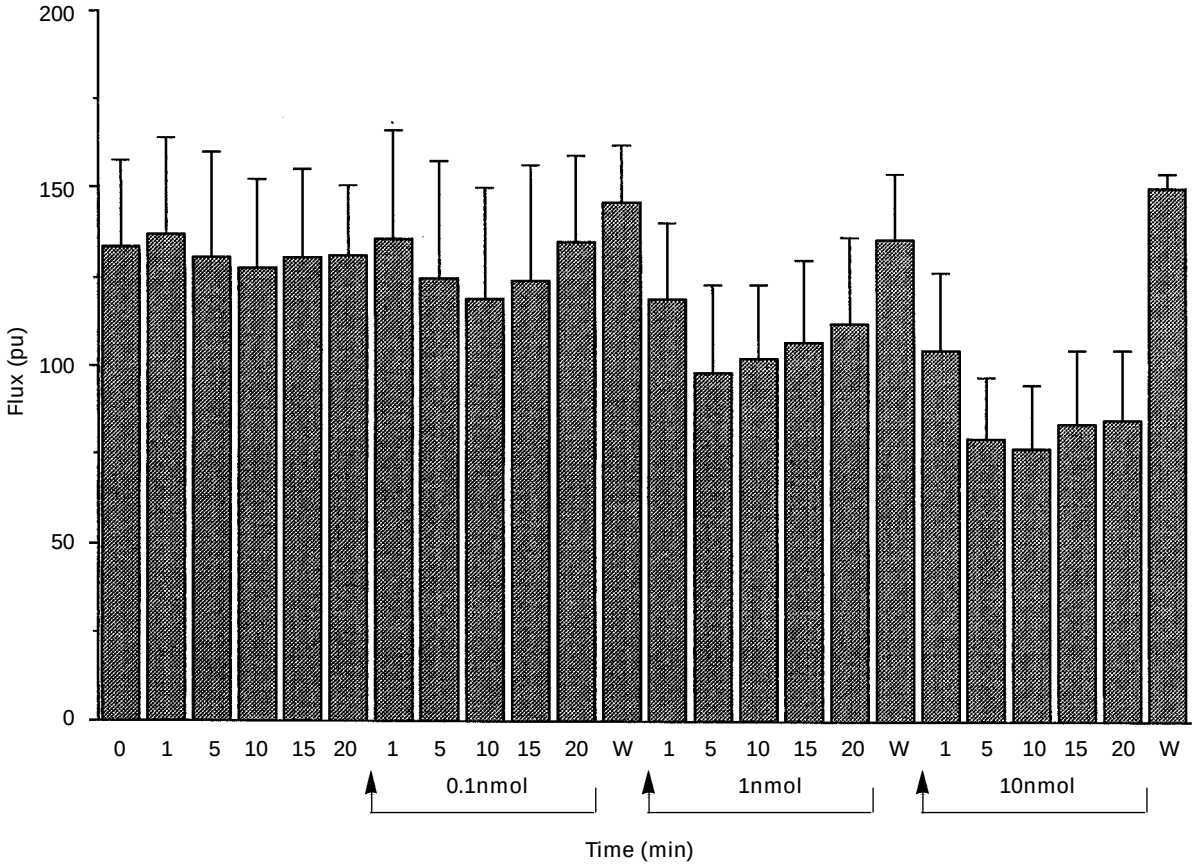

Figure 4 Perfusion values obtained from the FI site in response to application of $CGRP_{8-37}$ at arrows for 20 min. Scans taken at 1, 5, 10, 15 and 20 min following each dose. The first 20 min represents response to application of vehicle (0.9% NaCl). W: wash with 0.9% saline. Means ± s.e.mean are shown; $n = 5$.

Effect of co-administration of adrenaline and CGRP

In order to probe further the potency of CGRP acting at the different sites, the effect of co-administration of CGRP and a vasoconstrictor, adrenaline, was investigated. Adrenaline administration on its own (10^{-10} mol) led to clear vasoconstriction (Figure 7), which did not differ significantly between sites ($P = 0.1$, 1-way ANOVA). When CGRP (10^{-9} mol) was co-administered with adrenaline (10^{-10} mol), the constrictor response at the joint capsule site was greatly attenuated and dilatation occurred at all the ligament sites, this being greatest at the femoral insertion site. There were significant differences between the sites ($P = 0.006$, 1-way ANOVA) and *post hoc* testing (Student-Newmann-Keuls) revealed that the response at the femoral insertion site differed significantly from the tibial insertion and mid ligament sites (both $P < 0.05$) and the joint capsule site ($P < 0.01$).

Response to substance P

Topical application of SP was found to have no obvious effect on blood flow in either ligament or capsule. Scans obtained at 1, 5 and 10 min following application of 10^{-10} mol SP revealed little effect on ligament blood flow, the percentage change in perfusion (mean ± s.e. mean; $n = 3-4$) being 0.3 ± 4.3, -5.2 ± 6.2 and $-2.1 \pm 4.1\%$, respectively. Similarly, 10^{-9} mol SP failed to elicit vasodilatation at the same time points (-16.4 ± 0.9, -9 ± 5.5 and $-2 \pm 4.2\%$, respectively; $n = 3$).

Blood pressure

Blood pressure was monitored throughout the experiments and, as shown in Table 1, this parameter remained relatively constant during drug administration. Thus, the observations from this study cannot be ascribed to systemic uptake of the agents leading to alterations in blood pressure with consequent changes in joint perfusion. In any case, the changes in perfusion which did occur were in the opposite direction to what would have been expected had these agents exerted significant systemic effects.

Discussion

To date, the majority of techniques used to measure ligament blood flow have been unable to give any indication as to the regional differences in flow patterns which occur within this tissue. The observations from the present study provide clear evidence that blood flow in the epiligament is detectable with the LDI technique and that flow can be altered by vasoactive agents. The ability of this technique to generate spatial maps of tissue perfusion permitted examination of blood flow to different regions of the same tissue within the same time frame. Thus, the medial collateral ligament could be subdivided into three anatomically discrete regions and the results of this study make it clear that even in a tissue which appears to be relatively homogeneous, there are substantial differences between these regions in terms of their responsiveness to the drug-induced changes.

A surprising observation was the significantly greater response to CGRP of the femoral insertion of the medial collateral ligament. This was supported by the observation that administration of CGRP in the presence of adrenaline produced marked vasodilatation at the femoral insertion site which differed significantly from the other sites. It appears that even such an apparently homogeneous structure as the ligament shows heterogeneity in its responsiveness to neuropeptides, which must reflect functional differences in these regions. Buckland-Wright (1984) found that focal erosion of the subchondral bone in rheumatoid arthritic hands, originated at the

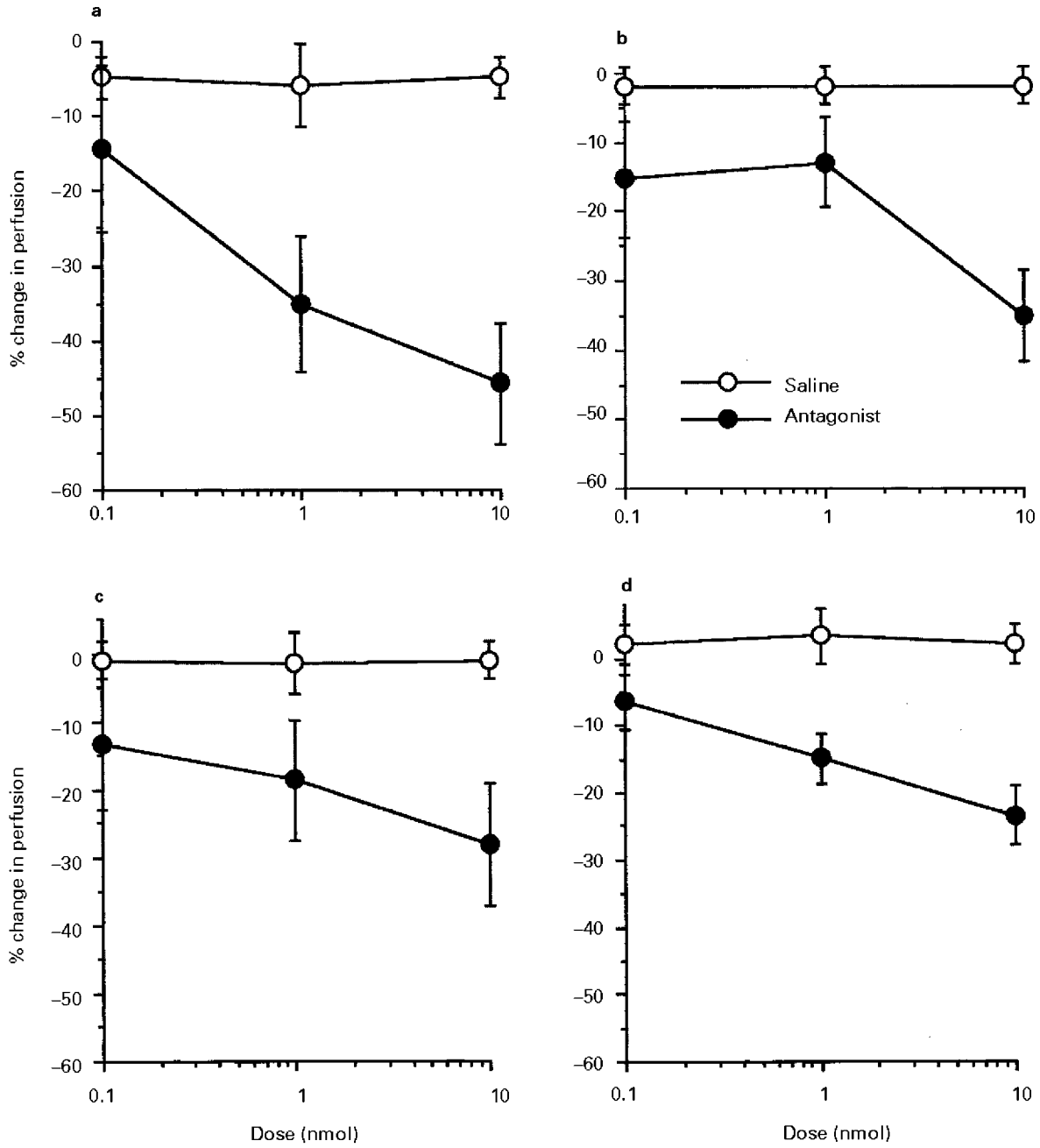

Figure 5 Response to individual doses of the CGRP receptor antagonist $CGRP_{8-37}$ at the medial collateral ligament femoral insertion site (a), the mid ligament site (b), the tibial insertion site (c) and the exposed external surface of the capsule (d). The corresponding saline time controls are also shown. Means are shown with vertical lines indicating s.e.mean; $n=5$ at each site.

insertion sites (entheses) of the articular ligaments. This finding emphasizes the importance of the insertion sites of ligament into bone and that altered vasoregulation in these areas could contribute to the early development of arthritis. The regional differences in perfusion may be important in tissue adaptive responses to joint loading. The femoral insertion and tibial insertion of the rabbit medial collateral ligament are distinct in their structural and functional behaviours (Frank *et al*., 1988; Matyas *et al*., 1990; Lam *et al*., 1995). The femoral insertion, for example, demonstrates the highest surface strain compared to other regions of the medial collateral ligament during application of an external tensile load (Lam *et al*., 1995). The wider range of vasoregulatory modulation by CGRP at the femoral insertion suggests flow may have more important influences on ligament adaptive responses to load bearing conditions, an intriguing physiological factor never previously described.

Topical application of adrenaline produced a constrictor effect, as anticipated from previous work (Najafipour & Ferrell, 1993), and the magnitude of this effect did not vary between different sites. Adrenaline was used in order to increase blood vessel tone at the different sites to allow assessment of how this would then be affected by CGRP co-administration. This intervention yielded marked differences between sites, with the greatest effect occurring at the femoral insertion site where the constrictor response to adrenaline was converted into a dilatation in the presence of CGRP. This, combined with the greater effect of exogenous CGRP occurring at this site argues for a greater density of CGRP receptors at this site. However, other possibilities which cannot be excluded include a difference in the transducer function or a difference in the intrinsic efficacy of the drug-receptor complex at the femoral insertion site. It is unlikely that the differential response to CGRP and $CGRP_{8-37}$ is due to the blood vessels at the femoral

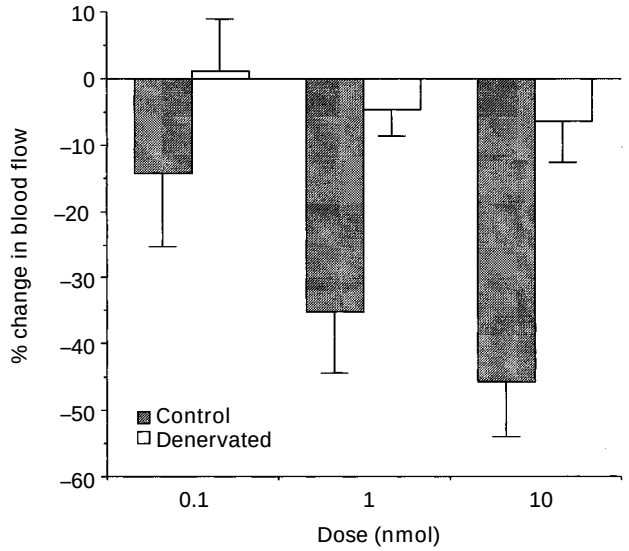

Figure 6 The effect of chronic surgical denervation of the knee on the response to topical administration of $CGRP_{8-37}$. In the normally innervated knee, $CGRP_{8-37}$ elicited dose-dependent vasoconstriction at the femoral insertion site. Ten days after transection of the saphenous nerve, the same doses of $CGRP_{8-37}$ produced a much smaller response which differed significantly from normal (see text). Means ± s.e.mean are shown; $n=5$ for normal, $n=4$ for denervated.

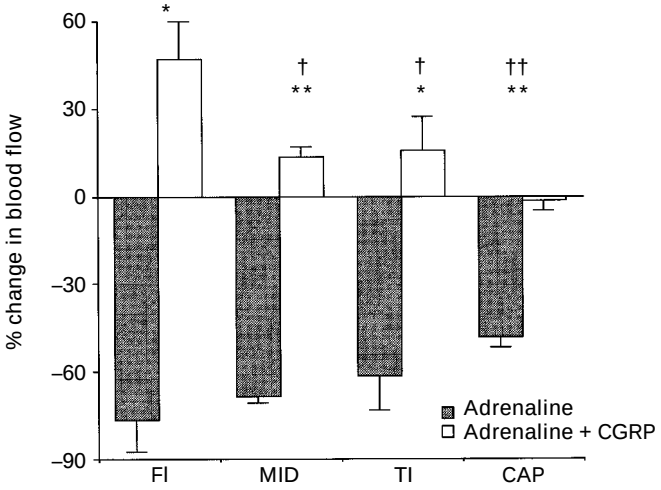

Figure 7 Response to topical application of 10^{-10} mol adrenaline, and to co-administration of 10^{-10} mol adrenaline and 10^{-9} mol CGRP for the various regions. The vasoconstrictor effect of adrenaline was abolished but at the femoral insertion (FI) site a clear dilator response to CGRP occurred. $^{*}P<0.005$; $^{**}P<0.0005$ compared to adrenaline response. $\dagger P<0.05$; $\dagger\dagger P<0.01$ compared to FI. Means ± s.e.mean are shown; $n=5$ at tibial insertion site, other sites $n=6$.

insertion site being more superficial and therefore more accessible to the drugs or the laser beam of the LDI, since the majority of ligamentous vessels occur in the epiligament and there is no histological evidence to suggest that the thickness of this layer is heterogeneous along its length. It may be possible that the observed differences in responsiveness to CGRP and $CGRP_{8-37}$ might be due to differences in the numbers of blood vessels at the different sites. However, once again there is no histological evidence to support this view.

The absence of a vasodilator effect of SP at the ligament is consistent with data indicating that SP does not produce vasodilator responses in the rabbit knee joint capsule (Yip & Lam 1995). This contrasts with the rat knee joint where administration of SP produces clear dose-dependent vasodilatation (Lam & Ferrell 1993b; Karimian *et al*., 1995), although with a much shorter timecourse than that obtained with CGRP administration. The lack of a vasodilator effect of exogenously administered SP on the ligament and joint capsule is surprising in view of the fact that SP-like immunoreactivity is found in nerves supplying the ligament (Gröndblad *et al*., 1991; McDougall *et al*., 1997) and the capsule (Gröndblad *et al*., 1988; Mapp *et al*., 1990), and may indicate an absence of tachykinin receptors in these tissues. Such a lack of effect of exogenously administered SP appears to be a species-specific feature as intradermal injection of SP in rabbits has extremely weak vasoactive effects (Brain & Williams, 1985). It is possible that SP and other endogenous tachykinins may have a neuromodulatory role in the rabbit, influencing the potent vasodilator action of CGRP. Previous work has shown that co-administration of SP reduces the vasodilator action of CGRP in both the skin (Brain & Williams, 1989) and the knee joint (Lam & Ferrell, 1993b) of rats.

The fact that basal blood flow is decreased by application of $CGRP_{8-37}$ suggests that there is tonic release of CGRP from sensory C fibres innervating the ligament. It could be argued that by exposing the joint capsule there was activation of these fibres and release of CGRP and that this would not occur under more physiological conditions. However, in experiments performed in the rat knee joint it was observed that the use of tachykinin receptor antagonists also resulted in decreased basal blood flow in the exposed joint capsule and that this effect still occurred when the overlying skin was left intact and changes in capsule perfusion measured by transcutaneous laser Doppler imaging (Ferrell *et al*., 1997). In the rabbit this transcutaneous approach is not feasible due to the less superficial location of the joint compared to the rat. However, there is no reason to believe that rabbit C fibres would behave differently to rat C fibres and it has been shown that, in the absence of inflammation, C nociceptive afferents show little spontaneous activity, even with the joint capsule exposed (Schaible & Schmidt, 1983).

The effect of surgical denervation of the medial aspect of the knee joint on the response to $CGRP_{8-37}$ provides strong evidence that there is tonic release of CGRP from nerve terminals in the joint. Surgical denervation was used in the present experiments as there have been few studies to investigate capsaicin-induced depletion of sensory neuropeptides in rabbits and of these, systemic capsaicin administration was found to be less effective than in other species (for review see Holzer, 1991). Even with periaxonal application of capsaicin to the saphenous nerve, SP depletion from the territory of skin innervated by this nerve was found to be only 46% (Lynn & Shakanbeh, 1988). This compares to 94% depletion of SP-like immunoreactivity from the urinary bladder following systemic capsaicin treatment in the rat (Maggi *et al*., 1988). In addition, at present there are no data on the effectiveness of any form of capsaicin administration in promoting CGRP depletion from rabbit peripheral nerve terminals. Thus, surgical denervation, although not selective for sensory fibres, provided the only means of assessing the likely source of CGRP in our experiments.

The results of this investigation have provided clear evidence that blood flow to the medial collateral ligament is influenced by CGRP, with considerable regional variation in responsiveness. Such variations are likely to be functionally significant and could be responsible for altering the mechanical properties of the ligament with injury or disease. A better understanding of these mechanisms could point to new therapeutic strategies aimed at minimising joint damage in arthritic diseases.

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