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THE EFFECT OF CARRAGEENAN-INDUCED INFLAMMATION ON BLOOD FLOW IN THE RABBIT KNEE JOINT

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ABSTRACT

Experiments were carried out on anesthetized adult New Zealand rabbits. Acute joint inflammation was achieved by intra-articular injection of 2% carrageenan. Radiolabelled microspheres were used to measure the effect of acute inflammation on blood flow in the knee joint and establish whether neural influences on blood vessel caliber were affected. Surgical exposure of the posterior joint "capsule" increased blood flow in the control knee, but subsequent section of the posterior articular nerve (PAN) supplying the knee produced no further increase. In the inflamed knee, blood flow declined progressively with each procedure. Electrical stimulation of PAN reduced blood flow in the posterior region of both knees, with somewhat less effects in the inflamed knee.

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INTRODUCTION

In a previous investigation it was shown that perfusion of the posterior region of the rabbit knee joint, measured by radiolabelled microspheres, was increased after surgical exposure of the joint capsule and section of the posterior articular nerve (PAN) which supplies this region.[1] However, it was not clear whether this increase was a consequence of surgery or loss of sympathetic vasoconstrictor "tone" following PAN sectioning. It was also observed that electrical stimulation of the nerves innervating the joint capsule resulted in vasoconstriction localized to the capsule. One of the aims of the present investigation was specifically to examine the influence of sympathetic tone on blood vessels in normal and acutely inflamed rabbit knee joints as well as the effect of sympathetic nerve stimulation, thereby establishing the significance of neural factors in regulating synovial blood flow in normal and inflamed joints.

METHOD

Adult New Zealand rabbits (2-3.5 kg) were deeply anesthetized, as judged by the absence of a flexor withdrawal response to a noxious stimulus applied to the forelimb, by an initial injection of hypnorm (0.15 mL/kg I. P.; Janssen) and diazepam (1.5 mg/kg I.P.; Roche). Acute inflammation of one knee was achieved by intra-articular injection of 2% λ carrageenan (Sigma) with the other (control) knee being injected with sterile saline (0.9%). Twenty-four hours later the animals were re-anesthetized with the same agents followed by a mixture of O_2/N_2O and 1% halothane. Arterial blood pressure was monitored throughout the experiment by a physiological pressure transducer (Statham P_{25}) and blood flow measurements taken only in the presence of stable cardiovascular parameters. Independent quantitative measurements of blood flow were obtained by intraventricular injection of radiolabelled microspheres

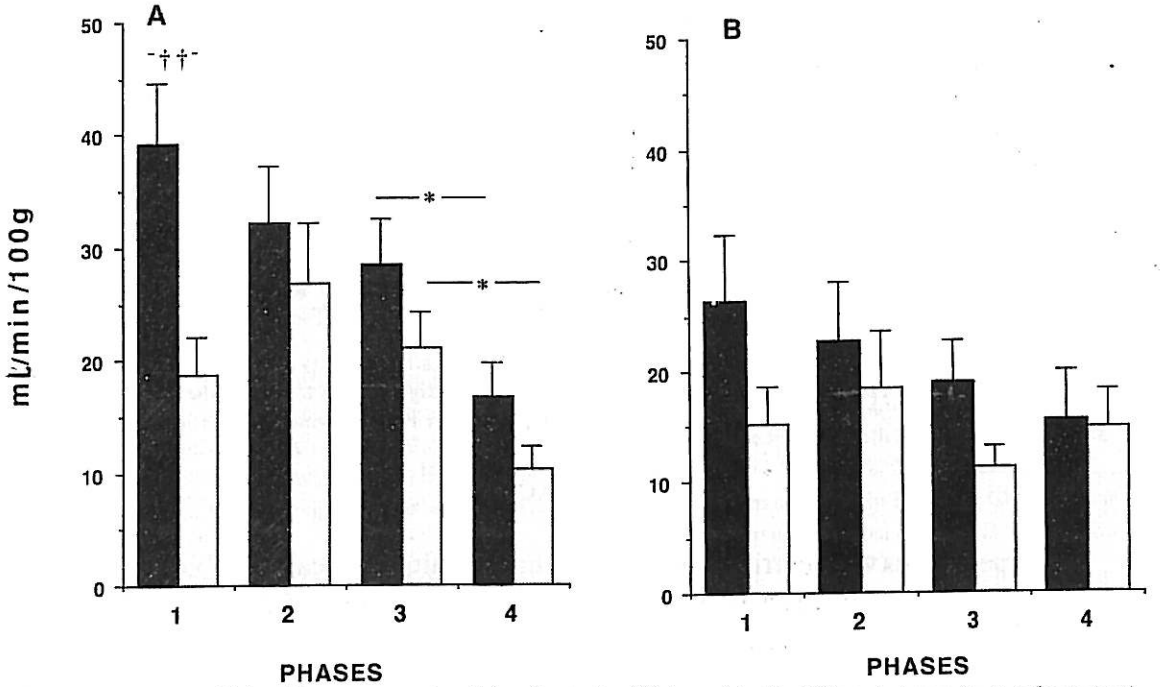

Fig. 1. The amount of blood flow in posterior (A) and anterior (B) knee joint in different phases (1-4; refer to text). †† $p<0.01$, significantly different between control □ and inflamed ■joint. * $p<0.05$, significantly different between phases 3 and 4.

^{113}Sn, ^{57}Co, ^{153}Gd and ^{46}Sc; 16.5±0.1 μm diameter, (NEN-TRAC, Dupont) with timed withdrawal of an arterial blood sample. The techniques employed in the present investigation have been described fully elsewhere.[1] The microspheres were administered in four phases: with both knees intact and before any surgical procedures (phase 1); after surgical exposure of the posterior region of the joint capsule and dissection of PAN (phase 2); immediately after transection of PAN (phase 3); and during maximal electrical stimulation of PAN (phase 4; stimulus parameters: amplitude 10V, width 1ms, train duration 1min and frequency 20 Hz). At the end of the experiment, cardiac arrest was induced by an intraventricular injection of 3M KCl and the following tissues harvested bilaterally: popliteus muscle, femoral condyles, tibial plateau, kidney, patellar ligament, anterior capsule and posterior capsule. The term "capsule" is imprecise in view of the regional heterogeneity of tissues overlying the synovium[3] but serves to indicate the tissue sampled.

The anterior capsule consisted of synovium, areolar tissue and overlying aponeurotic fibrous tissue whilst the posterior capsule comprised synovium and mixed areolar and fibrous tissues. After weighing, counts for the tissue samples and the reference blood samples were obtained with a gamma counter, and blood flow calculated as reference blood sample withdrawal rate multiplied by the ratio of tissue count to reference sample count. Data are presented as mean ±SEM, and non-parameteric statistical tests were used (Wilcoxon and Mann-Whitney U tests). Eleven animals were used but the numbers of observations per joint (in figure legends) were less as some values were excluded due to asymmetric kidney counts suggesting incomplete mixing of microspheres.

RESULTS

Intra-articular injection of carrageenan produced an inflammatory response with noticeable redness and swelling of the injected knee. The anterior capsules from the inflamed and control knees weighed 668±49 mg and 418±24 mg respectively, whilst the posterior capsules from these knees weighed 509±57 mg and 310±16 mg, respectively (mean ±SEM; n = 11). In both instances the inflamed capsules differed significantly from the control group ($p<0.01$; Wilcoxon test; Z=2.934).

In the carrageenan-treated knee, blood flow in the posterior capsule was significantly greater than in the control knee prior to any surgical procedure (phase 1), but after surgery (phase 2) this difference was no longer significant (Fig. 1). This was due to a reduction in the inflamed knee and an increase in the control knee following surgery, but neither of these differed significantly from their original phase 1 values. In the anterior capsule, blood flow in the inflamed knee was greater than the control knee during phase 1, but this difference failed to reach statistical significance. Subsequent surgical exposure of the posterior capsule (phase 2) did not alter blood flow in the anterior capsule of either knee. Throughout the remaining phases

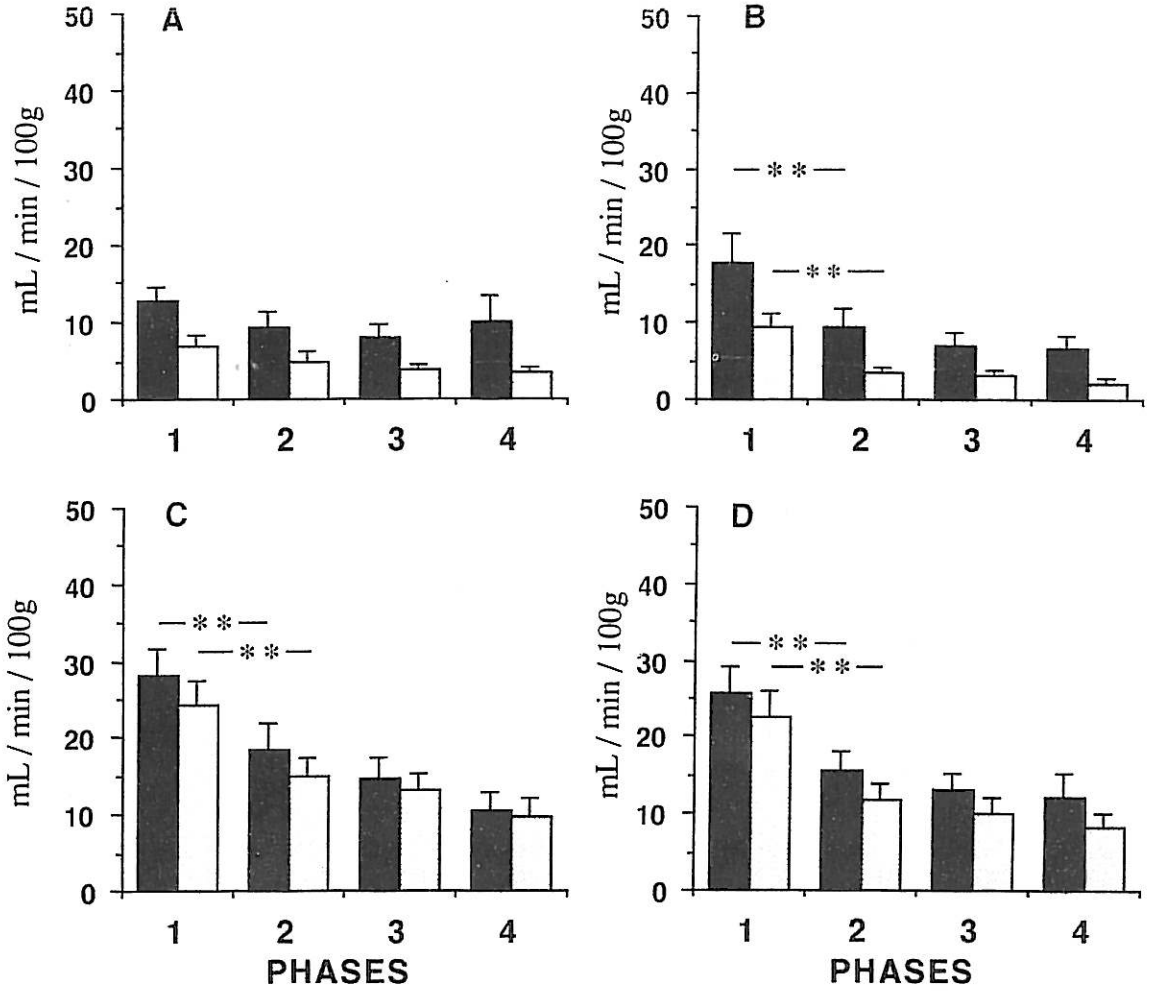

Fig. 2. The amount of blood flow in surrounding tissues such as the popliteus muscle (A), patellar ligament (B), distal femur (C), and proximal tibia (D) in different phases (1-4; refer to text). Control: □, Inflamed: ■.**$p<0.01$, significantly different between phases 1 and 2.

there was no significant difference in blood flow either between the inflamed and control knees or within each group. As anticipated from our previous study[1,6] transection of PAN (phase 3), which innervates the posterior capsule, did not significantly alter blood flow in the anterior region of either control or inflamed knees. However, the same was also true for the posterior region (Fig. 1), suggesting little basal sympathetic tone in the anesthetized animal. Nevertheless, subsequent electrical stimulation of PAN (phase 4) produced vasoconstriction in both control and inflamed knees (Fig. 1). PAN stimulation appeared to be more effective in saline-treated animals (55.5±6% reduction; n=7) than in carrageenan-treated knees (37.3±7.8%, n =7). However, this difference failed to reach significance. In the anterior region of the joint, PAN stimulation failed to produce vasoconstriction in either case.

Examination of other tissues showed that blood flow in the popliteus muscle from either limb remained constant throughout all phases (Fig. 2). But flow in the popliteus muscle was significantly higher in the inflamed knee compared to the control knee (Fig. 2; $p<0.02$ in all cases; Mann-Whitney; n=7-10). The patellar ligament also showed significant differences in blood flow between the inflamed and control knees throughout the phases ($p<0.05$; n =7-10). There was also a significant ($p<0.02$; n = 7-10) reduction between phases 1 and 2 for both knees, but no further significant decline in flow occurred in either case. The distal end of the femur and the proximal tibia showed a similar pattern of decline for both control and inflamed knees in flow between phases 1 and 2 from their initial values. In both tissues, there was no significant difference in flow between the control and inflamed knees during any of the phases.

DISCUSSION

The greater weight of the inflamed joint capsules

compared to the saline-injected knees resulted from tissue edema and thus tends to underestimate blood flow in the inflamed capsules relative to control. In the present study, the results in Fig. 1 show that blood flow is significantly different between control and inflamed posterior capsules but not anterior capsules. The area of carrageenan injection and accumulation of the drug in the posterior region seems to be the reason for this difference.[2] As shown in Figure 1, the significant differences of blood flow in control and inflamed posterior capsule–but not anterior capsule specimens–between phase 3 and 4 could be because of the anatomical innervation of the posterior capsule which is innervated by the posterior articular nerve (PAN).[1,6] Nerve mediated vasoconstriction in the carrageenan-inflamed knee is difficult to assess, as there was a clear trend for blood flow to decline in both the anterior and posterior regions with successive measurements. Thus, the reduction in blood flow observed during PAN stimulation may have been partly due to this downward drift and the actual nerve-mediated vasoconstriction was therefore correspondingly smaller.

The nerve mediated contrictor response failed to show a significant difference between inflamed and control knees and if drift is taken into account, then the difference is likely to become significant. Recent experiments using laser Doppler flowmetry[5] and laser Doppler perfusion imaging[4,6] have shown that nerve-mediated vasoconstriction is reduced in inflamed joints, indicating that inflammation alters the neural control of these vessels, perhaps contributing to the hyperemia of arthritis.

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