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STUDIES ON THE RELATIVE EFFECTS OF PROSTAGLANDINS, BRADYKININ, 5-HYDROXYTRYPTAMINE AND HISTAMINE ON THE SYNOVIAL MICROCIRCULATION IN DOGS

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1 The relative effects of prostaglandins E_1, E_2, $F_{1\alpha}$, $F_{2\alpha}$, bradykinin, histamine and 5-hydroxytryptamine (5-HT) on the canine synovial microcirculation were investigated using the rate of clearance of radioactive xenon (^{133}Xe) as an index of synovial perfusion.

2 All the compounds tested, except prostaglandin $F_{1\alpha}$, produced a vasodilator effect. The descending order of potency of the active compounds was (i) prostaglandin E_1, (ii) prostaglandin E_2 and bradykinin, (iii) histamine and 5-HT, (iv) prostaglandin $F_{2\alpha}$. The most potent compound tested, prostaglandin E_1 produced an effect in nanogram amounts in each joint.

3 Prostaglandin $F_{2\alpha}$ has been reported to have an anti-inflammatory action; however no evidence was found of antagonism of prostaglandin E_1 by two dose levels of prostaglandin $F_{2\alpha}$.

4 Preliminary studies showed that threshold doses of prostaglandin E_1 did not potentiate the vasodilator action of threshold or sub-threshold doses of bradykinin in the dog synovium.

Introduction

Current concepts of the pathophysiology of clinical joint inflammation include a role for the various chemical mediators of inflammation (Austen, 1972). Recently most interest has been displayed in the prostaglandins. Prostaglandin E-like activity has been found in increased amounts in the synovial effusions of experimentally induced arthritis in rabbits (Blackham, Farmer, Radziwonik & Westwich, 1974). Prostaglandins E and F have been shown to be present in rheumatoid synovial effusions (Giroud, Velo, Dunn, Timsit & Willoughby, 1974; Higgs, Vane, Hart & Wojtulewski, 1974). Kinins have also been detected in increased amounts in the synovial fluid of various arthritides (Melmon, Webster, Goldfinger & Seegmiller, 1967). Histamine is present in both normal and abnormal human synovium (Roth, Polley & Cohn, 1964; Zachariae & Zachariae, 1965) but this amine and 5-hydroxytryptamine (5-HT) have attracted less attention from investigators. The effects of these substances have been investigated on a variety of vascular beds (Majno & Pallade, 1961; Lewis, 1964; Zweifach, 1964; Viguera & Sunahara, 1969). As a help to the understanding of the relative importance of the various mediators in the pathogenesis of joint inflammation, we have investigated their activities on the normal synovial microcirculation. In these experiments we have compared the effects of prostaglandins E_1, E_2, $F_{1\alpha}$, and $F_{2\alpha}$, bradykinin, 5-HT and histamine on canine synovial perfusion, using the half-life ($T\frac{1}{2}$) of ^{133}Xenon clearance following intra-articular injection of the isotope.

Methods

These studies were carried out on adult mongrel dogs weighing between 20 and 30 kg and anaesthetized with thiopentone, nitrous oxide, O_2 and less than 1% halothane. Blood pressure and blood gases were monitored and maintained constant throughout all experiments.

Synovial blood flow was monitored indirectly by calculating the half-life ($T\frac{1}{2}$ min) of the clearance of the inert gas, radioactive xenon (^{133}Xe), from the knee and elbow joints following intra-articular injection of 0.1 ml of the gas dissolved in sterile 0.9% w/v NaCl solution (saline). This method provides an index of synovial perfusion (Dick, St. Onge, Gillespie, Downie, Nuki, Gordon, Whaley, Boyle & Buchanan, 1970), a decrease in the $T\frac{1}{2}$ value denoting a vasodilator response and an increase denoting a vasoconstrictor

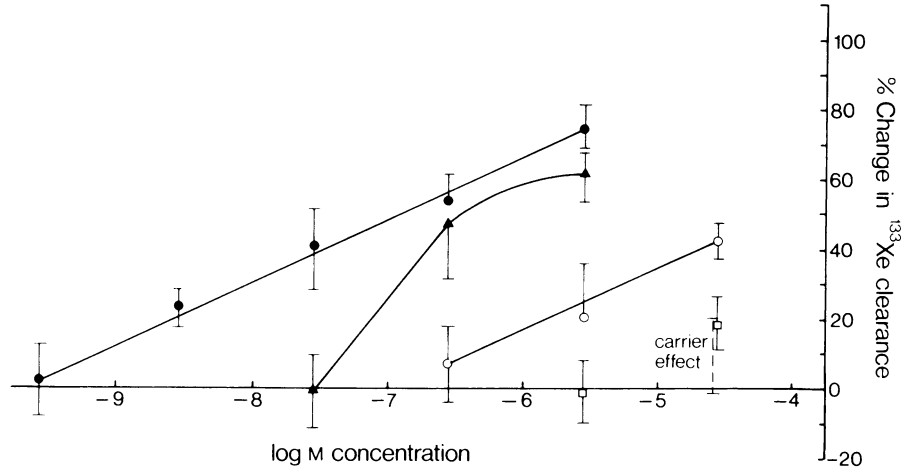

Figure 1 The effects of prostaglandin E_1 (●), E_2 (▲), $F_{1\alpha}$ (□) and $F_{2\alpha}$ (○) on percentage change of $T\frac{1}{2}$ values of ^{133}Xe clearance ($n \geqslant 5$). Vertical lines show s.e. mean.

response. There is considerable individual variation in the base-line xenon clearance from individual joints but repeated studies on the same joint have demonstrated acceptable reproducibility, provided strict attention is paid to critical aspects of the method. (Dick *et al.*, 1970). Clearance of the isotope was followed using a thallium activated sodium iodide scintillation crystal connected to a pulse height analyser, photomultiplier and rate meter recording on a potentiometric chart recorder. In each experiment a base-line recording from 15 to 20 min was taken, followed by further 15 to 20 min recordings following intra-articular injection of the agents used in the various doses (prostaglandins E_1 and E_2 0.1–100 ng; prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$ 1 ng–1 μg; histamine and 5-HT 10 ng–1 μg). The molecular weights of the various compounds tested are prostaglandin E_1 354, E_2 352, $F_{1\alpha}$ 356, $F_{2\alpha}$ 354, histamine acid phosphate 307, 5-hydroxytryptamine creatinine sulphate 405, and bradykinin hydrochloride 1060. All dose levels of each compound were tested five or more times. Statistical significance of alterations in response was determined using the Mann-Whitney *U* test.

The following drugs were used: prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ (Upjohn Co., Kalamazoo, U.S.A.); bradykinin hydrochloride and 5-hydroxytryptamine creatinine sulphate (Sandoz Products Ltd.) and histamine acid phosphate (Evans Medical). Prostaglandins were dissolved in ethanol (1 mg/ml) and diluted further in saline.

Results

Figure 1 compares the dose-response curves for prostaglandins E_1, E_2, $F_{1\alpha}$, and $F_{2\alpha}$. None of the prostaglandin $F_{1\alpha}$ effects shown differed significantly from the effects of the diluent when analyzed by the Mann-Whitney U test.

Prostaglandin $F_{2\alpha}$ produced a significant vasodilator effect in high dosage only and statistical analysis showed no difference between the carrier effect and all but the highest dose shown in Figure 1. Prostaglandins E_1 and E_2 were both extremely potent and produced dose-related vasodilator effects with prostaglandin E_1 being the more potent.

Figure 2 shows that the effects of bradykinin, 5-HT and histamine fell between those of prostaglandins E_1 and $F_{2\alpha}$, the order of potency on a molar basis being prostaglandin E_1, bradykinin, histamine and 5-HT, and then prostaglandin $F_{2\alpha}$.

The lowest effective molar concentrations of the various compounds are shown in Table 1.

Table 1 Approximate threshold concentrations of vasodilators producing change in $T\frac{1}{2}$ values of ^{133}Xe clearances

Mediator	*Threshold concentration (μM)*
Prostaglandin E_1	0.028
Prostaglandin E_2	0.28
Bradykinin	0.1
5-Hydroxytryptamine	2.4
Histamine	3.2
Prostaglandin $F_{2\alpha}$	28.0
Prostaglandin $F_{1\alpha}$	No effect at concentrations up to 28.0

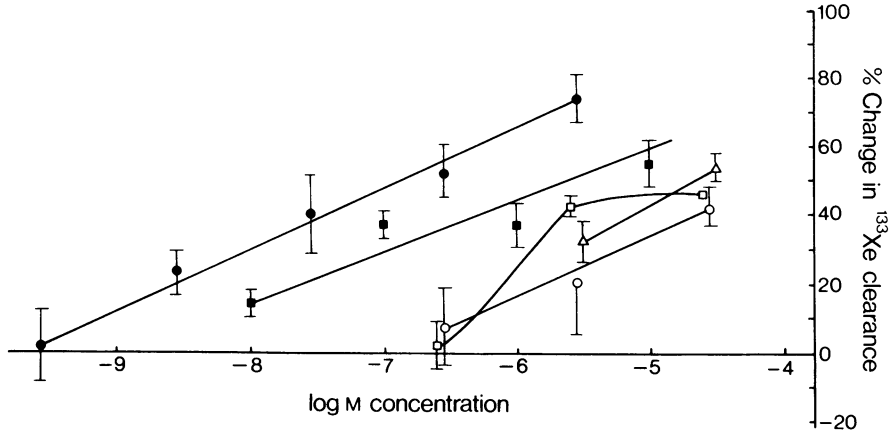

Figure 2 The effects of prostaglandin E_1 (●), $F_{2\alpha}$ (○), bradykinin (■), histamine (△) and 5-hydroxytryptamine (□) on $T\frac{1}{2}$ value of ^{133}Xe clearance ($n \geqslant 5$). Vertical lines show s.e. mean.

Prostaglandin E_1 was the most potent, prostaglandin E_2 and bradykinin approximately 5–10 times less effective than prostaglandin E_1, 5-HT and histamine approximately 50–100 times less effective than prostaglandin E_1 and prostaglandin $F_{2\alpha}$ approximately 500–1000 times less effective than E_1.

The mean percentage changes in ^{133}Xe clearance produced by prostaglandin E_1 28 nM given after pretreatment of the joint with 28 nM or 2.8 μM prostaglandin $F_{2\alpha}$ were 35.5% and 47.1% respectively. The responses were similar to the mean of 39.7% brought about by this dose of prostaglandin E_1 alone.

The mean percentage change in ^{133}Xe clearance produced when bradykinin 0.1 μM was injected just after pretreatment of the joint with prostaglandin E_1 28 nM was $37.2\% \pm 9.9$ which is not detectably different from the change produced by prostaglandin E_1 when injected alone (39.7%). The injection of the subthreshold dose of 10 nM bradykinin after the subthreshold dose of 2.8 nM prostaglandin E_1 produced no significant average vasodilator response ($-10\% \pm 7.3$).

Discussion

In these experiments the effects of various known inflammatory mediators on the normal synovial blood flow have been examined. Prostaglandin E_1 was the most potent vasodilator substance tested producing a response in nanogram amounts. Prostaglandin E_2 was also a potent vasodilator, approximately 5–10 times less potent than prostaglandin E_1. These results are of interest in that prostaglandin E_1 is reported to be the main prostaglandin in the synovial fluid of inflamed joints both in experimental animals (Blackham *et al.*, 1974) and in rheumatoid arthritis (Higgs *et al.*, 1974).

Bradykinin was about as active on a molar basis as prostaglandin E_2 in producing an effect on synovial perfusion. Histamine and 5-HT also produced a definite increase in synovial perfusion in certain doses. Their potency lay between that of prostaglandin E_2 and bradykinin on the one hand and prostaglandin $F_{2\alpha}$ on the other.

Prostaglandin $F_{2\alpha}$ produced a significant vasodilator effect in microgram dosage only and is thus a much weaker vasodilator than bradykinin, prostaglandin E_1 and E_2. Prostaglandin $F_{2\alpha}$ has been shown to inhibit the vascular permeability increasing action of prostaglandin E_1 (Willoughby, 1968) and Giroud *et al.* (1974) have suggested that prostaglandin $F_{2\alpha}$ may be associated with the waning of the naturally occurring inflammatory process and may in fact act as an anti-inflammatory mediator. We looked for an antagonistic effect of two doses of prostaglandin $F_{2\alpha}$ on the vasodilator effect of prostaglandin E_1 in the synovium but found no evidence for this.

Prostaglandin E_1 can in certain situations, potentiate bradykinin both in its pain producing effects (Ferreira, 1972) and in its effects on increasing vascular permeability (Williams & Morley, 1973). The synovial vasculature appears sensitive to small amounts of both prostaglandin E_1 and bradykinin but so far we have been unable to demonstrate potentiation by prostaglandin E_1 of the effects on synovial blood flow of a threshold or subthreshold dose of bradykinin.

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