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THE EFFECTS OF PROSTAGLANDIN E_1, BRADYKININ AND HISTAMINE ON CANINE SYNOVIAL VASCULAR PERMEABILITY

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1 The relative effects of prostaglandin E_1, bradykinin and histamine on canine synovial vascular permeability were investigated by a method based on the measurement of the amounts of radiolabelled dextran (molecular weight 20,000) leaking from the circulation into the synovial cavity.

2 Bradykinin, prostaglandin E_1 and histamine in that order of potency all increased synovial vascular permeability. Threshold concentrations were 0.3 μM, 3 μM and 30 μM respectively.

3 The effects of infusion of prostaglandin E_1 combined with either bradykinin or histamine were greater than mere summation.

Introduction

Prostaglandins and kinins have been demonstrated in increased amounts in the synovial fluid of various arthritides (Melmon, Webster, Goldfinger & Seegmiller, 1967; Higgs, Vane, Hart & Wojtulewski, 1974) and together with histamine may play a part in human clinical joint disease (Zeitlin & Grennan, 1976). We have previously shown that normal synovial blood flow is extremely sensitive to the vasodilator effects of prostaglandin E_1 and bradykinin, and reactive but to a lesser degree, to histamine (Dick, Grennan & Zeitlin, 1976).

In the present study the effects of synovial infusion of these mediators on synovial vascular permeability have been examined by a method based on the exudation of a radiolabelled dextran marker from the circulation into the synovial cavity.

Methods

Animals

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

Experimental procedure

Following intravenous injection of ^{131}I-labelled dextran dissolved in 0.1 ml of 0.9% w/v NaCl solution (saline), blood samples were taken at 5 min intervals from an in-dwelling catheter lying in the cubital artery. Samples of blood (1 ml) were subsequently counted in a Packard automatic gamma spectrometer. The stifle joint was perfused with sterile normal saline or with the test drug dissolved in saline, via a 25 gauge inflow needle inserted distal to the patella. The perfusing solution passed to the inflow needle via a plastic catheter coiled in a water bath kept at 37°C. The joint was drained continuously through a 19-gauge outflow needle placed distal to the patella with the hind limb arranged so that this needle was dependent and the perfusate could be collected. Flow rate was at 1 ml per min and the needles were arranged so that a free flow of fluid was obtained and the joint was not distended. Positioning of the needles was checked initially by aspiration of synovial fluid from the joint. Perfusion of the joint with saline or test solution was carried out after intravenous injection of radiolabelled dextran and 5 ml aliquots of perfusate were collected at the

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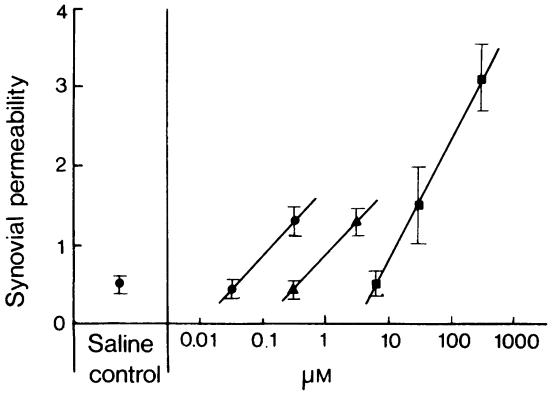

Figure 1 Dose-response relationships of the permeability increasing actions of bradykinin (●), prostaglandin E_1 (▲) and histamine (■) on dog synovial vasculature. Vertical lines show s.e. mean. Permeability to dextran 20 is expressed as: (counts min^{-1} ml^{-1} synovial perfusate) x 100/(counts min^{-1} ml^{-1} blood).

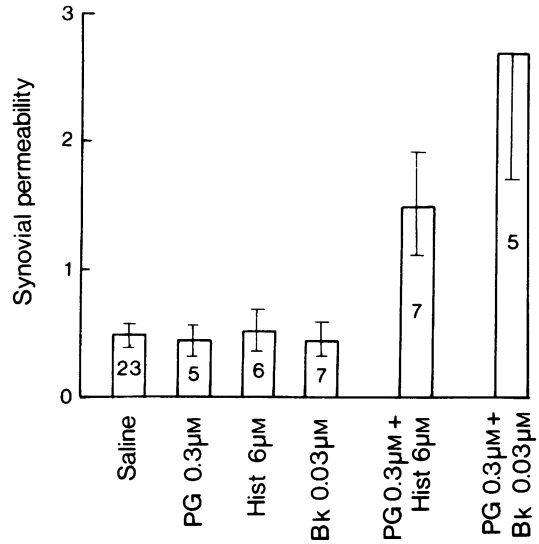

Figure 2 Interactions between the effects of threshold concentrations of prostaglandin E_1 (PG) and histamine (Hist) or bradykinin (Bk) on the permeability of dog synovial vasculature to dextran 20. Vertical lines show s.e. mean. Numbers of joints tested are shown. Permeability is expressed as: (counts min^{-1} ml^{-1} synovial perfusate) x 100/(counts min^{-1} ml^{-1} blood).

same time as blood samples every 5 minutes. If the fluid collected was blood-stained or cloudy the results for that particular joint were discarded. All samples were subsequently counted for 1 min in the Packard automatic gamma spectrometer and the radioactivity per ml of the isotope in the collected perfusate was calculated and expressed as a fraction of the isotope in the blood sample taken at the same time. The effects of various concentrations of mediators in the test joint were compared with those of saline in the control joint perfused for the same length of time and the dose-response relationships of the mediators were established.

The interactions of prostaglandin E_1 with bradykinin and histamine were investigated by perfusion of the joint with a sub-threshold concentration of either histamine or bradykinin in the presence and absence of sub-threshold concentrations of prostaglandin E_1. The opposite joints in these experiments were used as controls and were perfused with one of the mediators alone or with saline.

Statistical significance of alterations in response was determined using the Mann-Whitney U test. All doses were repeated on from 5 to 7 joints. The saline control was repeated on 23 joints.

Dextran

Dextran 20 (average molecular weight 20,000) was supplied by Pharmacia Fine Chemicals and labelled with ^{131}I using chloramine T and sodium metabisulphite. Radiolabelled dextran solutions were made up freshly before each experiment. An aliquot was retained after labelling and on completion of the day's experiment the stability of the label was checked by electrophoresis and by thin layer chromatography using a cellulose plate and 80% ethanol in water as solvent. A single peak was always obtained with both methods showing that the label had been stable for the duration of the experiments.

The effective diffusion radius of dextran 20 is approximately equivalent to that of serum albumin (Grotte, 1956).

Drugs

Histamine was supplied as histamine acid phosphate (Evans Medical). Synthetic bradykinin was obtained from Sandoz Ltd. Prostaglandin E_1 (Upjohn Co.) was stored in ethanolic solution at −20°C. Solutions of the various concentrations of drugs tested were made up freshly in saline before each experiment.

Results

Bradykinin, prostaglandin E_1 and histamine all caused increases in synovial permeability. The dose-response relationships are shown in Figure 1. The effects of perfusion with 6 μM histamine were similar to those of

the saline control ($P > 0.05$; $n = 6$) while the two higher concentrations 30 μM and 300 μM produced dose-related responses ($P < 0.05$; $n = 4$; $P < 0.001$; $n = 7$, respectively).

Responses to 0.3 μM solutions of prostaglandin E_1 were no greater than those to saline ($P > 0.05$; $n = 5$) while 3 μM solutions produced significant effects ($P < 0.001$; $n = 5$). Bradykinin in 30 nM solutions produced no significant effect ($P > 0.05$; $n = 7$) when compared to the saline response while 0.3 μM bradykinin significantly increased permeability ($P < 0.05$; $n = 4$).

The approximate threshold concentrations for these mediators in descending order of potency are bradykinin 0.3 μM, prostaglandin E_1 3 μM and histamine 30 μM.

Figure 2 shows the effects on synovial permeability of sub-threshold concentrations of either histamine or bradykinin when given together with a sub-threshold concentration of prostaglandin E_1. The effects both of histamine with prostaglandin E_1 ($n = 7$) and of bradykinin with prostaglandin E_1 ($n = 5$) appear to be greater than of either drug given alone ($P < 0.025$; $P < 0.001$ respectively). The effects of the two drug combinations were greater than the summation of the effects of the individual drugs by 56% and 167% respectively.

Discussion

Increased synovial vascular permeability with a resultant increase in synovial fluid volume is a feature of inflammatory joint disease (Boyle & Buchanan, 1971). In rheumatoid arthritis, synovial fluid has been shown to contain raised levels of bradykinin (Melmon *et al.*, 1967; Keele & Eisen, 1970) and prostaglandin (Higgs *et al.*, 1974). Mast cells are plentiful in the rheumatoid synovium (Norton & Ziff, 1966) and histamine and histidine-decarboxylase activity have been detected in the rheumatoid synovial membrane (Roth, Polley & Code, 1964).

Bradykinin (Sturmer & Cerletti, 1961; Lewis, 1964), prostaglandin E_1 (Kaley & Weiner, 1971) and histamine (Rocha e Silva, 1966) all increase vascular permeability in a variety of tissues and species and have now been shown to do so in the dog synovium. The most potent of these was bradykinin which increased synovial vascular permeability at an approximate threshold concentration of 0.3 μM. Bradykinin was 10 times more potent on a molar basis than prostaglandin E_1. This contrasts with the order of potency of these two mediators on dog synovial blood flow where the prostaglandin was nearly 4 times more active than bradykinin (Dick *et al.*, 1976). In the blood flow study, the drugs tested were administered as single injections into the synovium. The reversal of the potency order might possibly result from the greater lability of bradykinin in contact with biological fluids. This would not be an important factor in the present synovial perfusion method where the synovial level of bradykinin is kept constant.

Ferreira, Moncada & Vane (1974) have shown that the pain producing activity of bradykinin in the dog knee joint is potentiated by infusion of prostaglandins E_1 and E_2. In the experiments described here, the permeability response to synovial infusion of prostaglandin E_1 together with bradykinin or histamine also appears to be greater than a simple summation of effects. Prostaglandin E_1 has been shown to potentiate the rat paw oedema produced by bradykinin (Moncada, Ferreira & Vane, 1973; Lewis, Nelson & Sugrue, 1975) and to potentiate the actions of bradykinin and histamine in increasing cutaneous vascular permeability in guinea-pigs (Williams & Morley, 1973). No such potentiation was found in our earlier studies on dog synovial blood flow (Dick *et al.*, 1976).

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