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Histamine, 5-hydroxytryptamine, kinins and the anti-inflammatory activity of human plasma fraction in carrageenan-induced paw oedema in the rat

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In the carrageenan-induced paw oedema reaction in the rat, the swelling and accumulation of [^{131}I]albumin showed a similar time course over 4 h. Both aspects of the oedema were inhibited by a fraction prepared from human plasma. Treatment of the animals with substances which either deplete the levels or antagonize the actions of either histamine, 5-hydroxytryptamine or kinins showed that these mediators are concerned in the development of rat paw oedema after the injection of either carrageenan or yeast but not with the anti-inflammatory action of the plasma fraction.

A fraction showing anti-inflammatory activity in the carrageenan-induced rat paw oedema test has been isolated from human plasma (Ford-Hutchinson, Insley & others, 1973). In this acute inflammatory reaction it has been claimed (Leme, Hamamura & others, 1973) that a phase involving leakage of plasma proteins precedes and may be distinguished from a second phase concerned with an increased permeability to water. Conventional anti-inflammatory drugs, such as aspirin and indomethacin, were reported to act on the initial phase of the response. We have now studied the possible existence of these sequential aspects over the first 3 h of the carrageenan test by measuring both the swelling and the leakage of intravenously injected [^{131}I]albumin.

To determine if the anti-inflammatory activity of the plasma fraction could be due to its interaction with either histamine, 5-hydroxytryptamine (5-HT) or kinins, the effects of treating the animals with either compound 48/80, a mixture of mepyramine and methysergide, ellagic acid or aprotinin were investigated in the absence and presence of the plasma fraction. Additional experiments were carried out with yeast-induced rat paw oedema with and without the administration of a mixture of the histamine and 5-HT antagonists and the plasma fraction. The actions of the individual mediators on appropriate isolated tissues in the presence or absence either of known antagonists or of the plasma fraction were also investigated.

MATERIALS AND METHODS

Animals. Female albino Wistar rats (Oxfordshire Laboratory Animal Colonies, Southern Ltd), 150–200 g, were used for anti-inflammatory testing and as the source of isolated tissue preparations, uterus and fundus. Isolated preparations of ileum were obtained from guinea-pigs of the King's College Hospital strain.

Preparation of plasma fraction. The procedure was that described by Ford-Hutchinson & others (1973). The plasma fraction used in the present work was their combined fractions II and IV, prepared from pooled human plasma (Blood Transfusion

Centre, Tooting, London, S.W.17) by ultrafiltration in an Amicon 2L cell under an atmosphere of N_2 using a Diaflo PM 10 membrane followed by elution of the concentrated ultrafiltrate with distilled water after application to a Sephadex G 25 fine column.

Anti-inflammatory testing: carrageenan rat paw oedema

The method used was that of Winter, Risley & Nuss (1962). In the control groups each animal received either 1 ml of 0·9% (w/v) NaCl and in the experimental groups each rat received 1 ml of plasma fraction, prepared as described above. All the injections were filtered through Millipore Millex filter units, type GS 0·22 μm pore size, before being given intravenously into a tail vein 30 min before the injection of 0·1 ml of 1·0 g per 100 ml (w/v) carrageenan (Viscarin Marine Colloids) in 0·9% (w/v) NaCl in the plantar region of the right hind foot. Foot volumes were measured using a mercury plethysmograph (Arnold R. Horwell, Ltd., London) immediately after the injection of the carrageenan (0 h) and at hourly intervals for 4 h. The results were calculated as mean percentage increases in the volume of the injected paw compared to the value at 0 h.

Vascular response. Each rat received 3·3 μCi (120 μg protein) of [^{131}I]human serum albumin (Radiochemical Centre, Amersham, Bucks) mixed with either saline or plasma fraction, intravenously into a tail vein 30 min before the administration of the carrageenan. Groups (5 rats) were killed after 1, 2 and 3 h, both hind feet were cut off at the medial malleolus and the radioactivity in the excised paws counted using a γ spectrometer (Packard Instruments Co.). The results were calculated as the counts min^{-1} of the injected foot minus the counts min^{-1} of the control foot. This and all subsequent experiments with intact rats were repeated on at least two occasions.

Depletion and antagonism of histamine and 5-HT. Groups of 10 rats were depleted of their stores of histamine and 5-HT by repeated injections of compound 48/80 (Burroughs Wellcome and Co. Ltd.) according to Di Rosa, Giroud & Willoughby (1971). Either saline or plasma fraction was administered by intravenous injection to four groups, each of 5 rats; two of the groups had received the 48/80 treatment and two groups had not. Foot volumes were measured as described above. Similar experiments were performed with further groups of rats, some of which were given intravenous injections of mepyramine maleate (May & Baker) and methysergide bimaleate (Sandoz) to antagonize the actions of histamine and 5-HT. A mixture of the two drugs, both at doses of 2·5 mg kg^{-1}, was given by tail vein 15 min before the carrageenan injection and a further injection of the methysergide was given at 2 h according to the directions of Crunkhorn & Meacock (1971).

Depletion and antagonism of the kinin system. Similar experiments were performed in which the kininogen depletor, ellagic acid (Koch-Light Laboratories, practical grade) was injected slowly in a tail vein as a 0·2mM solution in 0·15M tris-HCl buffer, pH 7·35. Three injections, each of 0·5 ml, were performed at 5 min intervals, 30 min before the injection of carrageenan (Crunkhorn & Meacock, 1971). The liberation of kinins during the carrageenan reaction was blocked by the injection of aprotinin (Trasylol, Bayer Germany). Each treated rat received 2 ml of a preparation, containing a total of 20 000 kallikrein inactivating units, by intraperitoneal injection 30 min before the carrageenan (Di Rosa & Sorrentino, 1970).

Yeast paw oedema

The method was that described by Garattini, Jori & others (1965) in which each rat received 10 mg of yeast (Type II, Baker's Yeast, Sigma Chemical Co.) suspended in 0·1 ml of 0·9% (w/v) NaCl by subplantar injection. Foot volumes were measured as above. Other groups of rats were pretreated with a mixture of mepyramine maleate and methysergide bimaleate as described for carrageenan oedema.

Isolated tissue experiments

Guinea-pig ileum. A 2 cm strip of guinea-pig ileum was placed in a 10 ml organ bath containing Tyrode solution at 30°. A concentration of histamine (histamine acid phosphate B.P.) which produces a 70% of maximal contraction was chosen as the test system and after a reproducible series of responses were obtained several quantities, each of 1 ml, of the plasma fraction were added to the bath 1 min before further addition of the histamine. The effect of the addition of mepyramine maleate on the histamine stimulated preparation was also studied.

Rat fundus. Similar experiments were performed with the fundus of the rat stomach prepared by the method of Vane (1957). Contractions were induced by 5-HT (5-hydroxytryptamine creatinine sulphate complex, Sigma Chemical Co.) and the effects of the separate additions of methysergide and the plasma fraction were investigated.

Rat uterus. This preparation (Horton, 1959) was used to study the effects of the plasma fraction on contractions induced by synthetic bradykinin (bradykinin triacetate, Sigma Chemical Co.).

RESULTS

Vascular response in carrageenan oedema

The time courses of the swelling and leakage of the labelled albumin from the circulation into the inflamed paw are shown in Fig. 1. The two curves in the saline-treated animals are almost identical and there was no evidence of an initial phase in which leakage of vascular proteins predominated. Pretreatment of the animals with the plasma fraction significantly reduced both the oedema and extravasation of the labelled albumin in a similar fashion.

Effects of histamine and 5-HT depletion and blockade

The effects of compound 48/80 and mepyramine and methysergide on the development of the carrageenan oedema in either the presence or absence of the plasma fraction are shown in Fig. 2. Both the depleting agent and the combination of the antagonists significantly reduced the development of carrageenan oedema over the time period studied. The administration of the plasma fraction produced considerably more inhibition of the carrageenan oedema and pretreatment with 48/80 further reduced the swelling. This last effect was not observed in the mepyramine/methysergide experiment with the carrageenan reaction but was obtained when yeast was used as the irritant (Fig. 3).

The plasma fraction did not affect the contractions induced by either histamine or 5-HT in the guinea-pig isolated ileum and rat fundus preparations.

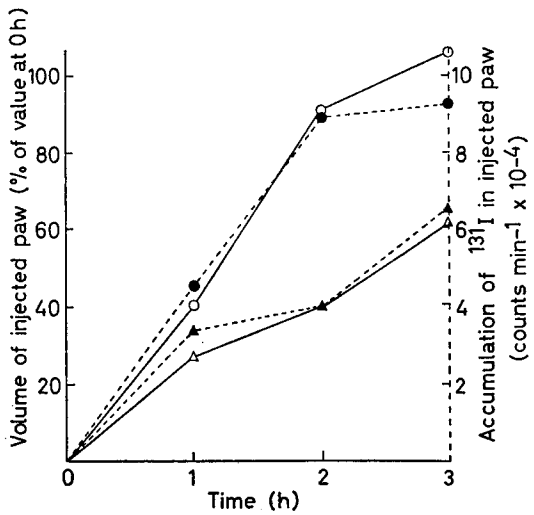

Fig. 1. Effects of plasma fraction on the swelling and the leakage of vascular protein in the carrageenan rat paw oedema test. Results calculated as either volume of paw as a percentage of corresponding value at 0 h or counts min^{-1} of radioactivity in injected paw minus counts min^{-1} in control paw. They are given as means, each experimental point representing 10 rats for the following groups, ○——○ saline control paw swelling; △——△ plasma fraction, paw swelling; ●- - - -● saline control ^{131}I accumulation; ▲- - - -▲ plasma fraction, ^{131}I accumulation. In this and the subsequent figures the values between the different groups have been analysed by the *t*-test. A statistically significant difference ($P < 0.02$) at one or more of the time intervals studied has been taken to represent a significant effect of the particular treatment. The plasma fraction inhibited both the swelling and extravasation of labelled albumin in the carrageenan paw reaction.

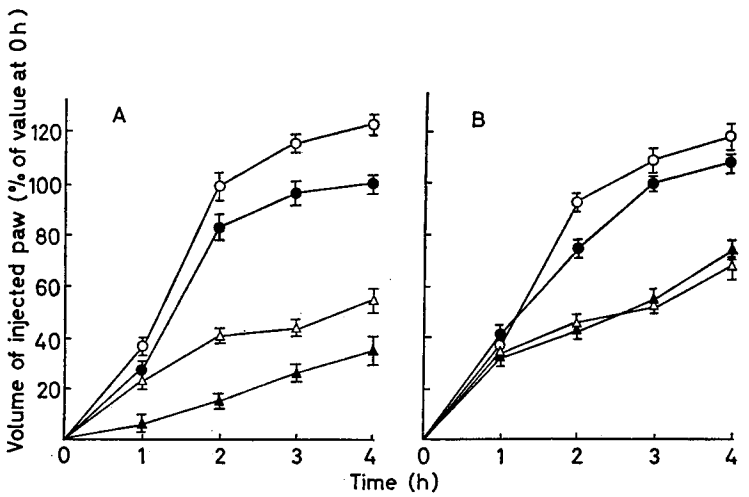

FIG. 2. Effects of plasma fraction in carrageenan rat paw oedema test in the presence or absence of either a depletor or antagonists of histamine and 5-HT. Results calculated as volume of paw as a percentage of corresponding value at 0 h. They are given as means ±s.e. for the following group, each of 10 animals: A; ○——○ saline control, △——△ plasma fraction, ●——● saline control pretreated with compound 48/80, ▲——▲ plasma fraction pretreated with compound 48/80, B; ○——○ saline control, △——△ plasma fraction, ●——● saline control treated with mepyramine and methysergide, ▲——▲ plasma fraction treated with mepyramine and methysergide. Pretreatment of the rats with 48/80 reduced the swelling of the saline controls and of the rats treated with plasma fraction. The plasma fraction alone caused a greater reduction of swelling than 48/80 alone. Similar effects were found with the mixture of mepyramine and methysergide except that the administration of the antagonists did not enhance the inhibitory effect of the plasma fraction.

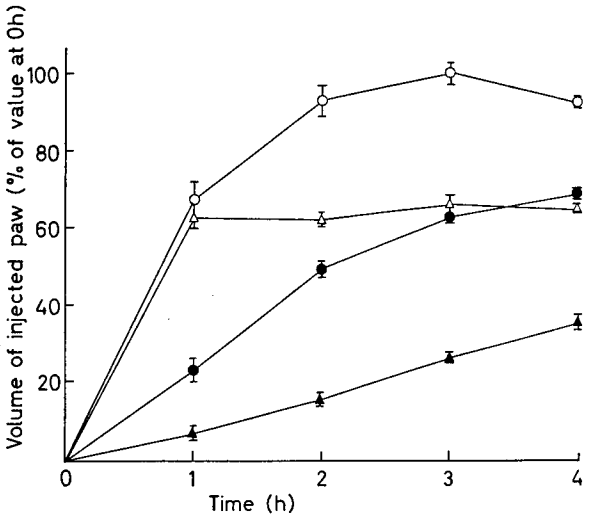

FIG. 3. Effects of plasma fraction and a mixture of mepyramine and methysergide in yeast induced paw oedema test. Results calculated and expressed as in Fig. 2 for the following group, each of 5 rats; ○——○ saline control, △——△ plasma fraction, ●——● saline control plus mepyramine and methysergide, ▲——▲ plasma fraction plus mepyramine and methysergide. The plasma fraction inhibited the yeast-induced paw oedema at 2, 3 and 4 h. Treatment with mepyramine plus methysergide alone caused a larger inhibition of the swelling from 1 h onwards and a further reduction of the oedema occurred when the rats were treated with plasma fraction and the mixture of antagonists.

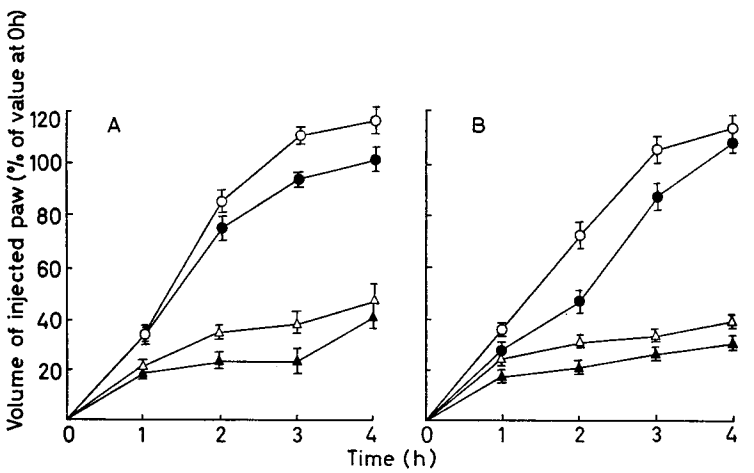

FIG. 4. Effects of plasma fraction in carrageenan rat paw oedema test in the presence or absence of either a depletor or inhibitor of the kinin system. Results calculated and expressed as in Fig. 2 for the following group: A; ○——○ saline control, △——△ plasma fraction, ●——● saline control pretreated with ellagic acid, ▲——▲ plasma fraction pretreated with ellagic acid, B; ○——○ saline control, △——△ plasma fraction, ●——● saline control treated with aprotinin, ▲——▲ plasma fraction treated with aprotinin. Pretreatment of the rats with ellagic acid or treatment with aprotinin reduced the swelling of the saline controls and of the animals which received the plasma fraction. The plasma fraction produced larger inhibitions of paw oedema than either the ellagic acid or aprotinin.

Effects of kininogen depletion or kinin blockade

The effects of ellagic acid and aprotinin on the development of the carrageenan oedema in the presence or absence of the plasma fraction are shown in Fig. 4. Both the kininogen depletor and the kallikrein inhibitor significantly reduced the development of the swelling over the period 2–4 h in either the presence or absence of the plasma fraction. In the rat uterus preparation the contractions induced by bradykinin were not affected by the plasma fraction.

DISCUSSION

The results of the present work show that the anti-inflammatory activity of a fraction isolated from normal human plasma affects both the development of swelling and the leakage of vascular proteins in the carrageenan-induced paw oedema test in the rat. Both aspects of the oedema and their reduction by pretreatment with the plasma fraction followed a similar time course. There was no evidence that a net dissociation existed between maximal extravasation of protein and maximal swelling. This finding agrees with the results of similar experiments involving the leakage of radioactively labelled albumin (Di Rosa & others, 1971) but not with those of Leme & others (1973) who used dye-labelled albumin and a coaxial perfusion technique to measure the extravasation of the proteins into the paw.

A number of mediators have been identified during the development of carrageenan-induced paw oedema in the rat (Di Rosa, 1972) and it has been suggested that several of these are released in an ordinate sequence (Willoughby & Di Rosa, 1971). In particular it has been stated that there is an initial release of histamine and 5-HT, the effects of which predominate during the first 90 min of the oedema, followed by a second phase, mediated by release of kinins, which persists over the period $1\frac{1}{2}$ to $2\frac{1}{2}$ h after injection of the carrageenan (Di Rosa & others, 1971). The experiments in the present work were designed to obviate the actions of these mediators as completely as possible so that the effects of the plasma fraction on the residual swelling in carrageenan paw oedema reaction could be tested. Compound 48/80 (Spector & Willoughby, 1959) was used to deplete the stores of histamine and 5-HT, a mixture of mepyramine and methysergide (Crunkhorn & Meacock, 1971) was employed to block the actions of the liberated amines, ellagic acid (Gautvik & Rugstad, 1967) was given as a kininogen depletor and aprotinin (Di Rosa & Sorrentino, 1970) was used to inhibit the formation of kinins by kallikrein. In each instance the depleting and blocking agents significantly reduced the development of the carrageenan oedema. The extent and time course of the inhibition observed with each agent was very similar. This finding suggests that histamine, 5-HT and kinins are liberated at the same time, rather than sequentially, and also that their effects run parallel over the first 4 h during the development of carrageenan paw oedema.

In each experiment the plasma fraction produced a much larger inhibition of the swelling than did any one of the depleting or blocking agents studied. However, these substances, either in the carrageenan or yeast oedema experiments, caused a further and significant reduction in the swelling when administered with the plasma fraction. Thus the inhibitory effect of the plasma fraction on these acute paw oedema reactions in the rat cannot be due to a specific interference with either the release or action of histamine, 5-HT or the kinins. Additional evidence that the plasma fraction does not exert its anti-inflammatory effects by interacting with histamine and 5-HT is that it is relatively inactive against dextran-induced rat paw

oedema (Elliott, Ford-Hutchinson & Smith, 1974). In this latter reaction it has been reported that the amines play a particularly prominent role in the production of the swelling (Garattini & others, 1965). The results of the *in vitro* experiments with histamine, 5-HT and bradykinin showed that the plasma fraction did not antagonize the effects of these mediators on appropriate isolated tissues.

It is possible that the plasma fraction exerts its anti-inflammatory action by interacting with other mediators thought to be concerned in the development of acute paw oedemas in the rat. These include the complement system, prostaglandins, slow reacting substances and factors affecting leucocyte emigration.

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