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Prostaglandins and the anti-inflammatory activity of a human plasma fraction in carrageenan-induced paw oedema in the rat

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A fraction prepared from normal human plasma inhibits the swelling of the acute carrageenan-induced paw oedema in the rat. Treatment of the animals with prostaglandin E_1, arachidonic acid or with substances which either block the formation of prostaglandins or antagonize their action showed that these mediators are concerned in the development of the paw oedema. The anti-inflammatory effect of the plasma fraction in the carrageenan reaction does not involve a specific interference with the prostaglandin system. The plasma fraction is not an inhibitor of the action of SRS-A on the guinea-pig ileum.

A fraction showing marked and reproducible anti-inflammatory activity in the carrageenan-induced paw oedema test in the rat has been prepared from normal human plasma (Ford-Hutchinson, Insley & others, 1973). A number of mediators have been identified during the development of this acute inflammatory reaction (Di Rosa, 1972), and previous work (Bolam, Elliott & others, 1974) showed that the anti-inflammatory action of the plasma fraction is not due to a specific interference with either the release or action of either histamine, 5-hydroxytryptamine or kinins. To determine if the plasma fraction interacted with the prostaglandin system the effects of treating the animals with eicosatetraynoic acid, a dibenzoxazepine derivative (SC-19220), prostaglandin E_1 or arachidonic acid were investigated in the presence or absence of the plasma fraction. The actions of prostaglandins E_1, E_2 and $F_{2\alpha}$ and of guinea-pig SRS-A on appropriate isolated tissues in the presence or absence of the plasma fraction were also studied.

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 the isolated fundus and colon. Isolated preparations of ileum and the SRS-A were obtained from guinea-pigs of the King's College Hospital strain.

Materials. Prostaglandins E_1, E_2 and $F_{2\alpha}$ (trimethamine salt) were a gift from Dr. J. E. Pike, Upjohn Co. Ltd., arachidonic acid (Grade 1) was obtained from the Sigma Chemical Co., 5,8,11,14-eicosatetraynoic acid was a gift from Dr. K. J. Stone, Roche Products Ltd., the dibenzoxazepine (SC-19220*) was a gift from Dr. J. H. Sanner, G. P. Searle & Co., U.S.A. and the SRS-A antagonist (FPL 55712†) was a gift from Dr. P. Sheard, Fisons Ltd. Carrageenan was obtained from Viscarin Marine Colloids, methysergide bimaleate from Sandoz Ltd. and atropine sulphate from the

* 1-Acetyl-2-(8-chloro-10,11-dihydrobenz[b,f][1,4]-oxazepine-10-carbonyl) hydrazine.

† Sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4*H*-chromene-2-carboxylate.

Sigma Chemical Co. The plasma fraction used was the combined fractions II and IV (Ford-Hutchinson & others, 1973) 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 G25 fine column.

Carrageenan rat paw oedema. The method used was that of Winter, Risley & Nuss (1962). In the control group each animal received 1 ml of 0·9 g per 100 ml (w/v) NaCl (saline) and in the experimental group 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 carrageenan in the plantar region of the right hind foot. Foot volumes were measured using a mercury plethysmograph (Arnold R. Horwell, Ltd.) immediately after the injection of the irritant (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.

5,8,11,14-Eicosatetraynoic acid experiments. Preliminary experiments were made with groups of 5 rats to determine the most effective dose and route of administration of the eicosatetraynoic acid in producing the maximum inhibition of the carrageenan-induced rat paw oedema. The best results were obtained with the intraperitoneal injection of 2 ml of a homogenized suspension containing 100 mg of the eicosatetraynoic acid and 1 mg of carboxymethylcellulose in saline given 45 min before the subplantar injection of the carrageenan. Either saline or plasma fraction was administered by intravenous injection to four groups, each of 5 rats, 30 min before the carrageenan; two of the groups had previously received the eicosatetraynoic acid-carboxymethylcellulose-saline suspension and two groups the carboxymethylcellulose-saline. Foot volumes were measured as described above. This and all subsequent experiments with intact rats were repeated on at least two occasions.

SC-19220 experiments. Similar experiments were performed in which the prostaglandin antagonist, SC-19220, was administered intraperitoneally 45 min before the carrageenan, as a suspension containing 10 mg of SC-19220 in 1 ml of 0·1% (v/v) of polyoxyethylene sorbitan mono-oleate (Tween 80, Sigma Chemical Co.) in saline (Sanner, 1972). Either saline or plasma fraction was administered by intravenous injection to four groups, each of 5 animals, 30 min before the carrageenan; two of the groups had previously received the SC-19220 treatment and two had received only the Tween 80 solution.

Prostaglandin E_1 and arachidonic acid experiments. Either saline or the plasma fraction was given intravenously to four groups, each of 5 rats, 30 min before the carrageenan. Each animal of two of the groups received 100 ng of prostaglandin E_1 by subplantar injection, together with the carrageenan, into the right hind paw and each rat in the other two groups received 100 μg of arachidonic acid in a similar manner (Lewis, Nelson & Sugrue, 1974). Foot volumes were measured every 30 min. Other experiments were performed by the method of Atkinson, Boura & Hicks (1969) to determine if either the prostaglandin or the arachidonic acid, in the doses used as described above, caused local swelling after subplantar injection. In addition the effects of pretreatment with either the eicosatetraynoic acid or SC-19220 on the potentiation of the carrageenan-induced paw oedema by either the arachidonic acid or the prostaglandin was studied in further groups of rats.

Isolated tissue experiments

Prostaglandins. Rat fundus preparations (Vane, 1957) were placed in a 10 ml organ bath containing Krebs solution at 37°. Concentrations of prostaglandins E_1, E_2 and $F_{2\alpha}$ which produced 70% of maximal contractions were chosen as the test system and after reproducible series of responses were obtained, several quantities, each of 1 ml, of the plasma fraction were added to the bath. Similar experiments were performed with isolated preparations of rat colon and guinea-pig ileum.

SRS-A. Guinea-pigs, 500 g, were sensitized with egg albumen (BDH Flake) given as 100 mg of a 10% (w/v) solution once by intraperitoneal injection and once by subcutaneous injection. Three weeks later the animals were killed, the lungs removed and SRS-A prepared according to the directions of Augstein, Farmer & others (1973). The activity of the preparation was tested on guinea-pig ileum, 3 cm strips in a 10 ml organ bath at 30° in Tyrode solution containing μM mepyramine bimaleate and μM atropine sulphate. The effects of concentrations of FPL 55712 up to 100 ng ml^{-1} and of amounts of the plasma fraction up to 1 ml on the contractions induced by the SRS-A preparation were studied.

RESULTS

Eicosatetraynoic acid experiments

The results given in Fig. 1 show that pretreatment of the animals with eicosatetraynoic acid significantly reduced the development of the carrageenan oedema over the time period studied. The administration of the plasma fraction caused a smaller, but still significant inhibition of the paw swelling. The combination of eicosatetraynoic acid and the plasma fraction produced inhibitions at 3 and 4 h significantly greater than were obtained with either treatment given separately.

SC-19220 experiments

The effects of pretreatment with this prostaglandin antagonist in either the presence or the absence of the plasma fraction are given in Fig. 2. The SC-19220 and the plasma fraction produced similar and significant inhibitions of the paw oedema over 2–4 h but the combination of treatments caused a significantly greater response.

Potentiation of carrageenan reaction by prostaglandin E_1 and arachidonic acid

The effects of prostaglandin E_1 and arachidonic acid on the development of carrageenan-induced paw swelling in groups of animals receiving either saline or plasma fraction are given in Fig. 3. Both the prostaglandin and its precursor significantly enhanced the response to carrageenan. This effect became evident with both substances at 30 min but disappeared more quickly with the prostaglandin. The plasma fraction showed significant anti-carrageenan activity in both sets of experiments, but the addition of either prostaglandin E_1 or arachidonic acid caused significant increases in the oedema. Thus the potentiating effects of the prostaglandin and its precursor on the carrageenan response were obtained in either the presence or the absence of the plasma fraction.

The specificity of eicosatetraynoic acid and SC-19220 as inhibitors of either prostaglandin formation or prostaglandin E_1 action in the carrageenan test was studied by repeating the above experiments and substituting pretreatment with the inhibitors

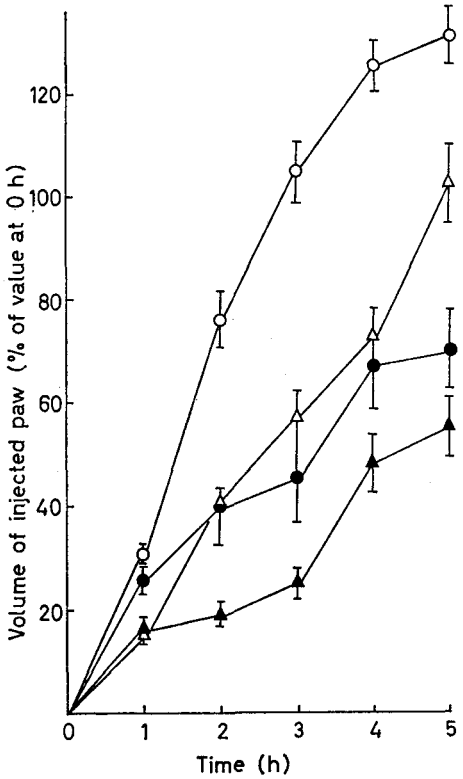

FIG. 1. Effects of plasma fraction in carrageenan-induced paw oedema in the presence or absence of eicosatetraynoic acid. Results calculated as volume of paw as a percentage of corresponding value at 0 h. They are given as means ± s.e. for the following groups, each of 10 animals; ○——○ saline control, △——△ plasma fraction, ●——● saline control pretreated with eicosatetraynoic acid, ▲——▲ plasma fraction pretreated with eicosatetraynoic acid. In this and the subsequent figures the values between the various groups have been analysed by the *t*-test. A statistically significant difference ($P < 0{\cdot}02$) at one or more of the time intervals studied has been taken to represent a significant effect of the particular treatment. Pretreatment of the rats with either eicosatetraynoic acid or the plasma fraction significantly reduced the swelling of the saline controls. The combination of the two inhibited the development of the foot swelling to a significantly greater extent than either treatment given separately.

for administration of the plasma fraction. Eicosatetraynoic acid blocked the potentiating effect of arachidonic acid and SC-19220 that of the prostaglandin.

The local irritant effect caused by the administration of either 100 ng of prostaglandin E_1 or 100 μg of arachidonic acid was both small and transient, the maximum increase in foot volume was $0{\cdot}23 \pm 0{\cdot}10$ ml occurring 30 min after the subplantar injection of either substance.

Isolated tissue experiments

The plasma fraction did not affect the contractions induced by either prostaglandins E_1, E_2 and $F_{2\alpha}$ in isolated preparations of the fundus of the rat stomach, rat colon and guinea-pig ileum or by a guinea-pig SRS-A preparation on the guinea-pig isolated ileum. The last response was completely blocked by the specific antagonist (FPL 55712).

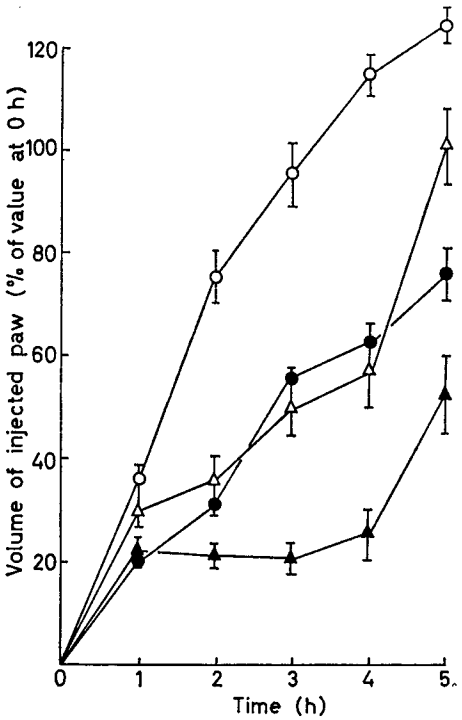

FIG. 2. Effects of plasma fraction in carrageenan-induced paw oedema in the presence and absence of SC-19220. Results calculated and expressed as in Fig. 1 for the following groups, each of 10 animals; ○——○ saline control, △——△ plasma fraction, ●——● saline control treated with SC-19220, ▲——▲ plasma fraction treated with SC-19220. Either the plasma fraction or the SC-19220 treatment caused significant reductions of the paw swelling but the effect was significantly greater when a combination of the two treatments was used.

DISCUSSION

One approach to the investigation of the mechanism of action of the fraction from human plasma in the carrageenan-induced rat paw oedema test is to study its possible interactions with known or suspected mediators of inflammation. In this accepted and widely used inflammatory reaction it has been postulated that the sequence of events leading to the production of the inflammatory response involves the release of histamine and 5-HT, the formation of kinins and the activation of the prostaglandin system and possibly slow-reacting substances (Di Rosa, Papadimitriou & Willoughby, 1971). Previous work has shown that the plasma fraction does not specifically interfere with either the release or action of either histamine, 5-HT or kinins during the development of carrageenan-induced paw oedema in the rat (Bolam & others, 1974).

Although prostaglandins have been implicated as inflammatory mediators in a number of experimental situations the evidence for their involvement in the carrageenan-induced paw oedema reaction is not extensive. It has been reported that the subplantar injection of prostaglandin E_1 in the rat induced local paw oedema (Arora, Lahiri & Sanyal, 1970). However, the increase in foot volume caused by the prostaglandin itself is both small, ranging from 0·1 to 0·2 ml, and transient (Moncada, Ferreira & Vane, 1973). Similar results were observed in the present work. An

increase in prostaglandin-like activity has been observed in the oedema fluid from rat feet inflamed by carrageenan (Willis, 1969). The interpretation of this finding is not straightforward since it was considered that some or all of this increased activity could have been released due to the physical damage which occurs when the oedema fluid was mechanically squeezed out of the affected paws.

One method of studying the possible role of the prostaglandin system in the carrageenan-induced paw oedema test itself is the administration of specific inhibitors of either prostaglandin formation or prostaglandin action. Such inhibitors are now available and their use is preferable to that of aspirin-like drugs which are known to exert multiple effects on many phases of cellular and tissue metabolism (Smith & Dawkins, 1971). A specific inhibitor of prostaglandin formation from the precursor, arachidonic acid, is the substance 5,8,11,14-eicosatetraynoic acid (Downing, Ahern & Bachta, 1970). It has been reported that treatment of rats with this substance reduces their ability to biosynthesize prostaglandins from arachidonic acid (Shaw, Jessup & Ramwell, 1972) and that both the carrageenan reaction and prostaglandin content of the rat paw are effectively reduced. A significant effect in reducing the paw swelling was found in the present work (Fig. 1). Further evidence that the eicosatetraynoic acid specifically blocked prostaglandin formation from arachidonic acid was obtained from the experiments in which the potentiating effect of the precursor in the carrageenan hind paw test was blocked by pretreatment of the animals with eicosatetraynoic acid.

These findings are presumptive evidence that the prostaglandin system is concerned in the development of carrageenan-induced paw oedema in this species. Confirmatory evidence was obtained by the use of a dibenzoxazepine hydrazide (SC-19220) which is a specific antagonist of prostaglandin actions, particularly those of the E

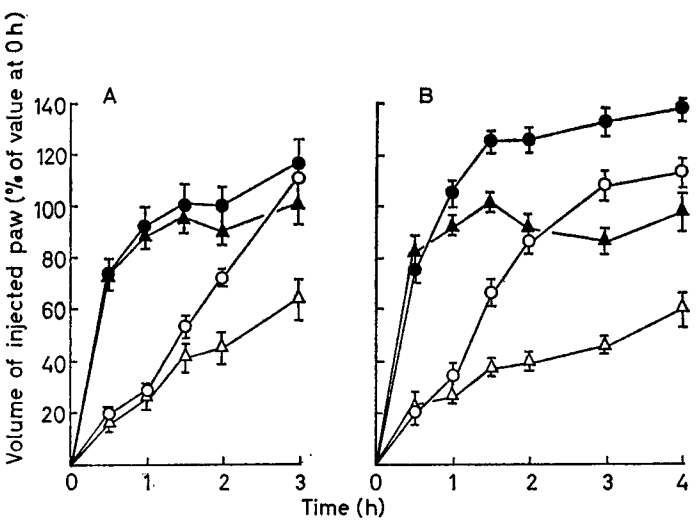

FIG. 3. Effects of plasma fraction in carrageenan-induced paw oedema potentiated by either prostaglandin E_1 or arachidonic acid. Results calculated and expressed as in Fig. 1 for the following groups, each of 10 rats: A; ○——○ saline control, △——△ plasma fraction, ●——● saline control plus prostaglandin E_1, ▲——▲ plasma fraction plus prostaglandin E_1, B; ○——○ saline control, △——△ plasma fraction, ●——● saline control plus arachidonic acid, ▲——▲ plasma fraction plus arachidonic acid. Both the prostaglandin E_1 and arachidonic acid significantly potentiated the carrageenan-induced paw swelling in the animals injected with either saline or plasma factor.

series (Sanner, Mueller & Schulze, 1973). The low water solubility of this substance initially limited its use *in vivo* but it has been shown to be much more effective when injected as a suspension in Tween 80 (Sanner, 1972). In the present work pretreatment of the rats with SC-19220 not only significantly reduced the development of the carrageenan-induced paw swelling (Fig. 2) but also blocked the potentiating effect of prostaglandin E_1 on the oedema.

The prostaglandin system is therefore concerned in the development of carrageenan-induced paw oedema in the rat. However, the anti-inflammatory action of the plasma fraction does not involve a specific interference with either the formation or action of the prostaglandins. Treatment of the rats with either eicosatetraynoic acid (Fig. 1) or SC-19220 (Fig. 2) caused a significant reduction in the foot swelling over and above that induced by the plasma fraction alone. This conclusion was confirmed by the results of further experiments in which the plasma fraction did not change the potentiating effects of either prostaglandin E_1 or arachidonic acid on the development of the carrageenan-induced swelling (Fig. 3). The results of the experiments with isolated tissues show that the plasma fraction does not block the action of prostaglandins *in vitro* and that it did not contain an inhibitor of SRS-A.

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