PRELIMINARY COMMUNICATIONS

Recovery of Prostaglandins in Human Cutaneous Inflammation*

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British Medical Journal, 1971, 2, 258–260

Summary

An in-vivo skin perfusion technique has been used to study the pharmacological activity in inflamed skin of patients with allergic contact eczema. Perfusates from 35 out of 45 patients contained a smooth-muscle-contracting agent with prostaglandin-like properties. Solvent partition followed by thin-layer chromatography revealed this activity to be due to a mixture of prostaglandins E and F. This direct evidence supports the view that prostaglandins mediate inflammation in man.

Introduction

Non-steroid anti-inflammatory drugs which are effective on topical application in skin diseases are urgently required. Failure to develop agents of this type is due at least in part to lack of knowledge of the identities of pharmacological substances which mediate inflammation. In an attempt to solve this problem we have developed a perfusion technique for direct recovery of pharmacological agents from inflamed human skin in vivo (Greaves and Søndergaard, 1970a, 1971).

In this paper we report the recovery of prostaglandins E_1, E_2, $F_1\alpha$, and $F_2\alpha$ from inflamed skin of patients with allergic contact eczema. This and other evidence indicates that prostaglandins may participate in the pathogenesis of inflammation in man.

Patients and Methods

Forty-five patients with proved allergic contact eczema were studied. With the criteria of Fisher (1967) positive patch tests showed that 16 of the patients were allergic to nickel, 10 to chromate, four to rubber, three to primula, two to cobalt, two to chloramphenicol, and one each to benzocaine, colophony, formaldehyde, neomycin, epoxyresin, Soframycin (framycetin sulphate), amethocaine, and paraphenylenediamine. Informed consent was obtained from all patients studied.

Detailed accounts of the techniques of perfusion and assay and of a quantitative evaluation of the method are reported elsewhere (Greaves and Søndergaard, 1970a, 1971). An area of contact eczema was induced by applying a patch test of area 6 cm^2 to clinically uninvolved skin of the flexor surface of the forearm. Inflamed eczematous skin was perfused 48 hours later for 60 to 90 minutes.

PERFUSION AND ASSAY

Two wide-bore needles with four holes perforated along each side of the shaft were inserted immediately subdermally in parallel 10 mm apart in opposite directions along the flexor aspect of the forearm. Warm sterile Tyrode solution was infused through one needle and recovered through the other into siliconized glass tubes in an ice-packed fraction collector. Recovery was assisted by confining the area of perfusion with elastic bands and by applying gentle suction with a peristaltic pump. Perfusion was continued for 60 to 90 minutes, the rate of infusion was 2 ml/min, and 40-70% of the infused Tyrode solution was recovered in the perfusate.

The perfusates were assayed for pharmacological activity with the following isolated organ preparations: stomach fundus strip, duodenum, colon, and uterus of the rat and the terminal ileum of the guinea-pig. The rat uterus and duodenum were mounted in a single organ bath. Prostaglandins E and F contract all these smooth muscle preparations (Von Euler and Eliasson, 1967). In order to exclude activity due to acetylcholine, histamine, and serotonin all bioassays were carried out in the presence of atropine 2 ng/ml, mepyramine 10 ng/ml, and bromlysergic acid diethylamide 500 ng/ml.

Further separation of prostaglandins was then carried out as suggested by Horton (1968). The perfusates were extracted by solvent partition between ethyl acetate and water at pH 3. The organic phase was dried in a stream of nitrogen, and the dried extract was reconstituted in a small volume of ethyl acetate. The redissolved extract was applied to a thin-layer silica gel G plate and co-chromatographed with prostaglandin standards in the AI and AII systems described by Green and Samuelsson (1964). Standards were visualized by application of 10% ethanolic phosphomolybdic acid followed by heating at 100°C for four to eight minutes. Distribution of activity on the chromatoplate was determined by elution with 95% acetone (3×5 ml) of bands corresponding to the Rf values of simultaneously developed standard prostaglandins. The origin and solvent-front areas of the plate were also eluted. Eluates from silica gel were dried in a stream of nitrogen and reconstituted in De Jalon's solution (Garcia De Jalon *et al.*, 1945) for bioussay on the single horn of the isolated uterus from an ovariectomized rat. Results were expressed in terms of prostaglandin E_1-equivalents.

Results

PROSTAGLANDINS

Perfusates from positive patch tests in 35 out of 45 subjects with allergic contact eczema contained a smooth-muscle-contracting agent which contracted the stomach fundus, duodenum, uterus, and colon of the rat and the guinea-pig ileum. Smooth-muscle-contracting agent did not contain detectable amounts of histamine, acetylcholine, 5-hydroxytryptamine, or catecholamines since the contractile responses were not antagonized by mepyramine, atropine, bromlysergic acid diethylamide, propranolol, or phentolamine. This pattern of smooth-muscle-contracting activity raised the possibility that prostaglandins might be present in the perfusates.

* A brief report of this work was presented to the British Pharmacological Society, London, on 7 January 1971.

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Prostaglandins, like other polar organic fatty acids, are totally ionized only at pH 8 or above and are almost completely in the non-ionized form below pH 4 (Horton, 1968). Accordingly solvent partition was carried out between ethyl acetate and water at pH 3. Perfusates from seven patients were studied and in all seven smooth-muscle-contracting agent was recovered in the organic phase, the aqueous phase containing no detectable smooth-muscle-contracting agent. By contrast solvent partition at pH 8 resulted in recovery of smooth-muscle-contracting agent almost entirely in the aqueous phase only. It was concluded, therefore, that smooth-muscle-contracting agent contained one or more fatty acids with prostaglandin-like properties.

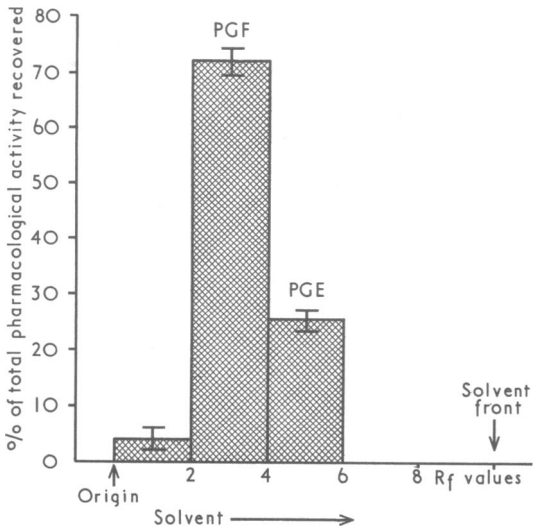

FIG. 1—Thin-layer chromatogram distribution of prostaglandin-like material recovered by ethyl acetate extraction from perfusates of three contact eczema patients using the AI solvent system of Green and Samuelsson (1964). Pharmacological activity, in terms of prostaglandin E_1 equivalents, expressed as percentage of total pharmacological activity present. Mean values shown with standard error of the mean.

Ethyl-acetate-extracted acidic lipid from perfusates of three patients was then submitted to thin-layer chromatography. These samples contained prostaglandin-like activity in concentration 0·4-1·6 ng prostaglandin E_1 equivalents (mean $0{\cdot}95 \pm 0{\cdot}36$ S.E. of mean) per ml of original perfusate. In the AI solvent system (Green and Samuelsson, 1964) 70% of the total pharmacological activity co-chromatographed with prostaglandin F and 25% with prostaglandin E (Fig. 1). The remainder, amounting to less than 5% of the total, chromatographed with an R_f value of less than 0·2. Further separation of prostaglandin E and F areas on the Green and Samuelsson AII solvent system showed the following approximate distribution of total activity in terms of prostaglandin E_1-equivalents: E_1 12%, E_2 12%, $F_1\alpha$ 24%, and $F_2\alpha$ 49%.

CONTROL EXPERIMENTS

Perfusates from healthy skin of 22 control subjects were studied. These were patients with various localized non-inflammatory skin conditions. In six of these who had negative patch tests to nickel a 6 cm^2 nickel patch test was applied to the forearm and perfusion of the underlying skin was carried out 48 hours later. No prostaglandin-like activity was detected when the perfusates were added to the rat uterus or duodenum preparations. Failure to detect prostaglandin activity in the perfusates, using the rat uterus and duodenum preparations, does not exclude the presence of extremely low concentrations. In order to concentrate any prostaglandin

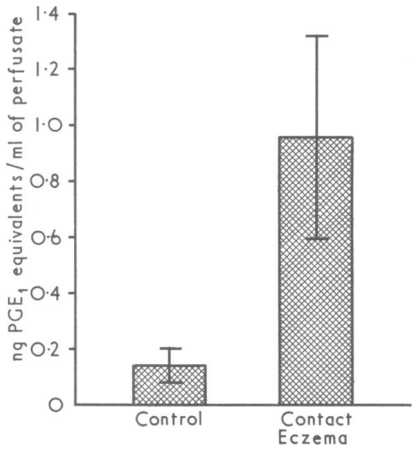

FIG. 2—Concentrations of prostaglandins expressed as prostaglandin E_1 equivalents in perfusates of seven patients with contact eczema and normal skin of four control subjects. Mean values shown with standard error of the mean.

present perfusates from four of the 22 subjects were subjected to ethyl acetate extraction at pH 3. Reconstituted dried extracts from the organic phase were assayed against standard prostaglandin E_1 with the rat uterus preparation. The results of these assays indicated that the original perfusates contained 0·05-0·3 ng prostaglandin E_1 equivalents (mean $0{\cdot}13 \pm 0{\cdot}06$ S.E. of mean) per ml, which is about one-tenth of the amount present in perfusates from skin of patients with contact eczema (Fig. 2).

Discussion

The present experiments show that perfusates from inflamed skin of allergic contact eczema contain nanogramme concentrations of prostaglandins. By contrast, prostaglandins could not be detected by direct examination of perfusates from normal skin, though ethyl acetate extraction revealed the presence of trace amounts.

The source of prostaglandins in inflamed eczematous skin is speculative. The increased concentrations of inflamed skin could be due to increased biosynthesis. Alternatively prostaglandins could be released from cell membrane lipids after activation of tissue phospholipases.

No prostaglandins have been detected in several types of human "weal-and-flare" reactions with the same skin perfusion technique (Greaves and Søndergaard, 1970a; Søndergaard and Greaves, 1971a). However, the presence of prostaglandins in inflamed skin may not be specific to allergic contact eczema since we have recovered a pharmacologically active fatty acid with prostaglandin-like properties from delayed cutaneous inflammation due to exposure to ultraviolet radiation (Greaves and Søndergaard, 1970b). Prostaglandin-like activity has also recently been identified in delayed inflammation in the rat (Willis, 1969; Giroud and Willoughby, 1970) and may be a mediator of inflammation in this species (Arora, *et al.*, 1970).

Prostaglandins are highly vasoactive in human skin, prostaglandin E being more potent than prostaglandin F (Juhlin and Michaëlsson, 1969; Søndergaard and Greaves, 1971b). Søndergaard and Greaves found that intradermal injections of prostaglandin E_1 in concentrations as low as 10 ng/ml caused pronounced erythema of a strikingly sustained quality.

The presence of a potent vasoactive substance in an inflammatory reaction does not permit conclusions to be drawn about its importance in the pathogenesis of the lesion. Nevertheless, our direct evidence raises a distinct possibility that prostaglandins may be mediators of human cutaneous inflammation, and should stimulate detailed observations on

cutaneous vascular tachyphylaxis (Greaves and Shuster, 1967) as well as attempts to recover prostaglandins in a wider range of inflammatory reactions.

We thank Professor Sam Shuster and Dr. J. S. G. Cox for helpful discussions. We also thank Mr. H. H. Poole and Mrs. A. Saunders, of the pharmacy department, Royal Victoria Infirmary, Newcastle upon Tyne, for preparing the Tyrode solution for infusion and Misses Maureen Davison and Helen Heiligstadt for technical help. The work reported in this paper was supported by grants from the Nuffield Foundation and Fisons Ltd. One of us (J.S.) is in receipt of a grant from Gillette Industries Ltd. The standard prostaglandins were a gift from Professor D. A. Van Dorp and Dr. P. F. Wilde, of Unilever Research Laboratories.

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Clinical Significance of 7s IgM in Monoclonal IgM Diseases

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British Medical Journal, 1971, **2**, 260–261

Summary

The sera from 117 patients with diseases associated with a high production of monoclonal IgM were analysed for the presence of low molecular weight (7s) IgM by using a simple thin-layer Sephadex technique. 7s IgM was found in the sera of patients with myelomata (66%), lymphomata (45%), and Waldenström's macroglobulinaemia (20%), but was absent from the sera of patients with benign monoclonal macroglobulinaemia.

This technique provides a cheap and practical test which may be valuable in selecting patients with lymphomata from those with benign lesions.

Introduction

A high production of monoclonal 19s IgM is a major feature of some lymphomata, Waldenström's macroglobulinaemia, IgM myelomatosis, and primary cold agglutinin disease and may also be associated with chronic lymphatic leukaemia, rheumatoid arthritis, and malignant diseases. The 19s IgM molecule is a polymer of five 7s IgM units, each 7s unit comprising two μ-heavy chains and two light chains. It has recently been shown that the production of low molecular weight (7s) IgM may also be seen in some of these diseases.

Solomon and Kunkel (1967) found 7s IgM in addition to 19s IgM in the serum of a patient with a neoplastic lesion of the plasma-cell/lymphatic system, and Bush, Swedlund, and Gleich (1969) and Hansson and Laurell (1969) also showed 7s IgM in the sera of patients with macroglobulin diseases. Separation of the 7s subunits from the 19s IgM was achieved by Hansson and Laurell using crossed-immunoelectrophoresis and by the other two groups using sucrose density-gradient ultracentrifugation.

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Though the presence of 7s IgM is a normal finding in the sera of some lower vertebrates (Marchalonis and Edelman, 1965; Clem and Small, 1967) and even in human neonates (Perchalski, Clem, and Small 1968), the significance of its presence in the serum of human adults is unknown.

In this paper the clinical importance of finding 7s subunits in adult sera in the presence of macroglobulin disease is investigated, and a simpler method for their detection than has hitherto been described is presented.

Patients and Methods

Sera were analysed in a blind trial of 117 patients with diseases associated wih excess monoclonal IgM production. They were patients in whom a definitive diagnosis had been possible through the kindness of our clinical colleagues, many in the Medical Research Council's Working Party on Leukaemia in Adults (see Hobbs, 1969), and many pathologists who had kindly sent material to Professor C. V. Harrison. The diagnostic criteria used were lymphomata, invasive destruction of lymph node architecture; Waldenström's macroglobulinaemia, infiltration of preserved architecture; myelomatosis, typical bone lesions; and cold haemagglutinin disease, typical serology (thanks to Drs. S. Worlledge and A. Cooper). Nine patients diagnosed as benign monoclonal macroglobulinaemia had all been followed up for at least six years during which time no increase in the IgM, nor any form of neoplasia, had been detected. The 117 patients were classified as in the Table.

Incidence of 7s IgM and Bence Jones protein (B.J.)

Underlying Disease	No. of Patients					% of Patients with IgM Subunits in Serum
	Total	B.J. in Urine	Type of IgM			
			19s	19+7s	7s	
Myeloma	3	3	1	2	—	66
Lymphoma	33	33	18	11	4	45
Waldenstrom's macroglobulinaemia	25	24	20	5	—	20
Primary cold agglutinin	45	4	41	4	—	9
Chronic lymphatic leukaemia	1	0	1	—	—	0
Rheumatoid arthritis	1	0	1	—	—	0
Benign	9	0	9	—	—	0

Spots of serum (0·25 μl) were run on horizontal microscope slides spread with 0·75 mm (0·03 in) of superfine Sephadex G200 along which 0·9% saline flowed from a reservoir via 3 MM chromatography paper wicks. The completion of the run was shown by the position reached by 2% blue dextran