

BIOSYNTHESIS OF PROSTAGLANDINS BY HUMAN INFLAMED SKIN

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Abstract. The biosynthesis of prostaglandins by human inflamed skin was studied in 8 patients with primary irritant dermatitis induced by benzalconium-chloride. Inflamed skin from these patients evidenced an increased ability to synthesize prostaglandins. In the presence of exogenous arachidonic acid in the incubation medium, the activity formed was about 80% greater in inflamed skin than in non-inflamed control skin. In prostaglandin E_1 -equivalents the concentration amounted to 8.09 ± 1.59 ng/mg protein nitrogen and 4.51 ± 1.24 ng/mg protein nitrogen, respectively (mean values $\pm$ S.E.). When inflamed skin was incubated without excess of exogenous precursor acids in the incubation medium the activity formed was about 80 times lower and the values were similar to those of non-inflamed skin. Thus, the present results support the view that the prostaglandin system is activated in primary irritant dermatitis, thereby providing a basis for future therapeutic attempts to control this disorder.

Key words: Prostaglandins; Biosynthesis; Primary irritant dermatitis; Arachidonic acid

It is now generally accepted that the prostaglandins participate in the development of sustained inflammation, including various pathological skin conditions, i.e. allergic contact dermatitis (3, 4, 12) and inflammation due to ultraviolet radiation (9).

Primary irritant dermatitis due to environmental toxic and irritative chemicals represents a major dermatological problem, contributing about 50% of occupational dermatoses and causing the patients great disability and long periods of sick-leave (2). Attempts to elucidate the role of chemical mediators in this condition have already been made (3, 16).

In this paper, which confirms and extends our previous results (16), we report that increased amounts of prostaglandins are synthesized by inflamed skin from patients with primary irritant dermatitis.

MATERIALS AND METHODS

Patients

Primary irritant dermatitis was induced in 8 subjects by patch tests with a 10% benzalconium-chloride aqueous solu-

tion. The findings were compared with those obtained in symmetrical non-affected areas of the skin in the same subjects. The 8 subjects had not previously been exposed to benzalconium-chloride. All subjects were volunteer patients with localized non-inflammatory skin conditions sparing the thighs.

Sampling

Punch biopsies, 6 mm in diameter, were obtained from areas with positive patch tests 24 hours after application of the patches, and from the clinically normal skin of the opposite thigh, ethyl chloride being used as local freezing anesthesia. The skin specimens were immediately frozen in liquid nitrogen and, if not used immediately, stored for up to one week at -20°C .

Reagents

The standard prostaglandins were a gift from Professor D. A. van Dorp and Dr P. F. Wilde, of Unilever Research Laboratories. ^3H -labelled PGE_1 was obtained from New England Nuclear Corp. and had a specific activity of 110 Ci/mmol. ^3H -labelled 5,8,11,14-eicosatetraenoic acid (arachidonic acid) was obtained from New England Nuclear Corp. and had a specific activity of 80 Ci/mmol.

Biosynthesis from endogenous precursor acids and from labelled arachidonic acid

The methods used were those described in detail by Jonsson & Ånggård (7). The punch biopsy was homogenized in a glass homogenizer in 10 ml medium (0.15 mol/l potassium phosphate buffer, pH 7.4, containing reduced glutathione 1.5 mmol/l and EDTA 30 mmol/l). Five ml of this homogenate was used for examination of the biosynthesis from endogenous precursor acids and the remaining 5 ml was used for determination of the biosynthesis using labelled arachidonic acid. The subsequent extraction procedures followed exactly those described by Jonsson & Ånggård (7).

After column chromatography on 0.5 g of silicic acid, further separation of prostaglandins was carried out as suggested by Horton (6) and previously described by one of us (4) using thin layer silica gel G plates and co-chromatography with prostaglandin standards in the Al solvent system described by Grén & Samuelsson (5). Bioassays were performed on eluates reconstituted in DeJalon's solution.

Bioassay

Bioassay was performed on the striated part of the ascending colon of gerbil and on rat stomach strip, as described by

Table I. Biosynthesis of prostaglandin in skin from patients with primary irritant dermatitis

	No. of patients	Biosynthesis of PG ^a from endogenous precursors (ng/mg prot.-nit.)	Biosynthesis of PG ^a from exogenous arachidonic acid (ng/mg prot.-nit.)
Inflamed	8	0.11 ± 0.07	8.07 ^b ± 1.59
Control	8	0.09 ± 0.04	4.51 ± 1.24

^a PG = Prostaglandin, expressed as PGE₁-equivalents

^b Significantly different from the controls at the 5% level of probability.

Weeks et al. (18). Three-point assays were done. The results were expressed in PGE₁-equivalents.

Determination of protein-nitrogen

The bromsulphthalein staining method adopted for determination of protein-nitrogen in human skin by Søndergaard & Glick (13) was used. The analyses were performed in duplicates on 20 µl of the original homogenates.

RESULTS

The 8 subjects all developed a positive primary irritant patch test reaction to benzalconium-chloride.

The biosynthesis of prostaglandins from endogenous precursor acids in the eight biopsies from the dermatitic skin was determined biologically using gerbil colon and rat stomach strip after incubation at 37°C for 20 min without addition of arachidonic acid in the incubation medium, and in PGE₁-equivalents the concentration (mean ± S.E.) was 0.11 ± 0.07 ng/mg protein-nitrogen. This value did not differ significantly from the results obtained in the corresponding control biopsies from non-inflamed skin, the mean values ± S.E. being 0.09 ± 0.04 ng/mg protein-nitrogen (Table I).

The inflamed skin of patients with primary irritant dermatitis had an increased capacity to synthesize prostaglandins in the presence of exogenous arachidonic acid in the incubation medium. In PGE₁-equivalents the concentration (mean ± S.E.) was 8.07 ± 1.59 ng/mg protein-nitrogen. The corresponding values obtained in non-inflamed skin were significantly lower ($p < 0.05$), mean ± S.E. 4.51 ± 1.24 ng/mg protein-nitrogen (Table I).

Further attempts to characterize the activity formed during the incubation period in the presence of exogenous arachidonic acid were then made after the column chromatography using thin-layer chro-

matography. In the AI solvent system (5) 95% of the total pharmacological activity co-chromatographed with prostaglandin E and the remainder, about 5%, with prostaglandin F. No difference was found in the ratio E/F prostaglandins by thin-layer chromatography of activity from inflamed and non-inflamed skin.

DISCUSSION

The results of the present study showed that inflamed skin from patients with primary irritant dermatitis had an increased capacity to synthesize prostaglandins in the presence of exogenous arachidonic acid in the incubation medium. On first exposure, benzalconium-chloride induces a primary irritant reaction (2), although on subsequent exposures, it may only rarely cause allergic contact dermatitis (11). All our patients were previously unexposed to benzalconium-chloride.

By contrast, when the inflamed skin from the patients with primary irritant dermatitis was incubated without excess of arachidonic acid in the incubation medium, the amount of activity formed was about 80 times lower, and the values obtained were similar to those of non-inflamed skin. These observations are in agreement with previous *in vivo* observations (4, 16). They were all conditions with free accessibility of precursor acids. Using an *in vivo* perfusion technique, Søndergaard et al. (16) recovered prostaglandins in the perfusate from 7 of 13 patients with primary irritant dermatitis. These findings agreed with the observations of Goldyne et al. (3), who demonstrated prostaglandin activity in blister fluid from four volunteers with cantharidin blisters.

The ratio of E to F prostaglandins that we found in the inflamed and normal skin of patients with primary irritant dermatitis is of interest. The results agree with previous *in vitro* studies. Thus, Jonsson & Ånggård (7) identified PGE₂ as the major prostaglandin in normal human skin and only trace amounts of PGF_{2α} were detectable. By contrast, our present *in vitro* results cannot be compared directly with previous *in vivo* studies, where conditions for biosynthesis and metabolism of prostaglandins may be completely different.

E prostaglandins are highly vasoactive in human skin (8, 14). Søndergaard & Greaves (14) found that intradermal injections of prostaglandin E₁ in concentrations as low as 10 ng/ml caused pronounced

erythema of a strikingly sustained quality. Furthermore, prostaglandin E_1 does not cause cutaneous vascular tachyphylaxis (10). Repeated injection of prostaglandin E_1 at the same site of the skin produces an inflammatory reaction, clinically characterized by redness, edema and tenderness, with biochemical changes in the glycosaminoglycans similar to those seen in the inflammatory reaction following tissue injury (15).

The evidence of a role of the prostaglandins in sustained inflammation is rapidly mounting up (1, 3, 4, 9, 10, 15–17). The results of the present study, taken together with results of our earlier studies (4, 16), support the view that the prostaglandin system in skin is activated during a variety of inflammatory conditions including primary irritant dermatitis.

The present work may provide a basis for future therapeutic attempts to control these disorders by using agents which inhibit the biosynthesis of prostaglandins.

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REFERENCES

1. Arturson, G., Hamberg, M. & Jonsson, C.-E.: Prostaglandins in human burn blister fluid. *Acta Physiol Scand* 87: 270, 1973.
2. Björnberg, A.: Skin Reactions to Primary Irritants in Patients with Hand Eczema. Oscar Isacson, Göteborg, Sweden, 1968.
3. Goldyne, M. E., Winkelmann, R. K. & Ryan, R. J.: Prostaglandin activity in human cutaneous inflammation: Detection by radio-immunoassay. *Prostaglandins* 4: 737, 1973.
4. Greaves, M. W., Søndergaard, J. & McDonald-Gibson, W.: Recovery of prostaglandins in human cutaneous inflammation. *Br Med J* 11: 258, 1971.
5. Gréen, K. & Samuelsson, B.: Prostaglandins and related factors: XIX. Thin layer chromatography of prostaglandins. *J Lipid Res* 5: 117, 1964.
6. Horton, E. W.: Recent Advances in Pharmacology, 4th ed. J & A Churchill Ltd., London, 1968.
7. Jonsson, C.-E. & Ånggård, E.: Biosynthesis and metabolism of prostaglandin E_2 in human skin. *Scand J clin Lab Invest* 29: 289, 1972.
8. Juhlin, L. & Michaëlsson, G.: Cutaneous vascular reactions to prostaglandins in healthy subjects and in patients with urticaria and atopic dermatitis. *Acta Dermatovener (Stockholm)* 49: 251, 1969.
9. Mathur, G. P. & Gandhi, V. M.: Prostaglandin in human and albino rat skin. *J Invest Dermatol* 58: 291, 1972.
10. Paulli Jørgensen, H. & Søndergaard, J.: Vascular responses to prostaglandin E_1 . *Acta Dermatovener (Stockholm)* 53: 203, 1973.
11. Shumes, E. & Levy, E. J.: Quaternary ammonium compound contact dermatitis from a deodorant. *Arch Dermatol* 105: 91, 1972.
12. Søndergaard, J. & Greaves, M. W.: Recovery of a pharmacologically active fatty acid from human allergic contact eczema using a skin perfusion method. *Fed Proc* 29: 972, 1970.
13. Søndergaard, J. & Glick, D.: Quantitative histochemistry of histamine and histidine decarboxylase activity in normal human skin. *J Invest Dermatol* 56: 231, 1971.
14. Søndergaard, J. & Greaves, M. W.: Prostaglandin E_1 : Effect on human cutaneous vasculature and skin histamine. *Br J Dermatol* 84: 424, 1971.
15. Søndergaard, J., Helin, P. & Paulli Jørgensen, H.: Human cutaneous inflammation induced by prostaglandin E_1 . *J Pathol* 109: 239, 1973.
16. Søndergaard, J., Greaves, M. W. & Paulli Jørgensen, H.: Recovery of prostaglandins in human primary irritant dermatitis. *Arch Dermatol* 110: 556, 1974.
17. Velo, G. P., Dunn, C. J., Giroud, J. P., Timsit, J. & Willoughby, D. A.: Distribution of prostaglandins in inflammatory exudate. *J Pathol* 111: 149, 1973.
18. Weeks, J. R., Schultz, J. R. & Brown, W. E.: Evaluation of smooth muscle bioassays for prostaglandins E_1 and $F_{1\alpha}$. *J Appl Physiol* 25: 783, 1968.

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