Release of prostaglandins in experimental immune-complex endophthalmitis and phacoallergic uveitis

AMJAD RAHI, PRIMAL BHATTACHERJEE, AND ROOPNARAIN MISRA

From the Department of Pathology and Experimental Ophthalmology, Institute of Ophthalmology, University of London

SUMMARY Prostaglandins-E were demonstrated in the aqueous humour, the anterior uvea, and the choroid of rabbits in which type III allergic reactions were produced by two different methods. In general the levels of prostaglandins were found to be higher in those animals in which the immune complexes were formed from autologous rather than heterologous tissue antigens.

Autocoids are endogenous autopharmacological agents which are intimately concerned with the general inflammatory processes in the eye and elsewhere in the body. One group of autocoids, the prostaglandins (PGs), belong to a family of 20-carbon unsaturated fatty acids, and although they were first discovered several decades ago (Goldblatt, 1933) it is only recently that prostaglandins have absorbed an enormous amount of research effort.

What roles prostaglandins play in ocular allergic and non-allergic inflammation in relation to well-established mediators such as histamine and bradykinin is a subject of current interest, but it seems likely that prostaglandins interact with other autopharmacological agents so that each potentiates the effect of the other (Sears *et al.*, 1973; Leopold, 1974; Eakins and Bhattacharjee, 1977). It is of importance to note that current interest in ocular prostaglandins was initiated by the studies of Ånggård and Samuelsson (1964) and Ambache *et al.* (1965), who extracted an inflammatory agent 'irin' from ocular tissues which was later identified as a mixture of prostaglandins.

Prostaglandins are released from tissues in response to a variety of neural, chemical, mechanical, and immunological stimulants. They do not seem to exist, however, in preformed stores but are synthesised *in situ* as needed. Prostaglandins act locally in the tissues when they are produced by the PG-synthetase system and are rapidly inactivated by a dehydrogenase.

There is no doubt that prostaglandins are released in ocular inflammation and that ocular tissues during this period show increased synthetase activity

(Bhattacharjee, 1977; Bhattacharjee and Phylactos, 1977). It is also well established that prostaglandins, particularly the E-type, are released in a variety of experimental and clinical allergic conditions (Giroud and Willoughby, 1970; Brocklehurst, 1975). Since at least 4 distinct types of allergic reactions, each with a different pathogenetic mechanism, have been recognised, it is not known whether prostaglandins are involved in 1 or all 4 categories of hypersensitivity states.

Intraocular injection of a foreign antigen leads to an immunogenical ocular inflammation (Silverstein and Zimmerman, 1959) in which prostaglandins are present in excessive amounts in the aqueous humour (Eakins *et al.*, 1972). Since in this study prostaglandins were estimated 2 weeks after the injection of the antigen, it is not certain whether the ocular inflammation was due to a type I or type II allergy, or if it was produced by antigen-antibody aggregates, or by sensitised T-lymphocytes or from a combination of all these 4 pathogenetic mechanisms. Furthermore, it is also not known if prostaglandins are increased in conditions in which immunogenic uveitis is produced by sensitisation with ocular antigens.

A type III allergic reaction is mediated by antigen-antibody aggregates in which the affected tissue is diffusely infiltrated by polymorphonuclear leucocytes during the early stages of the inflammation. Such a reaction is easily produced in the eye when a pre-immunised animal with high titres of precipitating antibodies in the circulation is injected intravitreally with a small dose of the same antigen. Estimation of prostaglandins in eyes inflamed in this manner would provide some insight into the pathogenesis of uveitis in man, since it seems likely that endogenous uveitis in general and lens-induced uveitis in

particular are manifestations of immune-complex disease (Rahi *et al.*, 1976; Marak *et al.*, 1976; Williams and Lehner, 1977).

The present study was undertaken to investigate the role of prostaglandins in type III ocular allergy (i.e., immune-complex endophthalmitis), and therefore it was decided to use both ocular and non-ocular antigens to induce uveitis in the experimental animals.

Materials and methods

Adult New Zealand rabbits were injected intramuscularly with 0.1 ml of human serum emulsified in equal volumes of Freund's complete adjuvant. After 6 weekly injections the animals were bled and the antibody titre was estimated by the agar-diffusion technique. Animals showing precipitating antibodies were injected intravitreally (in 1 eye) with 0.05 ml sterile human serum and killed 24 hours later by means of sodium pentobarbitone. Aqueous humour was withdrawn and both eyes were enucleated immediately and processed for prostaglandin estimation.

Another group of rabbits were immunised by multiple intramuscular injections of either heterologous or autologous lens proteins in Freund's complete or incomplete adjuvant. The antibody titre was measured both by agar diffusion and by passive haemagglutination techniques; the details have been published elsewhere (Rahi *et al.*, 1977a, b). Sera from only those rabbits which had haemagglutinin titre of 1:640 and over showed precipitating antibodies. At the end of the immunisation schedule 0.05 ml of sterile lens protein (20 mg/ml) was injected into the vitreous cavity of one eye of each animal. The other eye was used as control. These animals were similarly killed 24 hours later and the aqueous humour was collected for prostaglandin measurement. The eyes were then processed for histological examination.

A group of non-immunised rabbits were injected intravitreally with either 0.05 ml of human serum or lens homogenate and killed 20 hours later to obtain a base line for prostaglandins. One eye from each group was examined histologically for evidence of polymorphonuclear infiltration, which is pathognomonic of acute immune-complex disease.

PROSTAGLANDIN STUDY

The anterior uvea (iris and ciliary body) and the choroid were immediately dissected out and weighed separately. Tissues were homogenised in 2 ml of phosphate buffer (pH 7.4) at 4°C. Aqueous humour and tissue homogenates were then separately incubated at 37° for 30 minutes in a shaking water

bath. At the end of the incubation period the reaction was stopped with absolute alcohol. Aliquots of homogenates were also denatured with alcohol before incubation to determine the basal level of PGs.

Prostaglandin-like substances in aqueous humour and incubated tissue samples were extracted according to the method of Unger *et al.* (1971) and assayed against PGE₁ on the rat stomach strips (Vane, 1957), suspended in 10 ml of Krebs's solution at 37°C, and gassed with 5% carbon dioxide in oxygen. The solution contained the usual antagonists of histamine, catecholamine, serotonin, and acetylcholine (Gilmore *et al.*, 1968).

The extracts of aqueous humour, anterior uvea (iris and ciliary body), and choroid remaining after bioassay were pooled separately, re-extracted, and dissolved in chloroform. 50-ml samples were spotted on silica-gel plates (Eastman Chromatogram 6061). Pure PGE (mixture of E₁ and E₂ and F₂α (Upjohn)) was treated similarly. The plates were developed according to the method of Kiefer *et al.* (1975). Zones corresponding to the standard PGE and F₂α were scraped off, extracted, and bioassayed as described above. PGE recovered from ocular tissues was further separated into E₁ and E₂ using 3% silver nitrate treated silica-gel plates.

Statistical analysis of the differences in the mean values of prostaglandins were analysed by Student's *t*-test.

(i) SERUM-INDUCED ENDOPHTHALMITIS

The eyes of the immunised animals injected with human serum appeared inflamed, and histological examination showed marked polymorphonuclear infiltration suggesting an Arthus-type reaction. The eyes of the non-immunised animals injected with human serum showed, however, only mild reaction consisting of vascular dilatation and minimal cellular infiltration.

The levels of prostaglandins in the aqueous humour samples from the control and the test eyes of immunised and non-immunised rabbits are shown in Table 1. They were significantly raised in the injected eyes of the immunised animals (mean, 24.7 ng/ml, *P* < 0.001) as compared to the contralateral eyes (mean, 3 ng/ml) or the injected eyes of non-immunised rabbits (mean, 3.5 ng/ml). The levels of prostaglandins recovered from the incubates of the anterior uvea (iris and ciliary body) and choroid from the injected eyes of the immunised and non-immunised animals are compared in Table 2. After 30 minutes of incubation, a significantly large amount of prostaglandin was recovered from the inflamed anterior uvea of the immunised rabbits (mean, 6.3 µg/g wet weight, *P* < 0.001) as compared to the levels in the injected eyes of non-

Table 1 Prostaglandins in experimental immune-complex endophthalmitis

Rabbits	ng of PG-like substance per ml of aqueous humour		Significance
	Control eye Mean $\pm$ SEM	Test eye Mean $\pm$ SEM	
Immunised	3.0 $\pm$ 0.7 (4)	24.7 $\pm$ 1.7 (4)	P < 0.001
Non-immunised	3.5 $\pm$ 0.7 (2)	3.0 $\pm$ 0.7 (2)	

Figures in parentheses represent number of experiments. Each experiment represents pooled aqueous humour from 4 eyes. Significance of the differences between the means of the test eyes from immunised and non-immunised rabbits were calculated by Student's *t*-test

Table 2 Prostaglandins in experimental immune-complex endophthalmitis

Prostaglandins in $\mu\text{g/g}$ wet weight of the tissue							
Tissue	Non-immunised rabbits			Immunised rabbits			Significance
	Mean $\pm$ SEM			Mean $\pm$ SEM			
	0 min	30 min	Net	0 min	30 min	Net	
Iris + ciliary body	0.30	3.0	2.7	0.6	6.9	6.3	P < 0.001
	± 0.06 (4)	± 0.5 (4)		± 0.05 (4)	± 0.7 (4)		
Choroid	0.15	1.0	0.85	0.2	1.55	1.35	P < 0.2
	± 0.03	± 0.3		± 0.04 (4)	± 0.04 (4)		

Figures in parentheses denote number of experiments. Each experiment represents pooled tissue from 4 rabbits. Significance of the differences between the means of the control and the test eyes at 30 minutes were calculated by Student's *t*-test

immunised animals (mean, 2.7 μ g/g wet weight). The amount of prostaglandins recovered from the choroid of the test animals was only moderately in excess (mean, 1.55 μ g/g wet weight) of the control animals.

Chromatographic studies showed that approximately 90% of PGs obtained from the original samples of aqueous humour and tissue homogenates were of E-type. Further studies showed that the prostaglandin E in incubates of the anterior uvea consisted of 40% E₁ and 60% E₂.

(ii) PHACOALLERGIC UVEITIS

Histological examination of the injected eyes from animals immunised with heterologous or autologous soluble lens protein in Freund's complete or incomplete adjuvant showed an Arthus-type reaction. The intensity of the reaction, however, was proportional to the antibody titre in the serum, and only those with lens haemagglutinin titre > 1:640 showed marked polymorphonuclear infiltration. The serum antibody titre in rabbits injected with heterologous lens protein in Freund's complete

adjuvant was higher than those animals which received the same antigen in Freund's incomplete adjuvant. The levels of prostaglandins in aqueous humour from the test eyes of these animals showed a positive correlation with the serum antibody titre and the degree of inflammation (Table 3). The aqueous humour from immunised rabbits injected with autologous lens protein, however, contained larger amounts of prostaglandin-like substances, though the lens antibody titre in the serum was lower than in those rabbits which were immunised with heterologous lens proteins.

Discussion

The present communication confirms the earlier study of Eakins *et al.* (1972) that prostaglandins are raised in the aqueous humour in intraocular immunogenic inflammation. It also provides additional and perhaps important information regarding a possible increased synthetase activity in immunologically inflamed ocular tissues, particularly the iris and the ciliary body.

Since the antigen was injected into the vitreous of preimmunised animals with high levels of precipitating antibodies and the eyes were enucleated within 20 hours, the reaction produced resembled both temporally and histologically a type III hypersensitivity or an acute immune-complex disease. It has been possible for the first time, therefore, to demonstrate the involvement of prostaglandins in a specific type of ocular allergy.

It seems likely that certain forms of endogenous uveitis in man may represent a type III allergy resulting from the deposition of antigen-antibody aggregates in the uveal vessels (Rahi *et al.*, 1976; Williams and Lehner, 1977), because it is now known that a large proportion of these cases contain

Table 3 Prostaglandins in phacoallergic endophthalmitis

Antigen	Rabbits	ng of PG-like substance per ml of aqueous humour		Lens antibody titre (haemagglutination test)	
		Control eye	Test eye		
Heterologous Lens in FCA	H1	10	50	1:10	240
	H2	4	90	1:40	960
	H3	0	100	1:40	960
Heterologous Lens in FIA	H4	0	10	1:2560	
	H5	0	5	1:1280	
Autologous lens in FCA	A1	0	5	1:10	
	A2	0	180	1:640	
	A3	0	10	1:40	
	A4	0	75	1:640	

FCA = Freund's complete adjuvant; FIA = Freund's incomplete adjuvant

complement-fixing immune complexes in their blood (Holborow, 1974; Rahi *et al.*, 1977). By analogy, therefore, an immune-complex uveitis in man should also be associated with high levels of prostaglandin in the aqueous humour. That this is so is borne out by the study of Eakins *et al.* (1972), who found raised levels of PG-like activity in aqueous humour from patients with acute anterior uveitis.

Although it seems that prostaglandins are involved in heterologous immune-complex mediated ocular inflammation, it has so far been unknown if these autocoids are also concerned with ocular inflammation produced by deposition of autologous antigen-antibody aggregates. Since experimental phacoallergic endophthalmitis can be suppressed by antileucocyte serum and the inflamed uvea shows evidence of *in-vivo* complement fixation, it has been argued that lens-induced ocular inflammation is a typical example of type III allergy (Marak *et al.*, 1976). Attempts were made, therefore, to produce this lesion in rabbits using both heterologous and autologous lens antigens. It was encouraging to find that prostaglandins were also raised in aqueous humour from eyes with phacoallergic uveitis.

The present study not only shows for the first time that prostaglandins are involved in a type III allergic reaction in eyes where the antigen is of exogenous origin, but that they also play a role in conditions where the inflammation is due to the involvement of antigens resident in the ocular tissues.

The prostaglandins recovered from the eyes were of E-type. Although it is known that PGE₂ predominates in ocular tissues, particularly the iris, the reason why large amounts of PGE₁ were recovered from the inflamed iris may be related to the leucocytic infiltration of this tissue, because it is now generally believed that polymorphonuclear leucocytes (which infiltrated the eye in the present experiment) are the main source of PGE₁ (Higgs and Youten, 1972).

It is difficult to explain why rabbits immunised with autologous lens protein showed higher levels of prostaglandin in the aqueous humour than those which were immunised against heterologous lens protein, in spite of the fact that the latter group had a high titre of circulating antibody. It is possible that antigen-antibody aggregates consisting of autologous antigens are physico-chemically different and therefore more toxic than other complexes, and although complement-fixation studies were not performed, the possibility remains that endogenous immune complexes are more avid in activating the phospholipase enzyme of the complement system, which in turn may be directly involved in the elaboration of vasoactive prostaglandins (Giroud and Willoughby, 1970).

The reason why animals immunised with heterologous lens proteins in Freund's complete adjuvant showed higher levels of prostaglandins than those immunised with the same antigen in Freund's incomplete adjuvant is more likely related to the level of precipitating antibody in the blood of the immunised rabbits.

What role prostaglandins play in ocular allergy in general and immune-complex endophthalmitis in particular requires further research. It has been shown, however, that prostaglandins are released from guinea-pig lung during acute allergic reactions (Piper and Vane, 1971; Benzie *et al.*, 1975), and high levels of this substance are also found in allergic dermatitis in man (Greaves *et al.*, 1971).

Recent studies have shown that prostaglandins have little direct effect on cutaneous vascular permeability, but potentiate the activity of other autocoids such as histamine and bradykinin, leading to cellular exudation and inflammation (Williams and Morely, 1973; Williams, 1976); similar effects have been postulated for ocular tissues (Eakins and Bhattacharjee, 1977). It is of interest that prostaglandins can also act as an immunoregulator by controlling the activity of the T-lymphocytes and the production of lymphokines (Morley, 1974). They thus exert an anti-inflammatory effect by controlling the magnitude of tissue damage. It is possible, therefore, that raised levels of prostaglandins in immune-complex endophthalmitis and phacoallergic uveitis play a homeostatic rather than a pathogenetic role.

We are grateful to Professor Norman Ashton and Professor E. S. Perkins for their kind help and advice. We also wish to thank the Medical Research Council for financial help, Mr R. Alexander, Mr D. Clayton, Mr C. Higgins, Mr A. Phylactos, and Mr J. Prasad, who provided the technical assistance, and Mrs. A. Wood, who typed the manuscript.

Prostaglandins were generously supplied by Dr John E. Pike of Upjohn Co., Kalamazoo, Michigan, USA.

References

- Ambache, N., Kavanagh, L., and Whiting, J. (1965). Effect of mechanical stimulation on rabbit's eye: Release of active substance in anterior chamber perfusates. *Journal of Physiology*, **176**, 378-408.
- Änggård, I., and Samuelsson, B. (1964). Smooth muscle stimulating lipids in sheep iris. The identification of prostaglandins F₂α prostaglandins and related factors. *Biochemical Pharmacology*, **13**, 281-283.
- Benzie, R., Boot, J. R., and Dawson, W. (1975). A preliminary investigation of prostaglandin synthetase activity in normal, sensitized and challenged sensitized guinea-pig lung. *Journal of Physiology*, **246**, 80-81.

- Bhattacharjee, P. (1977). Stimulation of prostaglandin synthetase activity in inflamed ocular tissue of the rabbit. *Experimental Eye Research*, **24**, 215–216.
- Bhattacharjee, P., and Phylactos, A. (1977). Increased prostaglandin synthetase activity in inflamed tissues of the rabbit eye. *European Journal of Pharmacology*, **44**, 75–80.
- Brocklehurst, W. E. (1975). Pharmacological mediators hypersensitivity reactions. In *Clinical Aspects of Immunology*, ed. P. G. H. Gell, R. R. A. Coombs, and P. J. Lachmann, 3rd edn., pp. 821–846. Blackwell Scientific: Oxford.
- Eakins, K. E., and Bhattacharjee, P. (1977). Histamine, prostaglandins and ocular inflammation. *Experimental Eye Research*, **24**, 299–305.
- Eakins, K. E., Whitelocke, R. A. F., Bennett, A., and Martenet, A. C. (1972). Prostaglandin-like activity in ocular inflammation. *British Medical Journal*, **3**, 452–453.
- Eakins, K. E., Whitelocke, R. A. F., Perkins, E. S., Bennett, A., and Unger, W. G. (1972). Release of prostaglandins in ocular inflammation in the rabbit. *Nature (New Biology)*, **239**, 248–249.
- Gilmore, N., Vane, J. R., and Wyllie, H. J. (1968). Prostaglandin release by the spleen. *Nature*, **218**, 1135–1140.
- Giroud, J. P., and Willoughby, D. A. (1970). The interrelations of complement and a prostaglandin-like substance in acute inflammation. *Journal of Pathology*, **101**, 241–249.
- Goldblatt, M. W. (1933). A depressor substance in seminal fluid. *News Sheet: Society of Chemical Industry* (London), **52**, 1056–1057.
- Greaves, M. W., Søndergaard, J., and McDonald-Gibson, W. (1971). Recovery of prostaglandins in human cutaneous inflammation. *British Medical Journal*, **2**, 258–260.
- Higgs, G. A., and Youlten, L. J. F. (1972). Prostaglandin production of peritoneal polymorphonuclear leukocyte *in vitro*. *British Journal of Pharmacology*, **44**, 330P.
- Holborow, E. J. (1974). Pathogenesis of connective tissue disease. *Transactions of the Ophthalmological Societies of the United Kingdom*, **94**, 712–718.
- Kiefer, H. C., Johnson, C. R., Arora, K. L., and Kantor, H. S. (1975). Colorimetric identification of prostaglandins in subnanomolar amounts. *Analytical Biochemistry*, **68**, 336–340.
- Leopold, I. (1974). Advances in ocular therapy: non-corticosteroid anti-inflammatory agents. *American Journal of Ophthalmology*, **78**, 759–773.
- Marak, G. E., Font, R. L., and Alepa, F. P. (1976). Experimental lens induced granulomatous endophthalmitis. *Modern Problems in Ophthalmology*, **96**, 113–122.
- Morley, J. (1974). Prostaglandins and lymphokines in arthritis. *Prostaglandins*, **8**, 315–325.
- Piper, P. J., and Vane, J. R. (1971). The release of prostaglandins from lung and other tissues. *Annals of the New York Academy of Sciences*, **180**, 363–385.
- Rahi, A. H. S., Holborow, E. J., Perkins, E. S., Gungen, Y. Y., and Dinning, W. (1976). Immunological investigations in uveitis. *Transactions of the Ophthalmological Societies of the United Kingdom*, **96**, 113–122.
- Rahi, A. H. S., Misra, R. N., and Morgan, G. (1977a). Immunopathology of the lens: I Humoral and cellular immune responses to heterologous lens antigens and their roles in ocular inflammation. *British Journal of Ophthalmology*, **61**, 164–176.
- Rahi, A. H. S., Misra, R. N., and Morgan, G. (1977b). Immunopathology of the lens: II Humoral and cellular immune responses to autologous lens antigens and their roles in ocular inflammation. *British Journal of Ophthalmology*, **61**, 371–379.
- Rahi, A. H. S., Mohammed, I., Holborow, E. J., Perkins, E. S., and Dinning, W. (1977). Uveitis: a possible immune-complex disease. *British Journal of Ophthalmology* (in preparation).
- Sears, M. L., Neufeld, A. H., and Jampol, L. M. (1973). Prostaglandins. *Investigative Ophthalmology*, **12**, 161–164.
- Silverstein, A. M., and Zimmerman, L. E. (1959). Immunogenic endophthalmitis produced in guinea-pig by different pathogenetic mechanisms. *American Journal of Ophthalmology*, **48**, 435–447.
- Unger, W. G., Stamford, I. F., and Bennett, A. (1971). Extraction of prostaglandins from human blood. *Nature (New Biology)*, **233**, 336–337.
- Vane, J. R. (1957). A sensitive method for assay of 5-hydroxytryptamine. *British Journal of Pharmacology and Chemotherapy*, **12**, 344–349.
- Williams, B. D., and Lehner, T. (1977). Immune complexes in Behçet's syndrome and recurrent oral ulcerations. *British Medical Journal*, **1**, 1387–1389.
- Williams, T. J., and Morley, J. (1973). Prostaglandins as potentiators of increased vascular permeability in inflammation. *Nature*, **246**, 215–217.
- Williams, T. J. (1976). The pro-inflammatory activity of E-, A-, D-, and F-type of prostaglandins and analogues 16, 16-dimethyl-PGE₂ and (15S)-15-methyl-PGE₂ in rabbit skin; the relationship between potentiation of plasma exudation and local blood flow changes. *British Journal of Pharmacology*, **56**, 341–342.