PRELIMINARY COMMUNICATIONS

Prostaglandin-like Activity in Ocular Inflammation

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Summary

Aqueous humour samples from untreated patients with acute anterior uveitis were found to contain substantial amounts of prostaglandin-like activity. Little activity was found in aqueous from patients treated with steroids, and none (<2 ng/ml) was detected in aqueous from the uninflamed eyes of patients with cataract. Alkaline hydrolysis of the samples from inflamed eyes suggested the presence of both E and F prostaglandin-like activity. These results show that prostaglandins may be involved in acute anterior uveitis.

Introduction

Prostaglandins are present in ocular tissues (Ambache and Brummer, 1968) and can induce some of the characteristic changes associated with ocular inflammation, such as vasodilatation, increased vascular permeability, and a raised intraocular pressure (Beitch and Eakins, 1969; Eakins, 1970; Kelly and Starr, 1971; Starr, 1971).

Recently it has been observed that when severe uveitis was induced in rabbits with a single intravitreal injection of bovine serum albumin large amounts of a substance with activity like prostaglandin E_1 (PGE_1) were released into the aqueous, often together with small quantities of a material with activity like prostaglandin E_2 (PGE_2) (Eakins *et al.*, 1972). Since the ocular response produced in this experimental model of uveitis closely resembles the clinical condition we have examined aqueous humour taken from patients with acute anterior uveitis for the presence of prostaglandin-like substances.

Patients and Methods

Aqueous humour samples from 14 patients were examined for the presence of prostaglandins. Eight samples were from patients with acute anterior uveitis and six were taken from uninflamed eyes at cataract extraction. Patients with acute uveitis were given a subconjunctival injection of local anaesthetic. The anterior chamber was then carefully entered just anterior to the limbus with a 30-gauge needle attached to a sterile capillary tube and approximately 0·2 ml of aqueous was allowed to flow gradually into the tube. The samples were stored at — 20°C and refrigerated on solid carbon dioxide during transfer from Zürich to London for extraction and biological assay. The six samples obtained from uninflamed eyes were collected just before making the section at cataract extraction and were stored at — 20°C.

All except two of the uveitis samples were extracted for prostaglandins with ethanol acidified with formic acid, followed by chloroform (Unger *et al.*, 1971). They were then assayed against PGE_2 for prostaglandin-like activity on rat stomach strips (Vane, 1957) suspended in 5 ml of Krebs's solution at 37°C and aerated with 5% carbon dioxide in oxygen. The antagonist drugs methysergide, atropine, and mepyramine (all 0·1 μg/ml) were present in the bathing solution, and indomethacin (1 μg/ml) was added in some experiments. These drugs increased the selectivity and often the sensitivity of the assay.

In an attempt to identify the prostaglandin-like activity present, extracts and control solutions of PGE_2 and prostaglandin $F_2\alpha$ ($PGF_2\alpha$) were incubated with 0·2 N sodium hydroxide for 45 minutes at 37°C (PGE compounds are converted by this procedure to substances which do not contract rat stomach strips). Further examination of the prostaglandin-like activity—for example, by thin-layer chromatography—was not possible because of the small volumes available from each patient and the difficulty of obtaining these samples.

Results

No biological activity (< 2 ng/ml assayed as PGE_2) was detected in the extracts of aqueous humour removed from uninflamed eyes (Cases 1 to 6) (Fig. 1). In contrast, large amounts of prostaglandin-like activity (20-56 ng/ml assayed as PGE_2) were detected in extracts of aqueous removed from four patients with untreated acute anterior uveitis (Cases 7 to 10); one of these (Case 10) had received local steroid treatment three to four weeks previously and showed a lower level of activity than the other three. In extracts of aqueous from two patients receiving treatment with local corticosteroids at the time of sampling

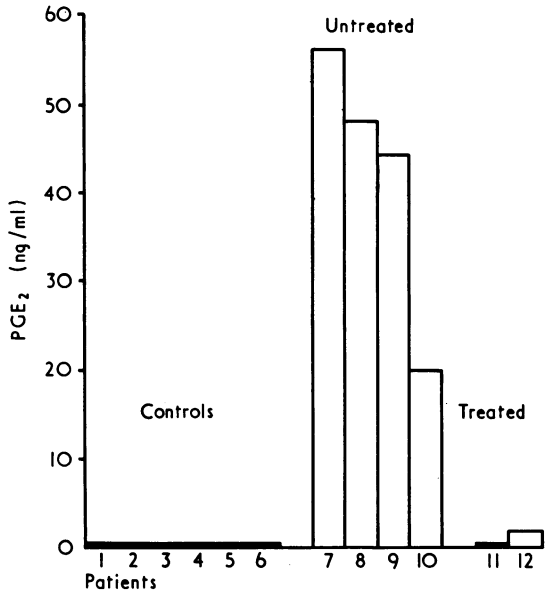

FIG. 1—Prostaglandin-like activity (ng/ml assayed as PGE_2) in extracts of aqueous humour from control subjects and patients with treated and untreated acute anterior uveitis.

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(Cases 11 and 12) prostaglandin-like activity was slight (2 ng/ml) or undetectable (< 2 ng/ml). The intensity of the inflammation seen clinically correlated well with the level of prostaglandin-like activity in the aqueous. The two unextracted samples of aqueous from inflamed eyes were also assayed; one from an untreated patient produced a small contraction of the rat stomach strip (equivalent to 3 ng/ml assayed as PGE_2), whereas the other, from a patient treated with local steroids, caused only a relaxation. It was clear that in the latter case biological material was present which would antagonize the response to prostaglandin-like material, and these two unextracted samples are therefore excluded from Fig. 1.

The remains of three extracts of aqueous from inflamed eyes which contained prostaglandin-like activity were pooled (there was not enough left for three separate experiments) and subjected to alkaline hydrolysis. The biological activity was roughly halved; whereas PGE_2 was inactivated, $PGF_{2\alpha}$ was not affected (Fig. 2).

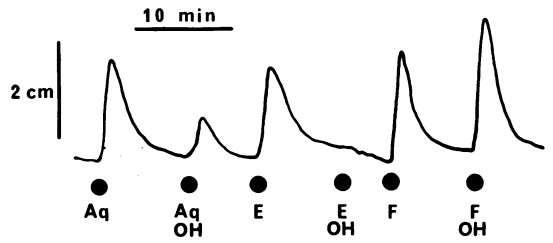

FIG. 2—Contraction of rat stomach strip in prostaglandin assay. Some activity in extracts of aqueous humour (Aq) was destroyed by alkaline hydrolysis (Aq OH). PGE_2 2 ng (E) was totally inactivated (E OH), whereas $PGF_2\alpha$ 20 ng (F) was not affected by alkali (F OH).

Discussion

The extraction characteristics of the samples of aqueous humour obtained from patients with acute anterior uveitis, together with the biological activity of the extracts, as measured on the rat stomach strip blocked with atropine, methysergide, and mepyramine, provide evidence for the presence of prostaglandin-like material. High amounts were present in the severe cases, and the levels correlated well with the clinical intensity of the uveitis. The reduction in biological activity after alkaline hydrolysis is consistent with the presence of both E and F prostaglandin-like activity. If this is so, then the amounts of prostaglandins present will be even higher than estimated, since the rat stomach is up to 10 times less sensitive to PGF than to PGE compounds. Other biological activity was present in one unextracted aqueous humour sample, since relaxation of the rat stomach strip occurred. This might have been due to a catecholamine; it was not due to 5-hydroxytryptamine, acetylcholine, or histamine.

Since no biological activity could be detected in the aqueous from control, uninflamed eyes it seems likely that raised levels of prostaglandin-like substances are an important feature of acute anterior uveitis. There is now evidence that prostaglandins are involved in acute inflammation in other parts of the body (Änggård *et al.*, 1970; Greaves *et al.*, 1971), and also in the rabbit eye (Eakins *et al.*, 1972).

The levels found in the untreated inflamed eyes were substantially higher than the slightly increased amounts occurring in open-angle glaucoma (Wyllie and Wyllie, 1971), a condition in which there is no inflammation and in which the levels of activity were reported to be up to 4·3 ng/ml assayed as PGE_1. (PGE_1 and PGE_2 were about equiactive on the assay tissue.) It is of particular interest to note that the two patients undergoing local steroid therapy at the time of sampling had little inflammation and negligible quantities of prostaglandin-like activity. It is possible that the steroids inhibited prostaglandin synthesis (Greaves and McDonald-Gibson, 1972), or they may just have suppressed the acute inflammatory process.

The release of PGE_1-like activity in induced uveitis in the rabbit eye (Eakins *et al.*, 1972) is of great relevance to the present findings. Of particular interest is the suggestion that the PGE_1-like material is released by the white blood cells which enter the inflamed eye. The present results suggest that prostaglandins may contribute to many of the clinical signs in acute anterior uveitis, though they do not show where they originate. It follows that substances which inhibit the action or synthesis of prostaglandins might be of value in treating this disease.

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