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RELEASE OF PROSTAGLANDIN-LIKE SUBSTANCES BY SHIGELLA ENDOTOXIN AND ITS INHIBITION BY NON-STEROIDAL ANTI-INFLAMMATORY COMPOUNDS

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1 The effects of intravenous and intraocular injections of Shigella endotoxin on the inflammatory response, the release of prostaglandin-like substances into the aqueous humour, and the effects of indomethacin on the inflammatory response were studied in the rabbit eye.

2 Both intravenous and intraocular injection of endotoxin released 12.8 ng to 72 ng of prostaglandin-like substances per ml of aqueous humour and increased the permeability of the blood-aqueous barrier as shown by the rise in aqueous humour protein (26-52 mg protein/ml of aqueous humour).

3 Indomethacin, 0.25% administered topically completely inhibited the release of prostaglandin-like substances but was found to have inconsistent inhibitory effects on the clinical signs of inflammation and on the blood-aqueous barrier. Indomethacin was less effective in eyes receiving the higher dose of endotoxin.

4 It is suggested that prostaglandins and possibly other chemical mediators are involved in endotoxin-induced ocular inflammation.

Introduction

Systemic injection of bacterial endotoxin produces a number of haemodynamic changes such as vasodilatation, hypotension and increased vascular permeability (Wiel, McLean, Visscher & Spink, 1956; Eckman, King & Brunson, 1958; Gilbert, 1960). The ocular effects of endotoxins injected either systemically or intraocularly have been studied by a number of workers (Howes, Aronson & McKay, 1970; Gamble, Aronson & Brescia, 1970). Intraocular injection of endotoxin results in a transient rise in intraocular pressure, vasodilatation, breakdown of blood-aqueous barrier and cellular infiltration. In a recent study, Bito (1974) reported migration of white blood cells, severe iritis, decreased ascorbate concentrations and increased protein concentrations (32 mg/ml) in the aqueous humour, 24 h after intravitreal injection of 10 μg of Shigella endotoxin. Chemical substances suggested as mediators of endotoxin reactions are histamine (Kellaway, Trethewie & Turner, 1940; Weil & Spink, 1957), 5-hydroxytryptamine (Armin & Grant, 1957; Davies, McQuarrie & Meeker, 1959), noradrenaline and adrenaline (Thomas, 1954; Howes & McKay, 1972).

In recent years, prostaglandins have been detected in inflammatory exudates from various tissues (Willis, 1969; Giroud & Willoughby, 1970; Greaves, Sondergaard & McDonald-Gibson, 1971; Higgs, Vane, Hart & Wojtulewski, 1974). Eakins, Whitelocke, Perkins, Bennett & Unger (1972) and Eakins, Whitelocke, Bennett & Martenet (1973) demonstrated the release of prostaglandins into the aqueous humour of rabbits and man with acute ocular inflammation.

Studies on the mechanism of action of nonsteroidal anti-inflammatory drugs showed that compounds such as indomethacin and aspirin inhibited prostaglandin synthesis, implying that prostaglandins are involved in the inflammatory process (Ferreira, Moncada & Vane, 1971; Smith & Willis, 1971; Vane, 1971).

The present study examines the release of prostaglandin-like substances by Shigella endotoxin administered intravenously and intraocularly, and the effect of indomethacin on prostaglandin release.

Methods

Adult albino rabbits, weighing 2.5-3.5 kg were used. Shigella endotoxin (Difco) and indomethacin were administered either intravenously or intraocularly.

Intravenous injection

Shigella endotoxin, 100 μg/kg, dissolved in 0.9% w/v NaCl solution (saline) was injected slowly over a period of 3-5 min into the marginal ear vein. Changes in pupil size and blood-vessels were observed for 3-4 h after which time the animals were killed and the aqueous humour removed for assay of prostaglandin-like substances and protein. In separate groups of rabbits, indomethacin, 10 mg/kg (solutions prepared by adding equimolar concentrations of Na_2CO_3 to aqueous suspensions of indomethacin) was injected intravenously 10 min before the endotoxin injection, and aqueous humour was removed as previously. White blood cells in diluted aqueous humour were counted in a haemacytometer.

Intraocular injection

Shigella endotoxin, 2 or 10 μg, was injected in 10 or 50 μl of saline into the anterior chamber or vitreous body respectively, using a No. 30 needle with an Agla micrometer syringe.

Treatment with 100 μl of 0.25% indomethacin, prepared as above, and administered topically, began 2 h before or 12 h after the endotoxin injection, at two hourly intervals for the first 24 h and then, three times a day.

Eyes from a separate group of rabbits, injected intravitreally with 50 μl of saline, were examined, and the aqueous humour was analysed for prostaglandin-like substances and for protein as described below.

Eyes were examined with a slit lamp and vasodilatation aqueous flare, conjunctival oedema and miosis were assessed and scored from 0-4 (0 being normal, 4 being the most severe change). These scores were summed up for each eye and the mean was taken as an 'Inflammatory index'. Photographs of control and treated eyes were taken on Tri-X film using a Nikon F Camera with an 80 mm lens, f 4.5 and a Xenon-flash tube energised by 250 J from a Bowen's Multilec 500.

Twenty-four hours after injection of endotoxin, rabbits were anaesthetized with sodium pentobarbitone and aqueous humour was withdrawn. Protein in the aqueous humour was determined according to the method of Lowry, Rosenbrough, Farr & Randall (1951). Prostaglandin-like substances in the aqueous humour were extracted according to the method of Unger, Stamford & Bennett (1972), assayed against PGE_1 using rat fundus strips suspended in 5 ml of Krebs solution at 37° C, gassed with 5% CO_2 in O_2, and containing methysergide, atropine and mepyramine (all 0.1 μg/ml) and indomethacin (1 μg/ml).

Results

Intravitreal injection of saline into the control eyes did not produce inflammation and did not release prostaglandin-like substances into the aqueous humour. The protein concentrations were in the normal range (0.6-1 mg/ml).

Within 30 min of an intravenous injection of endotoxin, the rabbits became very weak, did not move, and showed signs of endotoxin shock. Aqueous flare, due to exudation of protein into the anterior chamber, vasodilatation and miosis appeared simultaneously. These reactions, expressed as an 'Inflammatory index', reached their peak at about 2 h after the intravenous injection and were less intense than those observed after intraocular injections. Aqueous humour, withdrawn 3 h after the endotoxin injection contained a mean of 12.8 ng/ml prostaglandin-like substances and 26 mg/ml of protein (Table 1).

Table 1 Release of prostaglandin-like substances and increase of protein in aqueous humour following the administration of Shigella endotoxin by various routes.

Route of administration	*Doses of endotoxin*	*Prostaglandin-like substances* ng/ml of aqueous humour (mean ± s.d.)*	*Protein* mg/ml of aqueous humour (mean ± s.d.)*	*Inflammatory index (mean ± s.d.)*
Intravitreal	10 μg	72.6 ± 15 (5)***	50.0 ± 12 (5)	9.8 ± 2.0(20)
	2 μg	47.0 ± 6.5(3)	42.0 ± 8.5(3)	7.0 ± 1.5(12)
Intracameral	10 μg	52.0 ± 14 (3)	32.0 ± 9.9(3)	8.3 ± 1.5(15)
Intravenous**	100 μg/kg	12.8 ± 5.8(4)	26.0 ± 5.5(4)	4.0 ± 1.0(12)

* Each experiment represents pooled aqueous humour from 4-5 rabbits.
** Aqueous humour was withdrawn 3 h after endotoxin.
*** Figure in brackets denotes number of experiments.
s.d. = Standard Deviation.

Intraocular endotoxin

Intracameral .The inflammatory responses of the eye to 10 μg of endotoxin began to appear between 4 and 6 h after injection. These responses, characterised by vasodilatation, miosis and aqueous flare, were most severe after 16 hours. Slit lamp examination showed oedema of the iris, cells and fibrin in the aqueous humour. The mean concentration at 24 h of prostaglandin-like substances in the aqueous humour was 52 ng/ml and that of protein was 32 mg/ml.

Intravitreal. After intravitreal injection of 10 μg of endotoxin, ocular responses were qualitatively similar to those observed after intracameral injection, but the onset of reactions was between 8 and 12 h and the intensity of the responses was greater. In addition to miosis, vasodilatation and cellular infiltration, conjuntival oedema was also evident. The responses peaked after 20 h (Plate 1) and remained so for 48 to 72 h, after which time reactions began to subside very slowly and the eyes returned to apparent normality over 10-15 days. At 24 h the mean concentration of prostaglandin-like substances in the aqueous humour was 72.6 ng/ml. After 2 μg of endotoxin, inflammatory reactions were similar (Plate 2) but milder, and the return to normality was more rapid – over 3-5 days. The mean concentration of prostaglandin-like substances was significantly less than that after 10 μg of endotoxin. After either 2 or 10 μg of endotoxin, protein levels in the aqueous humour were very high, and white cells appeared in the aqueous humour 8 h after the injection.

Inhibitory effects of indomethacin on the responses to:

Intravenous endotoxin. Pretreatment with indomethacin 10 mg/kg (i.v.) 10 min before endotoxin gave almost complete protection against endotoxin shock. Although vasodilatation and miosis were prevented for the first 3 h, mild vasodilatation appeared later. The aqueous humour of these rabbits showed no prostaglandin-like activity, but contained some protein indicating that leakage through the blood-aqueous barrier had not been fully inhibited (Figure 1).

Intraocularly administered endotoxin. When the treatment commenced 2 h before the injection of endotoxin, the effect of indomethacin on inflammatory responses was greater than that of indomethacin treatment begun 12 h after the injection. The inhibition of the 'Inflammatory index' shown in Figure 1 represents the combined

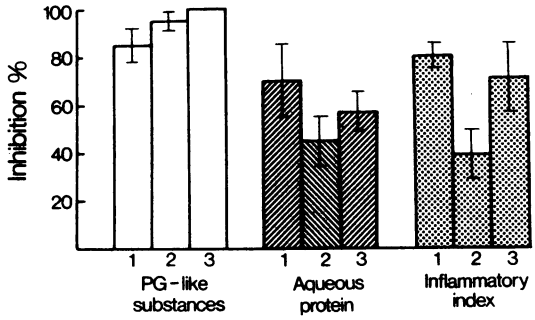

Figure 1 Percentage inhibition of inflammatory responses to endotoxin by indomethacin. Columns 1, 2, 3 represent inhibition of responses to 100 μg/kg (intravenously), 10 and 2 μg (intraocularly) of endotoxin, respectively. Note that although the release of prostaglandin-like substances, 10 and 2 μg of endotoxin was almost completely inhibited, the rise in aqueous humour protein was inhibited by 45-57%, and inflammatory indices by 39-70%.

results with both types of indomethacin treatment. The release of prostaglandin-like substances into the aqueous humour was completely inhibited by indomethacin. However, indomethacin inhibited the inflammatory response to 10 μg of endotoxin by only 39%, and to 2 μg of endotoxin by 70%; the rise in protein concentration was reduced by 45% and 57% respectively (Figure 1).

Comparison of Plates 1 and 3, and 2 and 4 shows that indomethacin suppressed the inflammation produced by 2 μg of endotoxin more effectively than that produced by 10 μg. White cell counts in aqueous humour (predominantly polymorphonuclear cells) were 6000-10,000/mm^3 and 3000-6000/mm^3 after 10 and 2 μg of endotoxin, respectively. Indomethacin had no effect on the migration of white cells into the aqueous humour after 10 μg of endotoxin, and inhibited by 10-15% the migration of white cells into the anterior chamber after 2 μg of endotoxin.

Discussion

Endotoxins do not produce inflammatory reactions by a direct action on target tissues, but through the release of chemical mediators (Gilbert, 1960) such as histamine, 5-hydroxytryptamine polypeptides and prostaglandins.

Histamine is released during the early phase of endotoxin, and other inflammatory reactions (Kun, 1947; Weil & Spink, 1957; Hinshaw, Jordan & Vick, 1961; Spector & Willoughby, 1963, 1968). Antihistamines inhibited changes in vascular permeability only in the initial stages of

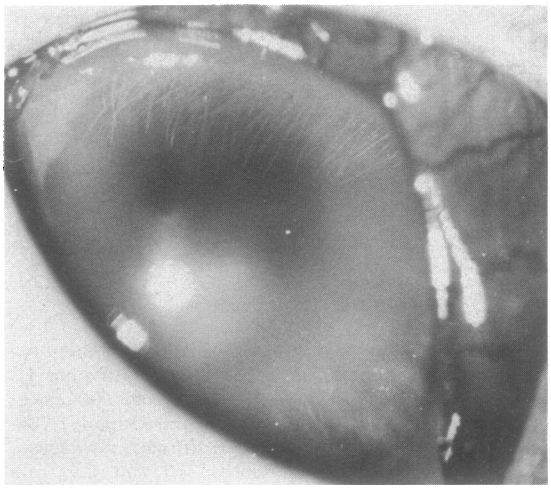

Plate 1 Rabbit eye, 24 h after 10 μg of intravitreal injection of Shigella endotoxin. Note: the detail of the iris cannot be seen due to iris oedema and inflammatory exudate in the anterior chamber.

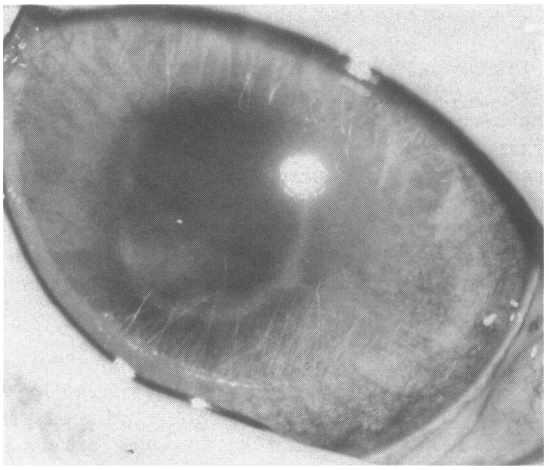

Plate 3 Eye injected as in Plate 1, but pretreated topically with 0.25% indomethacin. Although indomethacin has reduced inflammation, exudate and iris oedema are still present.

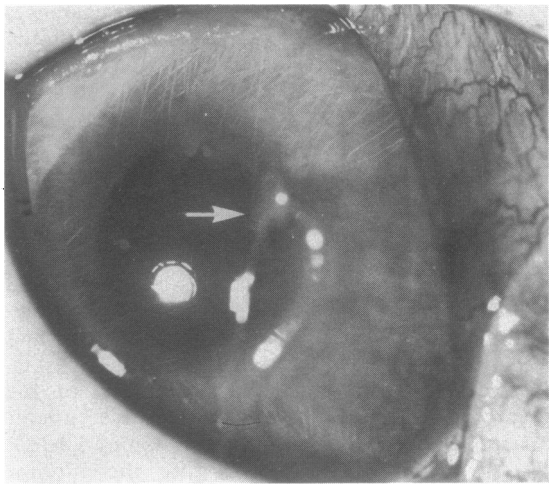

Plate 2 Rabbit eye 24 h after 2 μg of intravitreal endotoxin. Inflammatory exudate in the anterior chamber can be seen (at arrow).

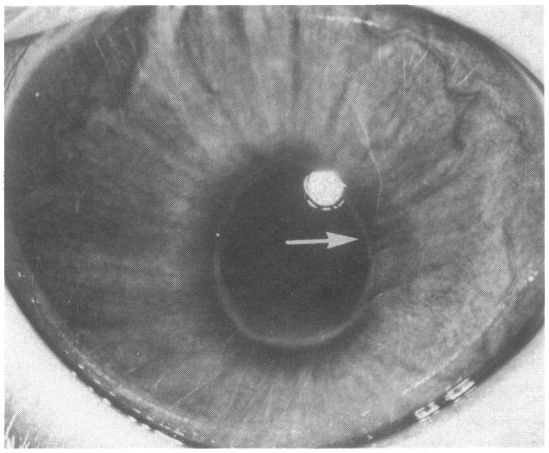

Plate 4 Eye injected as in Plate 2, but pretreated topically with 0.25% indomethacin. Note: almost normal iris and clear anterior chamber except that fibrin (at arrow) still present in the anterior chamber.

inflammation, and despite their repeated administration, increased vascular permeability at later stages could not be suppressed (Spector & Willoughby, 1957, 1959; Wilhelm & Mason, 1960). On the other hand, Schayer (1963) suggested, from studies on histidine decarboxylase activity, that stored histamine was not important, but that its continuous synthesis was responsible for changes in the later phase of inflammation.

Numerous mast cells are normally present in the posterior uvea, but not in the iris (Smelser & Silver, 1963). They migrate to the anterior uvea at an early stage of the inflammation induced by Shigella endotoxin and bovine serum albumin, and degranulate in the later phase of inflammation (Smelser, 1964; Segawa & Smelser, 1969). Histamine has been reported to be released in the initial stage of ocular inflammation (Kapuścínski, Baran & Sikorska, 1965), but its role is not yet known.

Activation of a kallikrein-kinin system (Nies, Forsyth, Williams & Melmon, 1968; Erdos & Miwa, 1968) and increased levels of circulating catecholamines after administration of endotoxin, have been reported (Egdahl, 1959). Kinins were found to play only a transitory role in the acute inflammatory process (Di Rosa, Giroud & Willoughby, 1971). Adrenaline is released from the adrenal medulla in the early stages of the reaction to endotoxin. Hinshaw, Brake, Emerson, Jordan & Masucci (1964) reported that adrenalectomized dogs were more sensitive to endotoxin, which suggested that catecholamines play a protective role. Adrenaline and noradrenaline have been reported to suppress inflammation (Setnikar, Subratorra & Temilcou, 1959; Northover & Subramanian, 1961). These studies on the release of chemical mediators by endotoxin were carried out after its intravenous administration.

Intraocular injections of endotoxin produce local inflammatory responses, but the role played by locally available histamine, kinins and noradrenaline is not known. Ocular responses to endotoxin are partially suppressed by superior cervical ganglionectomy and it has been suggested that a high local concentration of catecholamines in the uvea is partly responsible for making the eye unusually sensitive to the effects of endotoxin (Howes & McKay, 1972).

The release of prostaglandins into the aqueous humour of rabbits during acute immunogenic inflammation induced with bovine serum albumin has been reported by Eakins *et al.* (1972). The present study shows that non-immunogenic inflammation produced by endotoxin prostaglandin-like substances are also released into the aqueous humour. However, another mediator (or mediators) seems to be released as well since indomethacin, while inhibiting prostaglandin-release completely, only partially suppressed the clinical signs of inflammation and the increase in vascular permeability.

Indomethacin did not have the same effect on all parameters of the endotoxin response. The systemic hypotension, decreased central venous pressure and cardiac output and metabolic acidosis (Gilbert, 1960; Parratt & Sturgess, 1974) were inhibited by indomethacin as indicated by the good condition of the rabbit after intravenous endotoxin. The suppression of the clinical signs of ocular inflammation was incomplete and seemed to depend on the inhibition of vascular leakage. The fact that inflammatory responses to 2 μg of endotoxin were not much less than those to 10 μg indicated that the latter dose was a very strong stimulus which produced responses beyond effective control by non-steroidal anti-inflammatory compounds.

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