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INHIBITION OF THE PROSTAGLANDIN SYNTHETASE SYSTEMS IN OCULAR TISSUES BY INDOMETHACIN

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1 We have compared the sensitivity of the prostaglandin synthetase systems derived from microsomal fractions of rabbit ocular tissues (anterior uvea, conjunctiva and retina) with other rabbit tissues such as the kidney medulla and spleen, to inhibition by indomethacin.

2 Generation of prostaglandin-like activity by the microsomal fractions from added arachidonic acid varied with the tissue used. Highest activity was found in the kidney medulla, then in descending order, the conjunctiva, anterior uvea, spleen, retina and cornea.

3 Indomethacin was most potent in the spleen (ID_{50} 0.045 μg/ml) then in decreasing order in the kidney medulla, conjunctiva, anterior uvea and weakest in the retina, where the ID_{50} for indomethacin was 50 μg/ml.

4 The differential sensitivity to inhibition of the prostaglandin synthetase systems from different tissues is an important consideration in the development of new ocular anti-inflammatory agents.

Introduction

There is now much evidence to suggest that prostaglandins may contribute to many of the clinical signs of acute anterior uveitis both in the experimental animal and in man. Prostaglandin-like substances are present in ocular tissues (Ambache & Brummer, 1968) and low concentrations given either into the anterior chamber or onto the cornea can induce many of the characteristic changes associated with ocular inflammation (Beitch & Eakins, 1969; Eakins, 1970; Kelly & Starr, 1971; Starr, 1971a,b; Bethel & Eakins, 1972).

More recently, substantial amounts of prostaglandin-like activity have been detected in aqueous humour from rabbits with experimentally-induced uveitis (Eakins, Whitelocke, Perkins, Bennett & Unger, 1972a), and from human subjects with acute untreated anterior uveitis (Eakins, Whitelocke, Bennett & Martenet, 1972b). These observations led to the suggestion that substances which inhibit the action or synthesis of prostaglandins might be of value as ocular anti-inflammatory agents (Eakins *et al.*, 1972b).

Aspirin, indomethacin and other non-steroidal anti-inflammatory agents have been found to block the synthesis of prostaglandins in various systems (Vane, 1971; Ferreira, Moncada & Vane, 1971; Smith & Willis, 1971). Furthermore, the prostaglandin synthetase systems from different regions of the body have been shown to have different sensitivities to these inhibitory drugs (Flower & Vane, 1972). Vane (1971) has proposed that the study of prostaglandin synthetase systems from different tissues will lead to aspirin-like drugs with a greater specificity of action. In the present experiments we have compared the sensitivities of the prostaglandin synthetase systems from the rabbit eye (anterior uvea, conjunctiva and retina) and other tissues (kidney and spleen) to inhibition by indomethacin.

Methods

New Zealand white rabbits of either sex were killed with pentobarbitone sodium. The eyes, spleen and kidney were rapidly removed, and the iris-ciliary body and retina dissected from each eye. All tissues were cut with scissors and washed in ice-cold Krebs solution. For the ocular tissues, pooled samples from 10 eyes were used to prepare each batch of cell-free prostaglandin synthetase preparations according to the method of Flower, Gryglewski, Herbaczynska-Cedro & Vane (1972). The tissues were homogenized for 2 min in ice-cold 100 mM phosphate buffer (pH 7.4). After centrifugation at 10,000 *g* for 10 min at 2° C the precipitate was discarded and the supernatant

recentrifuged at 80,000 *g* for 1 h at 2° C. The pellets were then resuspended in phosphate buffer and used as the source of synthetase. Total protein was determined in this final solution by the method of Lowry, Rosenbrough, Farr & Randal (1951).

Microsomal fractions containing prostaglandin synthetase systems were incubated in mixtures of 2 ml 50 mM phosphate buffer, containing 20 μg arachidonic acid, 100 μg reduced glutathione, 10 μg hydroquinone and approximately 1 mg of protein from the pellet suspension. Indomethacin was added to the reaction mixtures in various concentrations. Samples were incubated aerobically with shaking at 37° C for 20 min, at which time the reactions were stopped by heating the tubes in boiling water for 1 minute. The contents of each tube were then extracted for prostaglandins with ethanol acidified with formic acid, followed by petroleum ether (BP37.5-52.8° C) and chloroform (Unger, Stamford & Bennett, 1971). They were then assayed against prostaglandin E_2 on rat stomach strips (Vane, 1957) suspended in 5 ml Krebs solution at 37° C gassed with 5% CO_2 in O_2 and containing methysergide, atropine, mepyramine (all 0.1 μg/ml) and indomethacin (1 μg/ml). In two experiments the reaction product was tentatively identified as prostaglandin E_2 by chromatography using the A-II system of Gréen and Samuelsson and paper impregnated with silica gel and silver nitrate (Stamford & Unger, 1972).

The following drugs were used, prostaglandins E_1, E_2 and $F_{2\alpha}$ (Upjohn) and indomethacin (Merck).

Results

Tissue homogenates and microsomal pellets were freshly prepared for every experiment. Generation of prostaglandin-like activity was determined for each tissue (activity in the 20 min sample minus activity in the zero-time sample). The results are seen in Table 1. Generation of prostaglandin-like activity by the microsomal fractions from the added substrate varied with the tissue used. Highest activity was found in the kidney medulla, then in descending order in the conjunctiva, anterior uvea (iris plus ciliary body), spleen, cornea and retina. Although the activity in the cornea and retina was low, control incubations with boiled protein indicated that it could not be accounted for by auto-oxidation of the arachidonic acid.

Inhibition of prostaglandin synthesis by indomethacin

Various amounts of indomethacin were added to the incubation flasks and the percentage inhibition of the control generation of prostaglandin-like activity was determined for each tissue after extraction. Dose-response curves were constructed for the inhibitory effect of indomethacin on each tissue studied (Figure 1). The potency of indomethacin as an inhibitor of prostaglandin synthesis in each tissue was calculated from the dose-response curves and expressed as the concentration (μg/ml) required to produce 50% inhibition (ID_{50}) of the control synthesis. The results are summarized in Table 2. Indomethacin was most potent in the spleen then in decreasing order the kidney medulla, conjunctiva, anterior uvea and retina. The latter required approximately 1,000 times more indomethacin than did the spleen.

Discussion

There is now accumulating evidence to support the proposal that inhibition of prostaglandin biosyn-

Table 1 Prostaglandin (PG) biosynthesis from added substrate by various rabbit tissues *in vitro*.

	*PG-like activity (ng/mg protein)**		
	Zero-time	*20 min*	*Increase in activity‡*
Spleen	18.9 ± 3.3 (9)	56 ± 10.5 (9)	38 ± 10 (9)
Kidney (medulla)	69 ± 12 (7)	648 ± 10 (7)	578.5 ± 96 (7)
Anterior uveat	60 ± 5 (15)	179 ± 14 (15)	117 ± 11 (15)
Conjunctiva	39 ± 7 (5)	244 ± 34 (5)	205 ± 28 (5)
Cornea	15 ± 6 (5)	30.5 ± 5 (5)	15 ± 1 (5)
Retina	18 ± 3 (6)	32 ± 5 (6)	14 ± 2 (6)

* ng prostaglandin-like activity assayed as prostaglandin E_2 generated by microsomes in incubation fluid containing 10 μg/ml arachidonic acid. Numbers in parentheses refer to the number of separate microsomal fractions from pooled tissue samples used for each determination. Results expressed as mean ± s.e. mean.

† Iris and ciliary body.

‡ Calculated from individual differences between zero time and 20 min samples.

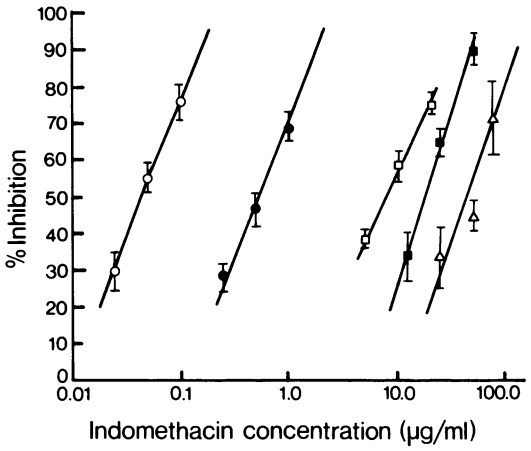

Fig. 1 Dose-response curves for the inhibitory activity of indomethacin on prostaglandin formation from added arachidonic acid. Each point represents the mean ± s.e. mean for at least four experiments. Spleen (○); kidney medulla (●); conjunctiva (□); anterior uvea (■); retina (△).

thesis by indomethacin and other aspirin-like drugs is the basis of their acute anti-inflammatory, anti-pyretic and analgesic actions (Vane, 1972). Furthermore, it has recently been demonstrated that paracetamol, which is analgesic and anti-pyretic but not anti-inflammatory, had little effect on prostaglandin synthetase derived from dog spleen, whereas it was active against rabbit brain synthetase (Flower & Vane, 1972). The present results lend further support to the hypothesis that synthetase systems from different tissues show different sensitivities to drugs. Indomethacin was found to be far less potent as an inhibitor of prostaglandin biosynthesis in ocular tissues than in spleen or kidney medulla. Some differential sensitivity was apparent even within the ocular tissues themselves; synthetase from the conjunctiva was the most sensitive to inhibition by indomethacin, whereas the retinal enzymes were the least susceptible.

There are few clinical reports on the effectiveness of indomethacin as an ocular anti-inflammatory agent. Perkins & McFaul (1965) demonstrated some beneficial effect of oral indomethacin especially in patients with acute uveitis, but Gordon (1970) reported little or no activity in cases of posterior or anterior uveitis. However, both studies reported side-effects such as headache or gastro-intestinal irritation. It is possible that the resistance of the prostaglandin synthetase systems in ocular tissues found in the present study may explain the relatively low potency of indomethacin as an ocular anti-inflammatory agent in man.

From our results it is apparent that the anterior uvea (iris plus ciliary body) has high enzyme activity, which is in agreement with Christ & van Dorp (1972) who also demonstrated a high conversion of substrate to prostaglandins in the rabbit iris. Retinal microsomes were also able to generate prostaglandin-like activity from arachidonic acid although at a rather low level. Previous work (van Dorp, Jouvenaz & Struijk, 1967) had shown that the pig retina could not convert all-cis-8,11,14-eicosatrienoic acid into prostaglandins. Two other points of interest arise from these studies: (a) microsomes from the rabbit spleen were much less effective than dog spleen (Flower *et al.*, 1972); (b) the observation that conjunctival tissue was able to generate substantial quantities of prostaglandin-like activity raises the possibility that prostaglandins may be involved in external as well as internal ocular inflammation.

There is now much evidence to support the idea that prostaglandins are involved in the pathogenesis of uveitis and it has been assumed that the source of the prostaglandins found in the aqueous humour is the iris. Recent work on paracentesis indicates that this may be the case for acute irritation of the eye (Miller, Eakins & Atwal, 1973). However, in experimental immunogenic uveitis the source of the bulk of the prostaglandins may be the invading leucocytes rather than the iris (Eakins *et al.*, 1972a and b). The sequence of events in uveitis could be: release of prostaglandins into the aqueous humour from the iris; invasion of leucocytes in part resulting from the leucotactic

Table 2 Comparison of the inhibitory activity of indomethacin on prostaglandin formation from added arachidonic acid by microsomal fractions of different rabbit tissues.

Tissue	*Indomethacin ID_{50}*	*Comparative ID_{50} (spleen = 1.0)*
Spleen	0.045	1
Kidney (medulla)	0.55	12
Conjunctiva	8.4	187
Anterior uvea	18.5	410
Retina	50.0	1,111

effects of the prostaglandins present in the aqueous; further prostaglandin release from the leucocytes (Higgs & Youlten, 1972). The problem is compounded by the fact that the normal uptake of prostaglandins from the aqueous (Bito & Salvador, 1972) does not function in the inflamed eye (Bito, L.Z., personal communication).

It is clear from the present experiments that the diverse sensitivity of the synthetase systems from different tissues to inhibition by indomethacin raises the possibility of developing other compounds which can selectively inhibit prostaglandin biosynthesis in some tissues with relatively little effect on others. For use as ocular anti-inflammatory agents, it would be necessary to consider their effectiveness against the synthetase systems of the anterior uvea, conjunctiva and leucocytes.

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