Inhibition of Prostaglandin Biosynthesis by 7-Oxa- and 5-Oxa-prostaglandin Analogues

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Some oxaprostaglandin derivatives have been shown to inhibit prostaglandin biosynthesis from arachidonate by a particulate prostaglandin synthetase preparation. The most potent inhibitor was 5-oxaprost-13-*trans*-enoate, and inhibition by this compound appeared to be competitive. Certain structure–activity relationships were ascertained.

Certain 7-oxaprostaglandin analogues are antagonists of prostaglandin $\S$ smooth-muscle-stimulating activity (Fried *et al.*, 1969; Flack, 1970). Some of these compounds had agonist properties and some had both agonist and antagonist properties. Because of these observations and because of the need for specific inhibitors of prostaglandin biosynthesis, we investigated a range of racemic analogues, made available by Dr. J. Fried, for effects on prostaglandin biosynthesis. Preliminary results on some of these have been reported (Fried *et al.*, 1971).

Materials and methods

A microsomal prostaglandin synthetase preparation was made from bovine seminal vesicles (Struijk *et al.*, 1967). The vesicles were homogenized in a Waring Blendor, all operations were performed at 4°C and the microsomal pellet was subjected to a further wash in 0.1M-sodium phosphate buffer, pH 8.0, and freeze-dried. Prostaglandin synthesis from arachidonate was measured by the incorporation of ^3H from [^3H]arachidonate. Reaction flasks contained unlabelled arachidonic acid (Sigma Chemical Co., St. Louis, Mo., U.S.A.) at the concentration indicated, 0.5 μCi of [5,6,8,9,11,12,14,15- ^3H]arachidonic acid (specific radioactivity 1.2 Ci/mmol; New England Nuclear Corp., Boston, Mass., U.S.A.), 0.325 mM-GSH, 0.275 mM-quinol and 1 mM-EDTA. After equilibration at 37°C the reaction was started by addition of enzyme (freeze-dried powder reconstituted in water; 0.5 ml $\equiv$ 0.5 mg of protein/assay). The final reaction volume was 2 ml. Reactions

were carried out for 30 min (during which time the reaction rate was linear) at 37°C in an atmosphere of $\text{O}_2 + \text{CO}_2$ (95:5) in a shaking water bath. The reaction was stopped by the addition of acetic acid to give pH about 3.0. After dilution with water lipids were extracted three times with diethyl ether. Ether was evaporated off and the residue was dissolved in a small volume of chloroform–methanol (2:1, v/v) for silica-gel t.l.c. in benzene–dioxan–acetic acid (50:50:1, by vol.) (Grn & Samuelsson, 1964). Prostaglandin standards were located by spraying with ethanolic 10% (w/v) phosphomolybdic acid and heating. Zones corresponding to prostaglandins E, F and other areas of the plate were removed and eluted three times with aq. 95% (v/v) acetone. After removal of solvent the residue was dissolved in 0.5 ml of de Jalon's solution (Garcia de Jalon *et al.*, 1945) and portions were removed for scintillation counting of radioactivity or bioassay. ^3H was determined by liquid-scintillation counting in a toluene-based scintillation fluid by using a Packard 3320 liquid-scintillation spectrometer. Counting efficiency was 30% and quench corrections were made by using the sample channels-ratio method. To calculate the conversion of [^3H]arachidonate into PGE_2 the prostaglandin radioactivity was multiplied by 8/7, since one of the eight ^3H atoms is lost during the biosynthesis (Klenberg & Samuelsson, 1965). Bioassay of prostaglandins was carried out by the bracketing method, with PGE_2 and $\text{PGF}_{2\alpha}$ as standards, with the ascending colon of the gerbil in aerated de Jalon's solution at 32°C. The variability of the bioassay was estimated to be $\pm 10\%$ of the amount measured. Prostaglandin recoveries were measured and corrected for by adding ^3H -labelled PGE_1 to all incubation mixtures after acidification to pH 3 if only bioassay was to be performed, or by adding ^3H -labelled PGE_1 to special control incubations containing no [^3H]arachidonate if biosynthesis was to be measured by ^3H incorporation. Interference from the oxaprostaglandin analogues in the final bioassay was not a problem, since all of these compounds moved well ahead ($R_F > 0.85$) of PGE_2 (R_F 0.5–0.6) in the t.l.c. system.

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$\S$ Prostaglandin nomenclature and abbreviations: PGE_1 , prostaglandin E_1 (11,15-dihydroxy-9-oxoprost-13-enoic acid); PGE_2 , prostaglandin E_2 (11,15-dihydroxy-9-oxoprost-5,13-dienoic acid); $\text{PGF}_{2\alpha}$, prostaglandin $\text{F}_{2\alpha}$ (9,11,15-trihydroxyprost-5,13-dienoic acid).

Table 1. *Effects of various racemic 7-oxa- or 5-oxa-prostaglandin derivatives on PGE₂ synthesis*

The arachidonate concentration in all cases was 0.04mM and the concentration of oxaprostaglandin derivatives was 0.15mM. PGE₂ biosynthesis was measured by ³H incorporation or by bioassay as indicated. For experimental details see the text. The specific activity of the enzyme preparation was approx. 280pmol of PGE₂ formed/min per mg of protein in the absence of oxaprostaglandin under these conditions. The results represent the means from two separate experiments in all cases. 100% inhibition by bioassay of the product indicates that no biological activity was detectable within the sensitivity limits of the bioassay used. % inhibition is the decrease in PGE₂ formed (relative to the appropriate control) expressed as a percentage of the control. % activation is the increase in PGE₂ formed (relative to the appropriate control) expressed as a percentage of the control.

Compound	Reference to compound	% inhibition of PGE ₂ synthesis		% activation of PGE ₂ synthesis By ³ H incorporation
		By ³ H incorporation	By bioassay
(I)	Fried <i>et al.</i> (1969)	23	100	—
(II)	Fried <i>et al.</i> (1971)	66	100	—
(III)	Fried <i>et al.</i> (1969)	31	19	—
(IV)	Fried <i>et al.</i> (1969)	42	57	—
(V)	Fried <i>et al.</i> (1971)	29	80	—
(VI)	Fried <i>et al.</i> (1970)	—	—	2
(VII)	Fried <i>et al.</i> (1970)	—	—	14
(VIII)	Fried <i>et al.</i> (1970)	—	—	10
(IX)	Fried <i>et al.</i> (1969)	—	—	30

Results

Certain of the oxaprostaglandin compounds effectively inhibited prostaglandin biosynthesis (Table 1). All inhibitors were compounds without oxo or hydroxy ring substituents and without a 15-hydroxyl group, whereas compounds with a 15-hydroxyl group and/or 9- or 11-oxo, -hydroxyl or -benzyloxy groups were not inhibitors. Indeed some of the latter compounds caused activation of prostaglandin synthesis.

Using one of the most potent inhibitors, as measured by bioassay for assessment of prostaglandin synthesis [compound (I); Table 1], we were able to show, in a separate experiment, an almost equal inhibition of both PGE_2 and $\text{PGF}_{2\alpha}$ synthesis. With 0.04 mM-arachidonate as substrate compound (I) (0.15 mM) caused about 90% inhibition of both PGE_2 and $\text{PGF}_{2\alpha}$ biosynthesis, both as measured by bioassay.

Kinetic studies were performed with the most potent inhibitor, as measured by both bioassay and ^3H incorporation [compound (II); Table 1]. The Lineweaver-Burk plot (Fig. 1a) shows that inhibition by this compound was apparently competitive. The K_m for arachidonate calculated from this plot was $2.3 \times 10^{-5} \text{ M}$ and the K_i for compound (II) was $3.2 \times 10^{-5} \text{ M}$. Alternative kinetic plots (Dixon, 1953;

Hofstee, 1956) yielded similar values for these constants. Fig. 1(b) illustrates how the degree of inhibition was dependent on the concentration of inhibitor.

Discussion

It is clear from these results that certain 7-oxa- or 5-oxa-prostaglandin analogues can inhibit prostaglandin synthetase. All inhibitors were compounds without 9- or 11-substituents on the ring and, perhaps more important, without the 15-hydroxyl group. This structure-activity relationship is shown most clearly and directly by comparing (in Table 1) compound (I) (no ring substituents or 15-hydroxyl group) with compounds (VI), (VII), (VIII) and (IX) [the same as compound (I) but with ring substituents and/or 15-hydroxyl group]. Compound (I) was inhibitory, whereas the other compounds had no effect or caused activation. Ring size did not appear to make any difference [compounds (I)–(V); Table 1]. These observations again emphasize the importance of the 15-hydroxyl group in structure-activity relationships among the prostaglandins. The importance of this group for various types of biological activity is well documented (Änggård, 1969; Kloeze, 1969; Ramwell *et al.*, 1969). Further, Flack (1970) has shown that with 7-oxa derivatives, such as those described here,

Fig. 1. Kinetic plots for inhibition of PGE_2 biosynthesis by compound (II) (Table 1)

(a) Lineweaver-Burk plot. (b) Effect of increasing inhibitor concentrations in the presence of 0.04 mM-arachidonate. Initial reaction rates were measured in all cases and all points represent the means of two assays. PGE_2 biosynthesis was measured by ^3H incorporation. For experimental details see the text.

the possession of a 15-hydroxyl group and/or 9- and 11-oxo or -hydroxyl groups gives the molecule smooth-muscle-agonist properties, whereas the absence of the 15-hydroxyl group and ring substituents makes the molecule an antagonist to prostaglandin actions on smooth muscle. These observations relate to those obtained with prostaglandin synthetase in the present work.

In most cases greater inhibition of PGE₂ synthesis was measured by bioassay of the product than by ³H incorporation. It is possible that additional ³H-labelled products are formed during incubation of the enzyme with arachidonate (see, e.g., Pace-Asciak & Wolfe, 1970*a,b*) and that these have chromatographic behaviour, in the system used, identical with that of PGE₂ but have little or no biological activity. Synthesis of PGE₂ may be inhibited by certain oxaprostaglandins, but formation of the additional products may not be. The net result would be an apparently greater diminution of biological activity (PGE₂) formed than of radioactivity (PGE₂ + additional products). This would provide one possible explanation for the discrepancy between inhibition assessed by ³H incorporation and by bioassay. However, in the absence of specific knowledge about the nature of such additional products, or their possible antagonistic effects to PGE₂ in the bioassay, or of the effect of the oxaprostaglandins on their formation, it is not possible to pursue this possibility any further on the basis of the current data alone.

On the basis of limited kinetic studies with the most potent inhibitor [compound (II); Table 1] inhibition appeared to be competitive. Some of these compounds appear to be more potent inhibitors of prostaglandin synthesis than oleate, linoleate or linolenate, since the ratio of inhibitor to substrate required to produce a given degree of inhibition in the present work is less than that reported (Pace-Asciak & Wolfe, 1968) to be required to produce a similar degree of inhibition by the above fatty acids. The present compounds, however, do not appear to be as potent inhibitors as the eicosa-8-*cis*,12-*trans*,14-*cis*-trienoate reported by van Dorp (1971) (K_i 1.2×10^{-7} M) or

eicosa-5,8,11,14-tetraynoic acid (Downing *et al.*, 1970).

The K_m value found in the present work for arachidonate (2.3×10^{-5} M) is close to that reported by van Dorp (1971) for dihomoy-linolenate.

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