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Polyphloretin phosphate (PPP) antagonists of prostaglandin action also inhibit prostaglandin biosynthesis in-vitro

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Abstract—Several polyphloretin phosphate (PPP) fractions (low mol. wt LC1259; high mol. wt LC1261; crude mixture, LC101) were confirmed in their established property as antagonists of the pharmacological actions of prostaglandins in a preparation of guinea-pig isolated ileum stimulated by prostaglandin (PG)E_2. Further samples of the same material were then compared in-vitro with indomethacin in their ability to inhibit prostaglandin biosynthesis from arachidonic acid by a microsomal enzyme preparation. All three PPP fractions potently inhibited prostaglandin generation, with the rank order of potency LC1259 = LC101 = indomethacin > LC1261. The oral LD50 in mice was 25 mg kg^{-1} for indomethacin and > 1 g kg^{-1} for LC101. PPP fractions (especially LC101) may therefore have therapeutic potential as anti-inflammatory agents.

Polyphloretin phosphate (PPP), a mixture of polyanionic polyesters of phloretin and phosphoric acid, inhibits several enzymes including alkaline phosphatase, hyaluronidase, urease (Diczfalusy et al 1953), swine lung 15-hydroxy-prostaglandin dehydrogenase (PGDH) in-vitro (Marazzi & Matschinsky 1972), and enzymes involved in metabolic inactivation of prostaglandins in guinea-pig lung (Crutchley & Piper 1973, 1974), phospholipase A_2 from rat isolated auricles (Giessler et al 1977) and cyclic AMP and cyclic GMP phosphodiesterases from guinea-pig heart and lung (Curtis-Prior & Chan 1981). PPP also reduces basal and insulin-stimulated glucose uptake by adipose tissue (Curtis-Prior 1974). The apparent enhancement by PPP of basal and hormone-stimulated adipose cell lipolysis (Curtis-Prior 1974, 1975) may be explained by simulation of glycerol by acting as an alternative substrate for ATP-glycerol transferase, so distorting biochemical estimates (Curtis-Prior & Jenner 1981) rather than by the earlier suggestion of prostaglandin (PG) antagonism. PPP was reported to be a selective and competitive antagonist of PG-induced contraction of various isolated smooth muscle preparations (Eakins & Karim 1970; Bennett & Posner 1971), and the topic is the subject of various reviews (Eakins 1971; Bennett 1974; Sanner & Eakins 1976; Sanner 1988).

Specific anti-inflammatory activities of PPP have also been recognized. Intra-arterial infusions in the rabbit reduced the intra-ocular pressure provoked by PGE and PGF compounds (Beitch & Eakins 1969; Bethel & Eakins 1971). PPP inhibited PGE-induced rat paw oedema (Blaszo & Gabor 1978) and the potentiation of bradykinin algesia induced by PGE_1 (Juan & Lembeck 1976). We now report that PPP inhibits the formation of prostaglandins from arachidonic acid.

Materials and methods

Chemicals. Lyophilized PPP fractions containing pre-weighed amounts of substance were supplied in hermetically sealed, sterile ampoules and used directly.

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Prostaglandin antagonism. Adult female Dunkin-Hartley guinea-pigs were stunned and exsanguinated, and segments of the removed ileum set up in 5 mL tissue baths containing Krebs solution (37°C; O_2/CO_2 95:5) to measure longitudinal muscle activity. The load on the tissue was 0·7 g. Isotonic contractions were registered with transducers and pen recorders. Submaximal consistent contractions were obtained to PGE_2 and acetylcholine (ACh), using a constant cycle time which varied from 5–10 min in different experiments, with an agonist contact time of 30 s. The PPP compounds were added to the bath fluid, and re-added when the bath fluid was changed to wash out the agonists. On each tissue three or four submaximal control contractions were obtained to PGE_2 and ACh. The means were used to calculate the changes with the antagonists. The test drugs were made up daily in 150 mM NaCl, and each experiment was on tissue from a different animal.

Inhibition of prostaglandin biosynthesis. Microsomes from bovine seminal vesicles were prepared as previously described (Curtis-Prior & Oblin 1979) modified from Takeguchi et al (1971), in which the final precipitate of microsomes, rather than being lyophilized, was further homogenized in ice-cold 100 mM Tris buffer at pH 8·2, and the homogenate stored in small volumes at −80°C under N_2. Samples of this microsomal enzyme source were allowed to warm in ice (from −80°C to +4°C) immediately before incubation for 5 min at 37°C with adrenaline bitartrate (5 mM) and reduced glutathione (10 mM) cofactors, in the presence of Tris (100 mM, pH 8·2) solutions of various concentrations of the PPP fractions, and following addition of arachidonic acid (final concentration 1 mM) containing a tracer dose of 365 μCi $mmol^{-1}$. The reaction was halted by addition of M HCl, and ethyl acetate extracts were separated by TLC on silica gel plates using 'cold' prostaglandins as authentic markers. The products were visualized with iodine vapour, cut from plastic-backed plates and counted in a liquid scintillation spectrometer using Instagel (Packard) as the scintillator.

Acute toxicity. Male mice of Charles River strain CD1, ten per group, were fasted overnight preceding drug administration. They were then observed daily during the seven subsequent days to monitor drug-induced behavioural anomalies or any mortalities.

Results and discussion

Prostaglandin antagonism. The results are summarized in Table 1. LC101 and LC1259 showed similar potency against PGE_2-stimulated ileum longitudinal muscle, producing approximately 70% inhibition at a concentration of 20 μg mL^{-1}, whereas LC1261 was somewhat less potent (only 40% inhibition) though more specific, being inactive against acetylcholine. These results confirm other previously reported data for the antagonism of prostaglandins by PPP.

Table 1. Inhibition by phloretin phosphate (PPP) of submaximal responses to prostaglandin (PG)E_2 or acetycholine (ACh) of isolated ileum from Dunkin-Hartley guinea-pigs.

PPP antagonist	LC101		LC1259		LC1261	
Agonist	PGE_2	Ach	PGE_2	ACh	PGE_2	ACh
PPP concn ($\mu g\ mL^{-1}$)						
20	63*	7	93	48	61	0
	37	46	40	−2	32	1
	96	79	73	36	31	8
Overall mean	65	44	69	27	41	3
10	—	—	32	6		
			39	−9		
Overall mean			36	−2		

*Results are shown to the nearest 1 per cent inhibition compared to control mean value. A negative value denotes responses that were greater than controls.

Inhibition of prostaglandin biosynthesis. LC1259 was the most potent against PGE_2 production, followed by LC101 and LC1261, with IC50 values of 15, 16 and 42 $\mu g\ mL^{-1}$, respectively (Fig. 1). A similar pattern of inhibition of synthesis occurred with $PGF_{2\alpha}$ production (Fig. 2) with IC50 values of 24, 26 and 72 $\mu g\ mL^{-1}$, respectively. The reference compound indomethacin exhibited IC50 values of 15 and 18 μM for PGE_2 and $PGF_{2\alpha}$, respectively.

It is noteworthy that the PPP inhibition of prostaglandin biosynthesis reported here, occurred at concentrations of drug that were not dissimilar to those required for prostaglandin antagonism, whereas Crutchley & Piper (1973, 1974) reported that the concentration of PPP required for inhibitory activity against prostaglandin-metabolizing enzymes, was much lower than that required for pharmacological antagonism of prostaglandins in guinea-pig isolated, perfused lung.

Acute toxicity. Indomethacin gave an LD50 value of 25 mg kg^{-1} whereas LC101 was for all practical purposes without toxicity (LD50 value > 1 g kg^{-1}, Fig. 3).

The true average molecular weight of PPP fraction LC101 is thought to be approximately 2000 (Fex, personal communication). Thus the IC50 values as an inhibitor of prostaglandin biosynthesis, may be calculated as 8 μM for PGE_2 production and 13 μM for $PGF_{2\alpha}$ production. This type of molecule therefore has potential as a therapeutic anti-inflammatory, particularly in view of its ability to block PG receptors in addition to PG synthesis.

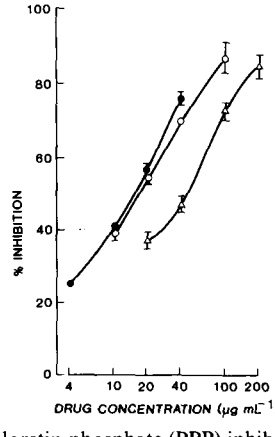

FIG. 1. Polyphloretin phosphate (PPP) inhibition of PGE_2 production from arachidonate by seminal vesicle enzyme preparation. PPP drugs (LC1259, ●——●; LC1261, △——△; LC101, ○——○) were incubated with bovine seminal vesicle microsomal enzyme (protein 14 mg mL^{-1}) in the presence of arachidonate (final concentration 0·5 mM), 5 mM adrenaline bitartrate and 10 mM reduced glutathione at 37°C in 100 mM Tris buffer at pH 8·2 for 5 min, in a total volume of 500 μL. The reaction was stopped by addition of 250 μL of 1 M hydrochloric acid. Ethyl acetate extracts were developed in a solvent system of ethyl acetate:acetone:aqueous acetic acid (90:10:1, v/v) and products detected by visualization with iodine vapour.

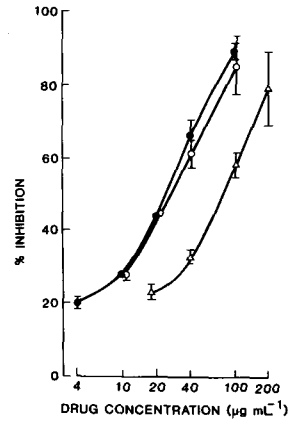

FIG. 2. Effects of polyphloretin phosphate (PPP) on PGF_2 production from arachidonate by a bovine seminal vesicle enzyme preparation. (LC1259, ●——●; LC1261, △——△; LC101, ○——○). (Detail as for Fig. 1.)

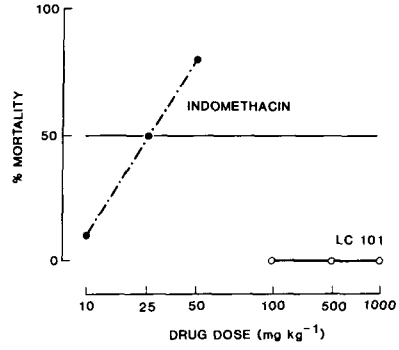

FIG. 3. Results of acute toxicity testing in grouped male mice. Fasted mice were administered, either indomethacin (10, 25 or 50 mg kg^{-1} p.o.) or LC101 (100, 500 or 1000 mg kg^{-1} p.o.) and observed during the subsequent seven days. From the graph it is evident that the LD50 for indomethacin was approximately 25 mg kg^{-1} whereas LC101 was without toxic effects up to a dose of 1000 mg kg^{-1}.

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Cortisone: a potent $GABA_A$ antagonist in the guinea-pig isolated ileum

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Abstract—In the guinea-pig isolated ileum, cortisone at 0·001–10 nM induced a non-competitive, dose-dependent antagonism of $GABA_A$-receptor-mediated contractile responses to applied GABA, depressing the maximum contractile response to GABA (100 μM), without affecting contractile responses to acetylcholine or cholinergic twitch contractions. At higher concentrations (> 10 nM), cortisone depressed contractile responses to acetylcholine (10–100 nM) and cholinergic twitch responses to transmural stimulation. Cortisone is thus the most potent non-competitive antagonist at $GABA_A$-receptor complexes in the guinea-pig ileum. From molecular modelling, sterically there appeared little difference between cortisone and cortisol, the latter being an enhancer of $GABA_A$-receptor-mediated action in the ileum. However, there were significant differences in electrostatic potentials between the two steroids, due to the different levels of oxidation at C_{11} which may contribute to such opposing actions.

Several endogenous steroids are now known to be potent modulators at $GABA_A$-receptor complexes. In particular, A-ring reduced metabolites of progesterone such as 3α-hydroxy-5α-tetrahydroprogesterone potentiate GABA-activated chloride conductance (Majewska et al 1986), whilst the neurosteroid pregnenolone sulphate appears to be an endogenous antagonist at $GABA_A$-receptor complexes (Majewska & Schwartz 1987; Ong et al 1987a). Indeed this 3α-hydroxy substituent and saturated A-ring in the 5α configuration have been considered essential for modulation at $GABA_A$-receptor complexes (Gee et al 1987). However, cortisol, with a 3-oxo substituent on a 4-pregnene ring and a 17α-hydroxy group, is a highly potent modulator of $GABA_A$-receptor-mediated contractile responses in the guinea-pig isolated ileum, potentiating at low concentrations and inhibiting at higher doses (Johnston et al 1987; Ong et al 1987b; Andres-Trelles et al 1989). Majewska (1987) has found similar interactions of 17-hydroxy glucocorticoids at $GABA_A$-receptor complexes in rat brain synaptosomes. We now show that cortisone, which differs from cortisol only in the oxidation level at C_{11} (Fig. 1), does not potentiate, but instead is only a highly potent antagonist of GABA-induced ileal contractions, as previously reported in brief (Johnston et al 1987).

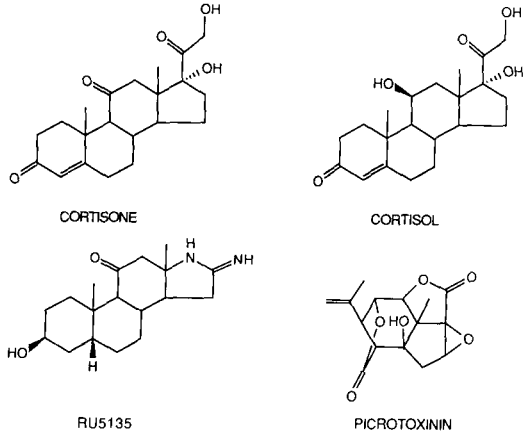

FIG. 1. Structures of cortisone, a potent non-competitive antagonist, cortisol, a potent potentiator, RU 5135, a steroid analogue which is a potent competitive antagonist and picrotoxinin, a non-competitive antagonist at $GABA_A$-receptor complexes in the guinea-pig isolated ileum.

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