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The character of the antagonism by polyphloretin phosphate of contractions to prostaglandins E_1 and $F_{2\alpha}$ in guinea-pig ileum

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Polyphloretin phosphate (PPP) produced a dose-dependent decrease in the tone and reduction of the spontaneous phasic contractions of the longitudinal muscle of guinea-pig isolated ileum. PPP (100 μg ml^{-1}) after a 2 min contact with the ileum decreased the contractile effects of PGE_1 0·1 μM by 40·6 ± 7·4%, of PGE_1 0·01 μM by 86·7 ± 3·3% and of $PGF_{2\alpha}$ 0·1 μM by 62·2 ± 8·6%. After 10 min contact of PPP the contractile effect of PGE_1 0·1 μM was decreased by 47·7 ± 4·7% and that of $PGF_{2\alpha}$ 0·1 μM by 89·6 ± 1·7%. When the contact was longer, PPP showed a pronounced after-effect in respect to the effects of PGE_1 and particularly of $PGF_{2\alpha}$. PPP significantly reduced contractions to 5-HT and $BaCl_2$, but not to acetylcholine, histamine or substance P. The type of antagonism of PGE_1 by PPP was examined using cumulative concentration-effect curves for PGE_1 in the presence of increasing concentrations of PPP. We conclude that on guinea-pig ileum PPP acts as a non-competitive antagonist of PGE_1 and $PGF_{2\alpha}$.

Several types of drugs interfere with prostaglandin (PG) release or action: inhibitors of PG synthetase (Vane, 1971; Kuehl, Oien & Ham, 1974; Gryglewski, 1976), glucocorticoids, which block PG formation or release probably by stabilizing cell membranes (Herbaczynska-Cedro & Staszewska-Barczak, 1974; Gryglewski, Dembinska-Kiec & others, 1975; Lewis & Piper, 1975) and phospholipase inhibitors, which decrease the amount of substrate for the PG biosynthesis (Flower, Blackwell & Parsons, 1975). There is also a mixed group of drugs which block PG action (Bennett, 1974) including polyphloretin phosphate (PPP) (Eakins & Karim, 1970; Fredholm, 1976).

The mechanism of PPP action is not clear, and the actions can vary depending on the species and the organs used.

The main purpose of the present work was to examine the selectivity of PPP action on PGE_1- and $PGF_{2\alpha}$-induced contractions of guinea-pig ileum.

MATERIALS AND METHODS

Segments, 20 mm long, of ileum from male guinea-pigs aged about 3 months, were placed under a 1 g load in a bath at 36° with 10 ml modified Krebs solution containing (in mM): NaCl 120; $CaCl_2$ 2·5; KCl 5·9; $NaHCO_3$ 15·4; $MgCl_2$ 12; NaH_2PO_4 1·2; and glucose 11·5 bubbled with O_2. Isotonic contractions of the longitudinal muscle were recorded at a magnification of 15 or 20 using a transducer.

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The following series of experiments were made.

1. *Effect of PPP on tone and spontaneous activity of the ileum.* After a 30 min equilibration the tone and the phasic contractions of the ileum were recorded for 10 min. PPP (20 or 100 μg ml^{-1} bath concentration) was added and the mechanical activity recorded for a further 2 or 10 min.

2. *Influence of PPP on the contractile effects of PGE_1 and $PGF_{2\alpha}$.* After a 30 min equilibration, spontaneous activity was recorded for 10 min. A submaximal contraction to PGE_1 or $PGF_{2\alpha}$ was recorded for 1 min, and usually repeated 5 min later. After 10 min, PPP 100 μg ml^{-1} was added to the organ bath and 2 or 10 min later PGE_1 or $PGF_{2\alpha}$ was applied again for 1 min. The PG was tested again after thoroughly washing out the PPP. In the experiments with a 10 min contact of PPP, PG doses were applied every 5 min until the responses returned to normal.

In other experiments cumulative concentration-effect curves for PGE_1 were obtained before starting and 1 min after adding PPP, and again after washing out the PPP. During the 15 min pause between each curve the segment was washed out several times with Krebs solution. A total of 60 cumulative curves on 7 isolated tissues was obtained. The ED50 of PGE_1 in the presence of increasing PPP concentrations and the dose ratio (DR) were determined.

3. *Effect of PPP on the contractions induced by 5-hydroxytryptamine, acetylcholine, histamine, substance P or $BaCl_2$.* The experimental procedure resembled that described above using contractions

nearly 80% of maximum, and usually 10 experiments with each spasmogen.

The drug concentrations used throughout were chosen from preliminary experiments.

The data were analysed statistically using Student's *t*-test.

Substances used were: PG E_1 and $F_{2\alpha}$ tromethamine salt (Upjohn Co.), polyphloretin phosphate (Leo Research Labs), 5-HT creatinine phosphate (Merck), acetylcholine chloride (Berlin Chemie), histamine dihydrochloride (Polfa), substance P (Institut für Wirkstofforschung, Berlin), $BaCl_2$ (Reanal).

RESULTS

1. *Effect of PPP on tone and spontaneous activity of the ileum.* In almost all experiments PPP decreased muscle tone and spontaneous activity. These effects were much greater at a PPP concentration of 100 than at 20 μg ml^{-1}.

2. *Influence of PPP on contractions to PGE_1 and $PGF_{2\alpha}$.* The decreases in the contractile effects of PGE_1 and $PGF_{2\alpha}$ induced by PPP 100 μg ml^{-1} applied 2 min before the respective prostaglandin, were as follows: PGE_1 0·1 μM, 40·6 ± 7·4% (s.e. n = 11, P <0·01); PGE_1 10 μM, 86·7 ± 3·3% (n = 10, P <0·001); $PGF_{2\alpha}$ 0·01 μM, 62·2 ± 8·6% (n = 11, P <0·002) (Fig. 1). In the experiments with a 10 min contact of PPP with the ileum the PGs contractile effects were decreased as follows: PGE_1 0·1 μM, 47·7 ±4·7% (n = 8, P <0·02) (Fig. 2); $PGF_{2\alpha}$ 0·1 μM, 89·6 ± 1·7% (n = 9, P <0·001). After a 2 min contact with PPP, contractile effects of PGE_1 and $PGF_{2\alpha}$ were usually restored to their initial values

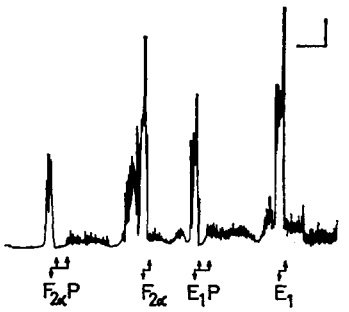

FIG. 1. Influence of PPP 100 μg ml^{-1} (P) on the tone and the spontaneous phasic contractions during a 2 min contact with the isolated segment of guinea-pig ileum and on the contractile effect of PGE_1 0·1 M (E_1) and $PGF_{2\alpha}$ 0·1 μM ($F_{2\alpha}$). ↑—administration of the substance tested; ↓—wash out. Chart movement—from right to left. Vertical scale: 0·5 mm. Horizontal scale: 5 min.

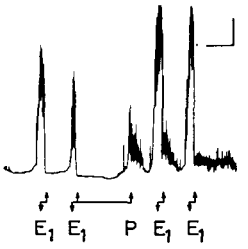

FIG. 2. Influence of PPP 100 μg ml^{-1} (P) on the tone and the spontaneous phasic contractions during a 10 min contact with the isolated segment of guinea-pig ileum and on the contractile effect of PGE_1 0·1 μM (E_1). Designations are as in Fig. 1.

several minutes after washing out the preparation. After a 10 min contact with the antagonist the responses to PGE_1 returned to the initial values 30 min after PPP was washed out (Fig. 3), while with

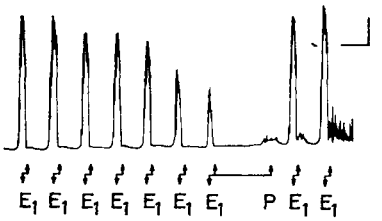

FIG. 3. Effect of PPP 100 μg ml^{-1} (P) on the tone and the phasic contractions during a 10 min contact with the isolated segment of guinea-pig ileum. Effect and after-effect of PPP on the PGE_1 0·1 μM (E_1)-produced contractions. Designations are as in Fig. 1.

$PGF_{2\alpha}$ the responses were still reduced (Fig. 4). PPP, 10 μg ml^{-1} was ineffective, while 30 μg ml^{-1} exerted but a slight effect on the PGE_1 cumulative concentration-effect curves. PPP, 70 μg ml^{-1}, influenced the curves in a manner similar to 100 μg ml^{-1}. A further increase in the antagonist concentration (100, 300 and 700 μg ml^{-1}) resulted in the curves

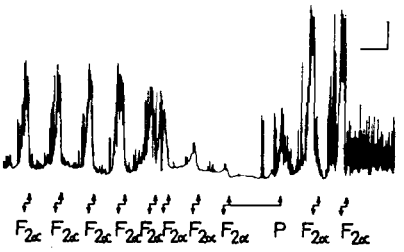

FIG. 4. Effect of PPP 100 μg ml^{-1} (P) on the tone and the phasic contractions during a 10 min contact with the isolated segment of guinea-pig ileum and the responses to $PGF_{2\alpha}$ 0·1 μM. Designations are as in Fig. 1.

being moved to the right without the maximum responses changing (Fig. 5). The ED50 values of PGE_1 after increasing the PPP concentration as well as the dose-ratios are shown in Table 1.

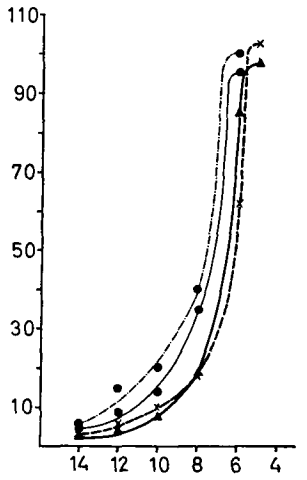

FIG. 5. Cumulative log concentration-effect curves for PGE_1. Experiments on isolated segments of guinea-pig ileum. (●—·—●) control; (●—●) after PPP 100 μg (▲—▲) after PPP 300 μg ml^{-1}; (× - - ×) after PPP 700 μg ml^{-1}. Ordinate: % of maximum contraction. Abscissa: −Log concn PGE_1 (M).

3. *Effect of PPP on the contractions induced by 5-HT, acetylcholine, histamine, substance P and $BaCl_2$.* The results are summarized in Table 2. PPP 100 μg ml^{-1} decreased contractions to 5-HT (0·1 μM) or $BaCl_2$ (0·1 mM) by 49·1 ± 7·5 and 53·9 ± 6·0% respectively (Figs 6 and 7), but responses were restored completely or almost completely after PPP was washed out. The effects of the other spasmogens (acetylcholine 10 nM, histamine 0·1 μM and substance P 10 and 100 nM) were unaffected.

DISCUSSION

The dose-dependent decrease by PPP of the tone and the spontaneous activity of guinea-pig ileum might be explained by antagonism of endogenous PGs. The important role of the endogenous PGs in maintaining the tone of the intestinal muscle was established by Ferreira, Herman & Vane (1972, 1976) and Eckenfels & Vane (1972) in rabbit isolated jejunum and rat stomach. Our earlier work (Petkov & Radomirov, 1977) showed that cat isolated jejunum kept for 30–180 min in Krebs solution showed gradual increase of the tone, and sometimes of phasic contractions. These changes were not observed when the bathing solution contained the

Table 1. *Effect of PPP on the ED50 PGE_1.*

Conc. of PPP (μg ml^{-1})	ED50 of PGE_1 (nM)	Dose ratio
—	20·9	1
100	45·7	2·2
300	195·0	9·3
700	589·8	28·2

Table 2. *Effect of PPP on guinea-pig ileal contractions induced by 5-HT, acetylcholine, histamine, substance P or $BaCl_2$.*

Spasmogens μM	No expts.	Contractions in mm ± s.e.m. Before PPP	After PPP (100 μg ml^{-1})	% decrease ± s.e.m. of effects of spasmogens after PPP
5-HT 0·1	9	66·8 ± 3·8	34·9 ± 6·2	49·1 ± 7·5*
Acetylcholine 0·01	10	63·3 ± 4·9	57·4 ± 5·1	8·9 ± 4·4**
Histamine 0·1	8	79·0 ± 3·2	72·8 ± 4·9	7·8 ± 5·0**
Substance P 0·1	5	84·4 ± 1·3	84·6 ± 1·6	0·2 ± 1·3**
Substance P 0·01	5	83·8 ± 2·8	83·2 ± 3·5	0·8 ± 1·3**
$BaCl_2$ 100	9	73·3 ± 6·2	34·7 ± 5·3	53·9 ± 6·0*

* Statistically significant difference ($P < 0.05$).
** Statistically insignificant difference.

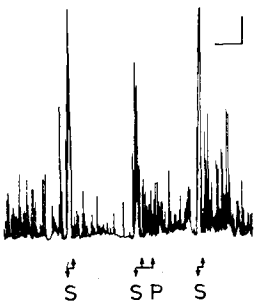

FIG. 6. Effect of PPP 100 μg ml^{-1} (P) on 5-HT-induced (0·1 μM) contractions of guinea-pig ileum. Designations are as in Fig. 1.

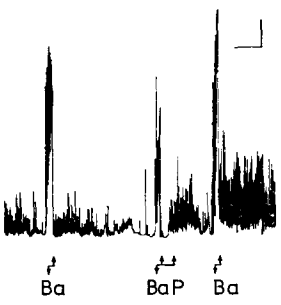

FIG. 7. Effect of PPP 100 μg ml^{-1} (P) on the contractions induced by $BaCl_2$ 100 μM (Ba). Designations are as in Fig. 1.

prostaglandin synthetase inhibitors indomethacin or aspirin (30 μM).

To clarify the mechanisms of the PPP interaction with PGs we wished to know whether the antagonistic action was specific and the type of antagonism concerned. Thus we performed experiments with spasmogens acting through different mechanisms. PPP 100 μg ml^{-1} did not affect contractions to acetylcholine (thus confirming the results of Bennett & Posner, 1971), histamine or substance P. However, effects of 5-HT or $BaCl_2$ were reduced to about the same extent as PGE_1 and $PGF_{2\alpha}$. PPP antagonized nicotine-induced contractions (Bennett, 1974; Bennett, Eley & Stockley, 1976). These results suggest that the antagonistic action of PPP on PGE_1 and $PGF_{2\alpha}$ is not selective, or that PGs modulate responses to indirectly-acting agonists.

Acetylcholine release from the intestinal cholinergic nerve terminals plays a role in the spasmogenic action of some PGs on intestinal muscle (Bennett, Eley & Scholes, 1968; Levine, 1973; Radmanović, 1975 for the action of PGE_1 and E_2 on guinea-pig ileum; Bennett, Eley & Stockley, 1975 for the $PGF_{1\alpha}$ and $PGF_{2\alpha}$ action on guinea-pig colon; Petkov & Radomirov, 1977 for the $PGF_{2\alpha}$ action on cat jejunum). However, the antagonism of PGs by PPP is clearly not due to an effect on the gut's response to acetylcholine. The action of PPP was not typical of a competitive antagonist since increasing the concentration greatly increased the degree of block. The precise length of the PPP chain and the extent of its branching have not yet been established, so we cannot discuss the PGE_1-PPP interactions on a molecular basis.

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