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Polyphloretin phosphate temporarily potentiates prostaglandin E_2 on the rat fundus, probably by inhibiting PG15-hydroxydehydrogenase

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Polyphloretin phosphate (PPP), an antagonist of some smooth muscle action of prostaglandins, potentiates the effect of prostaglandin E_2 on the rat isolated stomach fundus preparation. No such potentiation could be demonstrated for prostaglandin 15(S) 15 methyl E_2 methyl ester, a synthetic analogue of prostaglandin E_2. PPP also stimulates the longitudinal muscle of human isolated jejunum to contract. Evidence is produced to show that the potentiation of prostaglandin E_2 and the direct stimulant action of PPP are due to inhibition of prostaglandin 15-OH dehydrogenase, an enzyme which inactivates prostaglandin E_2 but not 15(S) 15 methyl E_2 methyl ester.

Polyphloretin phosphate (PPP), a phosphorylated polyanionic phloridzin derivative is now known to antagonize the effects of prostaglandins E_2 and $F_{2\alpha}$ (PGE_2 and $PGF_{2\alpha}$) on several *in vitro* systems including the isolated preparations of jird colon, rabbit jejunum and rabbit uterus (Eakins & Karim, 1970; Eakins, Karim & Miller, 1970; Eakins, Miller & Karim, 1971; Eakins, 1971). These findings have subsequently been confirmed (see Eakins & Sanner 1972 for references). Bennett & Posner (1971) showed that PPP selectively abolishes the stimulant effect of prostaglandins on human and guinea-pig isolated smooth muscle preparations. These authors mentioned, however, that PPP also produced small dose-dependent contractions of the rat stomach strips, and that contractions to PG or 5-hydroxytryptamine were either unaffected or slightly reduced in the presence of PPP. Villanueva, Hinds & others (1972) recently found that PPP (200 mg $kg,^{-1}$ i.v.) in anaesthetized cats, initially stimulated gastrointestinal activity before antagonizing the stimulant effects of PGE_2 and $F_{2\alpha}$.

Naturally occurring PGs (e.g. E_2 and $F_{2\alpha}$) are susceptible to enzymatic inactivation. Important points in the metabolism of these prostaglandins include (a) dehydrogenation at carbon 15, (b) β-oxidation of the carboxylic acid side chain, (c) reduction of the carbon-13 double bond and (d) ω-oxidation of the alkyl side-chain (Samuelsson, Granström & others, 1971). The dehydrogenation at C-15, which takes place with particular ease, is brought about by the enzyme prostaglandin 15-hydroxyl dehydrogenase. The resulting keto prostaglandins exhibit a greatly reduced biological activity. Polyphloretin phosphate is known to have an inhibitory action on many different enzymes (Diczfalusy, Ferno & others, 1953), and Marrazzi & Matschinsky (1972) have recently reported that PPP is also a potent inhibitor of the enzyme prostaglandin 15-hydroxyl dehydrogenase.

In the present investigation the mechanism involved in the stimulant action of PPP on smooth muscle and the potentiation of the effect of prostaglandins has been investigated.

METHODS

Rats of either sex, 150 to 200 g, were killed by a blow on the head. The fundus of the stomach was cut into a strip and suspended under a load of 2 g in a 25 ml organ bath containing Krebs solution at 37° and gassed with 5% carbon dioxide in oxygen. Responses were recorded on a kymograph by a frontal writing lever, magnification 8–12. A dose cycle of 8 to 10 min and a contact time of 2·5 min were used for prostaglandins.

The effects of different doses of PPP on the stimulant action of PGE_2 was studied on strips from 12 rats. In a further three preparations PG 15(S) 15 methyl E_2 methyl ester, a synthetic analogue of PGE_2 (Fig. 1), was substituted for PGE_2. A dose response curve of these two prostaglandins was first obtained. A dose which gave a 30% response of the maximum was chosen as control and repeated until three constant responses were obtained. This selected dose was repeated in the presence of various doses of PPP. In each case, PPP was added to the bath and left in contact with the tissue for 1 min befor the addition of PG.

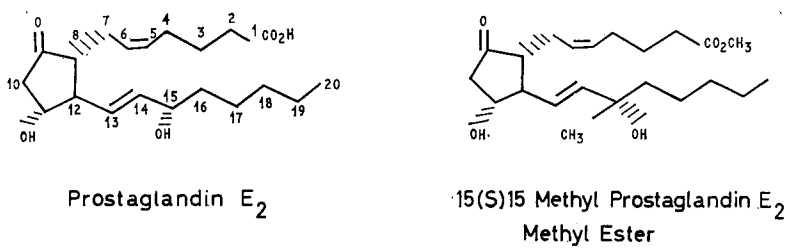

FIG. 1. Chemical structures of PGE_2 and its synthetic analogue 15(S) 15 methyl E_2 methyl ester.

In another set of experiments, the separated longitudinal muscle of excised portions of human jejunum, free from any pathological lesion, was cut into strips and set up in the same way as the rat fundus preparations. The effect of various doses of PPP on the spontaneous activity was studied.

RESULTS

A threshold dose of about 0·2 ng ml^{-1} of PGE_2 was required to contract most rat fundus strips. In all the strips from 12 animals PPP (50–800 ng ml^{-1}) caused temporary dose dependent potentiations of the stimulant action of PGE_2 (Figs 2 and 3). With higher doses of PPP (1–50 μg ml^{-1}) there was either potentiation or partial antagonism of the effect of PGE_2. With even higher doses (up to 640 μg ml^{-1}) PPP completely abolished the stimulant effect of PGE_2, probably non-selectively (Bennett & Posner, 1971). The dose of PPP required to potentiate the action of PGE_2, was about 1500 times smaller than that required for its blocking action on the same strip. After removing PPP from the bath there was a gradual but complete recovery from the blockade.

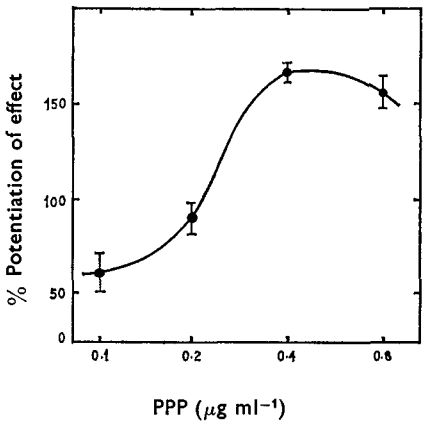

FIG. 2. The relation between different doses of polyphloretin phosphate and percentage potentiation of response of the isolated rat fundus preparation to repeated single submaximal dose of PGE_2. Maximal potentiation occurred at 0·4 μg ml^{-1} PPP. Each point is a mean of 12 observations with standard error.

In stomach strip preparations from three rats the threshold stimulant dose of the analogue 15(S) 15 methyl E_2 methyl ester was around 2 ng ml^{-1}. PPP (50 ng ml^{-1} to 640 μg ml) did not potentiate responses to the analogue. At the higher does (50–640 μg ml^{-1}) PPP antagonized the effect of 15(S) 15 methyl E_2 methyl ester on all three preparations.

The human jejunum preparations exhibited marked spontaneous activity. PPP in a dose of 20 μg ml^{-1} stimulated the jejunum strip. With a higher dose (160 μg ml^{-1}, PPP) there was a gradual decrease and complete abolition of the spontaneous activity of the jejunum (Fig. 4).

DISCUSSION

The results of the present investigation show that PPP has two types of effects on the rat fundus and human jejunum. At low dose levels PPP potentiates the stimulant

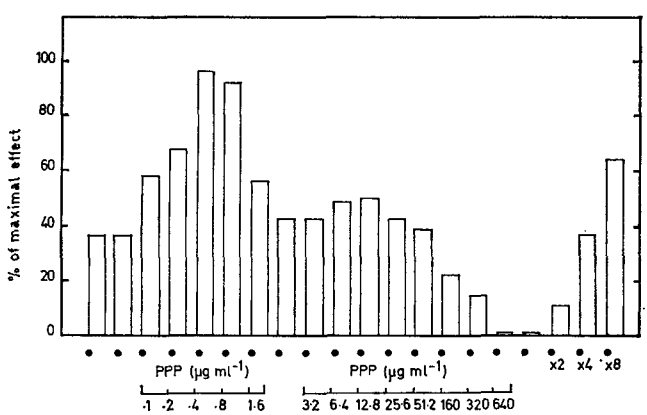

FIG. 3. Results from a single preparation of rat fundus strip showing the stimulant effect of PG E_2 (●, 0·4 ng ml^{-1}) in the presence of different doses of polyphloretin phosphate. Note the dual action of PPP: potentiation at low doses and blockade with higher doses. The blockade is reversed by increasing the dose of PGE_2 (i.e. ×2, ×4, ×8).

action of PGE_2 on the fundus and increases the tone of the longitudinal muscle of human jejunum. These effects are reproducible in different preparations, and the degree of potentiation depends on the dose of PPP. The amount required to antagonize the effect of PGE_2 is about 1500 times higher than that required to potentiate the effect of PGE_2.

PPP is known to antagonize a variety of enzymes. Eakins & others (1971) have shown that the parent dihydrochalcone (phloretin) and the corresponding glucoside (phloridzin) have very little prostaglandin-blocking activity. Furthermore, other polymeric phosphates with enzyme-inhibitory properties similar to those of PPP do not possess significant prostaglandin blocking activity. Thus the ability of PPP to antagonize the effects of prostaglandin does not seem to be related to its inhibitory actions on various enzymes including alkaline phosphates, hyaluronidase and urease (Diczfalusy & others, 1953). However, the action of PPP in potentiating the smooth muscle action of prostaglandin seems to be through enzyme inhibition. Marrazzi & others (1972) have shown that PPP is a potent inhibitor of PG 15-OH dehydrogenase, a widespread enzyme which inactivates PGs. Prostaglandin 15(S) 15 methyl E_2 methyl ester is not a substrate for the dehydrogenase (Bundy, Lincoln & others, 1971), and

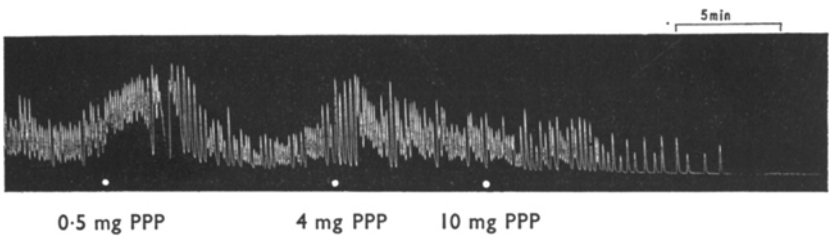

FIG. 4. Effect of different doses of polyphloretin phosphate on the spontaneous activity of the longitudinal muscle of human jejunum *in vitro*. Doses of 0·5 and 4 mg PPP increased the tone whereas 10 mg caused inhibition.

in this study it has been found that PPP does not potentiate the action of this analogue. It appears likely, therefore, that PPP inactivates the PG 15-OH dehydrogenase in the rat stomach, thus potentiating the exogenously added prostaglandin by preventing its metabolism.

The stimulation of the human gastrointestinal tract by PPP in our findings and previously by Villanueva & others (1972) could be explained by the ability of PPP to prevent inactivation of endogeneous PGE_2. Karim (1972) has shown that PPP orally in doses of up to 2 g had no effect on prostaglandin-induced diarrhoea in healthy male volunteers. With doses of 4 g and over PPP itself produced several episodes of watery diarrhoea similar to that produced by oral PGE_2 and $F_{2\alpha}$. Since PGE_2 is present in the gastrointestinal tract, the diarrhoea with high doses of PPP could be due to prevention of metabolism of endogenous PG.

Ferreira, Herman & Vane (1972) have shown that the tone of the rabbit isolated jejunum is maintained by a continuous generation of prostaglandins. This conclusion is supported by the observations of Bennett & Posner (1971) that PG antagonists (PPP and others) lower the tone in many isolated smooth muscle preparations. The inhibition of spontaneous activity in the human jejunum preparation by PPP (Fig. 4) is consistent with the possibility that PG release plays a role in maintaining the spontaneous activity of this preparation.

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