Papaverine stimulation of prostaglandin E₂ production by cultured rabbit gastric mucosa

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SUMMARY Prostaglandins (PGs) are synthesised by gastric mucosa, and have been shown to inhibit gastric acid secretion and ulcer formation in man and experimental animals. Recently exogenous PGs, mainly of the E group, have been used for the treatment of peptic ulcer disease. We therefore searched for a drug that would stimulate endogenous gastric prostaglandin E₂ (PGE₂) synthesis. Rabbit gastric mucosa slices were cultured for 22 hours at 37°C. PGE₂, measured by radioimmunoassay, was found to be linearly secreted into the culture medium. PGE₂ accumulation in the medium during 22 hours of culture was 7.9 ± 0.5 (SE) ng/mg tissue (N=20). Addition of papaverine (100 µg/ml), a cyclic nucleotide phosphodiesterase inhibitor, resulted in a significant increase (250% of control) in PGE₂ accumulation in the medium: 24.3 ± 1.8 ng/mg tissue (N=25). Isobutylmethylxanthine (IBMX 100 µg/ml), another phosphodiesterase inhibitor, only slightly increased PGE₂ accumulation, while 8 bromo-cyclic AMP (1 mM) had no effect. Under these conditions IBMX increased by 20-fold mucosal cyclic AMP levels: 3.9 ± 0.3 pmol/mg tissue (N=8) as compared with control levels: 0.2 ± 0.03 pmol/mg tissue (N=8). Papaverine, however, did not alter mucosal cyclic AMP accumulation. These results indicate that papaverine stimulates PGE₂ production by cultured rabbit gastric mucosa and that this stimulation is not related to the inhibition of phosphodiesterase activity and accumulation of mucosal cyclic AMP. Papaverine induced stimulation of PGE₂ production should be further evaluated regarding its possible beneficial effects in protecting gastric mucosa and in reducing acid secretion in peptic ulcer patients.

Prostaglandins (PGs) E₂ and F_{2α} have been shown to be synthesised by human gastric mucosa. PGE₂-like material was also shown to be present in gastric juice (Bennett *et al.*, 1968). The synthesis of PGE₂ exceeds that of the PGF_{2α} by a factor of 10 (Peskar, 1977). Their role in gastric physiology has not yet been clearly established. Administration of PGE₂ analogues significantly decreases gastric acid secretion and enhances ulcer healing in animals and human subjects (Robert *et al.*, 1967, 1968; Fung, *et al.*, 1974; Wilson *et al.*, 1977). In addition, PGs have gastric cytoprotective properties (Robert, 1976; Dousa and Dozois, 1977; Chaudhury and Jacobson, 1978). Stimulation of endogenous gastric PGE₂ synthesis by hypertonic solutions introduced into the rat stomach inhibited gastric secretion and protected

against ulceration in pylorus-ligated rats (Assouline, 1977). On the other hand, aspirin-like drugs which inhibit PGs synthesis (Grossman *et al.*, 1961; Hamberg, 1972) induce gastric mucosal damage and bleeding. The aim of the present study was to search for an agent that would stimulate endogenous gastric PGE₂ synthesis and thus may be beneficial in protecting gastric mucosa and in reducing gastric acid secretion.

Methods

Male and female rabbits weighing 2–3.8 kg were killed by intravenous air injections. The stomach was removed, opened along the lesser curvature, and washed with normal saline. The mucosa of the body was separated carefully from the underlying layers and cut into slices (weighing 3–10 mg each). The slices were kept at 4°C and cultured within 20–30 minutes.

Organ culture was performed according to the

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method of Browning and Trier (1969). The culture medium used was RPMI 1640 (Bio-Lab, Israel) containing 10% fetal calf serum (Gibco Inc., Grand Island, NY), penicillin (100 U/ml), and streptomycin (100 μ g/ml). The biopsies were gently mounted, epithelial surface up, and cut surface down, on a stainless steel wire screen which rested on a small well in the centre of a sterile plastic culture dish (Falcon Plastics, Los Angeles, California). A ring of porous paper surrounding the central well was saturated with 0.15 M NaCl. One millilitre of the organ culture medium was added to the central well to barely float the mesh and biopsy. The culture dishes were placed in an incubator at 37°C gassed with 95% O_2 +5% CO_2 . The medium was changed after the first hour of incubation (preincubation) and the culture was carried out for 22 hours. In some cultures, after preincubation, one of the following agents was added to the medium: papaverine (Sigma Chemical Co., St Louis, Missouri, USA) 1, 10 or 100 μ g/ml, 3 isobutyl-11-methyl xanthine (IBMX) (Aldrich Chemical Co. Inc., Milwaukee, Wisconsin, USA), 100 μ g/ml, 8 Bromo cyclic AMP (Sigma Chemical Co.) 1 mM, secretin (Boots Pure Drug Co. Ltd., Nottingham, England) 1 U/ml, insulin (Calbiochem, San Diego, California, USA) 1 U/ml, or indomethacin (Assia Chemical Lab., Tel-Aviv, Israel) 10 μ g/ml. Aliquots of the medium were collected at one, three, seven and 22 hours and kept at -20°C for PGE_2 determination. At the end of the culture, the mucosa was boiled for three minutes in 50 mM acetate buffer (pH 4) and then homogenised. The homogenate was centrifuged at 2500 RPM for 15 minutes. The supernatant thus obtained and aliquots of the culture medium were kept at -20°C for cyclic AMP determination.

PGE_2 was determined by radioimmunoassay as previously described (Bauminger *et al.*, 1973). Cyclic AMP was determined by a modification of the method described by Gilman (1970).

Statistical evaluation of the data is based on Student's *t* test for unpaired data.

Results

PGE_2 accumulation in the culture medium was found to be linear during 22 hours of culture: 0.56 ± 0.11 (SE, $N=5$), 0.39 ± 0.07 ($N=5$), and 0.33 ± 0.02 ($N=5$) ng/mg wet wt/hour in the first to third, fourth to seventh, and eighth to 22nd hours respectively (Figure). Addition of papaverine (100 μ g/ml), a known cyclic nucleotide phosphodiesterase (PDE) inhibitor, resulted in two to three-fold increase in PGE_2 accumulation. The addition of papaverine (10 μ g/ml) also stimulated PGE_2 accumulation, whereas the addition of 1 μ g/ml had no effect.

Figure 1 PGE_2 accumulation by rabbit gastric mucosa cultured in drug-free medium and in medium containing either papaverine (100 μ g/ml) or IBMX (100 μ g/ml). PGE_2 accumulation was determined by radioimmunoassay on aliquots of the culture medium obtained at various time intervals.

Results are mean $\pm$ SE of five cultures performed with each drug.

IBMX (100 μ g/ml), another PDE inhibitor, only slightly increased PGE_2 accumulation. 8 bromo cyclic AMP, secretin, and insulin did not affect the accumulation of PGE_2 whereas indomethacin completely inhibited it (Table 1). Cyclic AMP content was determined at the end of the incubation in the tissue as well as in the medium (Table 2). c-AMP content in tissue culture with IBMX (100 μ g/ml), was 20 times higher than in mucosa cultured in drug-free medium. A thirty-fold increase in c-AMP content was also noted in the medium. Papaverine (100 μ g/ml) did not affect c-AMP accumulation.

Table 1 PGE_2 accumulation by cultured gastric mucosa*

Treatment	No. of cultures	PGE_2 accumulation (ng/mg tissue/22 h)
None	20	7.9 ± 0.5
8 Bromo cyclic-AMP (1mM)	8	6.3 ± 0.8
Secretin (1 u/ml)	8	7.3 ± 0.8
Insulin (1 u/ml)	5	7.4 ± 0.9
Indomethacin (10 μ g/ml)	5	$0.5 \pm 0.06^\dagger$
IBMX (100 μ g/ml)	7	$11.5 \pm 0.6^\ddagger$
Papaverine (1 μ g/ml)	5	$5.6 \pm 0.7^\ddagger$
Papaverine (10 μ g/ml)	10	$14.5 \pm 1.5^\ddagger$
Papaverine (100 μ g/ml)	21	$24.3 \pm 2.5^\ddagger$

*Organ culture of rabbit gastric mucosa and PGE_2 accumulation in the medium was determined as described in the Methods section. Results are mean $\pm$ SE. Significantly different as compared with cultures performed without drug.

$^\dagger P < 0.01$. $^\ddagger P = 0.05$.

Table 2 Cyclic-AMP accumulation in cultured gastric mucosa and medium*

Treatment	Tissue	Medium
	(pmol/mg tissue/22 h)	
None	0.20 ± 0.03†	0.26 ± 0.02
Papaverine (100 µg/ml)	0.24 ± 0.04	0.20 ± 0.02
IBMX (100 µ/ml)	3.90 ± 0.60	8.60 ± 0.80

*Organ culture of rabbit gastric mucosa and c-AMP concentration in the cultured tissue and medium were performed as described in the Methods section.

†Results are mean ± SE of eight to 10 cultures.

Discussion

In the present study organ culture was used to study endogenous PGE₂ production by rabbit gastric mucosa. This system has been used to study various aspects of intestinal physiology and pathophysiology (Eastwood and Trier, 1973a; b) and lately we found that it was also valid in determining PGE₂ synthesis by human rectal mucosa (Sharon *et al.*, 1978). The validity of organ culture to study synthesis of gastric mucosal PGs is apparent from the fact that PGE₂ accumulation was linear during the culture period and was completely inhibited by the addition of indomethacin, a PG synthetase inhibitor.

The addition of secretin, which inhibits gastrin stimulated acid secretion (Barrington and Dockray, 1976), was found to have no effect on PGE₂ accumulation by cultured gastric mucosa. Insulin also did not affect PGE₂ production in this system. As it was previously reported that cyclic AMP enhances PG accumulation in several tissues (Burke *et al.*, 1973; Hamprecht *et al.*, 1973; Zor *et al.*, 1977), the effect of 8 bromo cyclic AMP and PDE inhibitors such as papaverine and IBMX (Poch and Kukovetz, 1971; Triner *et al.*, 1970) on PGE₂ synthesis was tested. Papaverine was found to stimulate significantly PGE₂ accumulation, IBMX, a PDE inhibitor of the xanthine group, had only slight stimulatory effect, while 8 bromo cyclic AMP had no effect on PGE₂ accumulation.

Cyclic AMP failed to stimulate PGE₂ accumulation in this study, suggesting that it does not mediate PG synthesis by rabbit gastric mucosa. This assumption is additionally supported by the fact that IBMX, although inducing a significant increase in gastric cyclic AMP levels, only slightly increased PGE₂ accumulation. On the other hand, papaverine, which effectively stimulated PGE₂ accumulation, did not increase cyclic AMP content in either the tissue or medium. The dissociation between papaverine-induced stimulation of PGE₂ synthesis and its effect on the cyclic AMP system is additionally supported by the observation that papaverine released catecholamines from rat brain synaptosomes, while theo-

phylline and exogenous cyclic AMP did not (Eitan and Hershkowitz, 1977). It therefore seems that papaverine exerts its effect on gastric PGE₂ either directly or via mediators other than cyclic AMP.

Papaverine is commonly used as an antispasmodic drug to relieve colicky pains. It is sometimes used, in combination with other drugs, to relieve ulcer pain, though its effect on gastric acid secretion was not established. The results reported in this study indicate that papaverine effectively stimulates endogenous PGE₂ production by cultured rabbit gastric mucosa. Augmentation of PGE₂ content in gastric mucosa might decrease gastric acidity and contribute to gastric cytoprotection and thus help the stomach to withstand various insults. More *in vivo* studies are needed further to establish this assumption.

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