Br. J. Pharmac. (1976), **58**, 193–199

STIMULATION OF PROSTAGLANDIN BIOSYNTHESIS BY DRUGS: EFFECTS *in vitro* OF SOME DRUGS AFFECTING GUT FUNCTION

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1 Low concentrations of several emetic, purgative or irritant drugs in the absence of added co-factors stimulated conversion of arachidonic acid to prostaglandin E_2 and $F_{2\alpha}$ by prostaglandin synthetase extracted from bull seminal vesicles (BSV prostaglandin synthetase). Their effect was dependent on concentration and time.

2 Stimulation of BSV prostaglandin synthetase by apomorphine, aloes, tyramine or zingerone was increased several-fold by addition of reduced glutathione to the incubation medium, whereas hydroquinone, a phenolic co-factor of prostaglandin synthetase caused slight depression.

3 From this finding and from the observation that many of the stimulant drugs possess a phenolic group, whereas their inactive relatives lack such a group, it is suggested that these stimulant drugs act as co-factors for prostaglandin synthetase in place of hydroquinone.

4 Aloes, tyramine, ethanol and quipazine also produced a dose-related increase in resting tone of the isolated fundus of the rat stomach. This increase occurred at concentrations comparable to those effective in stimulating BSV prostaglandin synthetase, and was abolished by acetylsalicylate.

5 These findings support the view that certain drugs exert some of their pharmacological effects by stimulating prostaglandin synthetase.

Introduction

Many parts of the digestive tract release prostaglandins in response to various stimuli (Coceani, Pace-Asciak, Volta & Wolfe, 1967; Bennett, Friedmann & Vane, 1967; Collier, 1974; Herman & Vane, 1975). Administration of exogenous prostaglandins causes emesis, bile reflux, accumulation of water and electrolytes in the lumen of the small intestine, abdominal pain and diarrhoea (Horton, Main, Thompson & Wright, 1968; Misiewicz, Waller, Kiley & Horton, 1969; Matuchansky & Bernier, 1973; Milton-Thompson, Cummings, Newman, Billings & Misiewicz, 1975; Main & Whittle, 1975). These facts led us to investigate the possibility that some drugs exert their effects on the digestive tract through stimulation of prostaglandin biosynthesis in the gut wall or elsewhere in the body. Preliminary experiments, some of which have been briefly reported (Butt, Collier, Gardiner & Saeed, 1974; Collier, McDonald-Gibson & Saeed, 1974; 1975), showed that some emetics, purgatives or flavouring agents stimulate prostaglandin biosynthesis in homogenates of bull seminal vesicles incubated with arachidonic acid. We now describe further studies on the more potent stimulants, and investigations of other drugs.

Methods

Enzyme preparation

Bull seminal vesicles from the slaughterhouse were used fresh or after not more than four weeks storage at −20°C. Vesicles were trimmed of fat and connective tissue, cut into small pieces and homogenized for 1–2 min at full speed in a Waring Blendor in ice-cold 50 mM phosphate buffer at pH 7.4 (referred to subsequently as buffer) containing 1 mM disodium edetate (EDTA). The suspension was strained through cheesecloth and centrifuged for 20 min at 600 *g*. The supernatant was used as the synthetase preparation.

Standard test

After the optimal conditions for prostaglandin biosynthesis and the time during which production

increase was linear had been determined, using a procedure already outlined by Collier *et al.*, 1974, the following standard test was adopted. To 0.5 ml of the synthetase preparation arachidonate was added to give a final concentration as sodium salt of 61 μM (20 μg/ml). Appropriate concentrations of test drugs were included in the reaction mixture which was made up to 2 ml with buffer and incubated with shaking at 37°C for 15 minutes. Two ml 0.2 M citric acid and 16 ml ethylacetate were added, and after thorough mixing and centrifugation for 5 min at 600 *g*, 10 ml of the ethylacetate layer was removed, evaporated to dryness *in vacuo* and the residue dissolved in Krebs solution for bioassay of total prostaglandin-like activity. All experiments included controls in which boiled BSV homogenate was used.

For separate assay of E and F prostaglandin-like activities, the dried residue was redissolved in 50 μl of ethanol and spotted quantitatively onto thin-layer silica gel plates, with markers of 2 μg prostaglandin E_2 and $F_{2\alpha}$. The dried plate was developed to a distance of about 16 cm in a modified AI (Gréen & Samuelsson, 1964) solvent system (benzene: dioxane: acetic acid: 50:50:1). After chromatography, the marker spots were visualized by spraying with 10% phosphomolybdic acid in ethanol and exposing to hot air. The areas corresponding to prostaglandins E_2 and $F_{2\alpha}$ were scraped into test tubes and eluted with acetone or ethanol. The eluates were dried in a stream of N_2 and taken up in Krebs solution for bioassay.

In four experiments, 0.125 μCi [^{3}H]-prostaglandin E_2 and 0.125 μCi [^{3}H]-prostaglandin $F_{2\alpha}$ were incubated with unboiled or boiled enzyme for 15 min, extracted, separated by thin-layer chromatography and assayed. In this procedure, <8% of the prostaglandins E_2 and $F_{2\alpha}$ was lost during incubation with unboiled enzyme preparation. It was concluded that the increased net prostaglandin production in the presence of test drugs could be attributed largely to increased prostaglandin biosynthesis and not to protection from breakdown.

Bioassay

Bioassay of prostaglandins was performed, with two to four samples of each test solution, on rat stomach fundus strip, superfused at 5 ml/min with Krebs solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.6, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 5.6, gassed with 95% O_2 and 5% CO_2 and containing a mixture of selective antagonists: hyoscine 0.33 μM, mepyramine 0.35 μM, methysergide 0.57 μM, phenoxybenzamine 0.33 μM and propranolol 11.57 μM. As the product of the enzyme in the conditions of these experiments was largely prostaglandin E_2-like, this prostaglandin was used for reference. Some results were expressed as the production ratio, which is the ratio of total prostaglandin production in the presence of drug to that in controls. When prostaglandins E_2 and $F_{2\alpha}$ were assayed separately, the rat stomach strip and rat colon were used respectively.

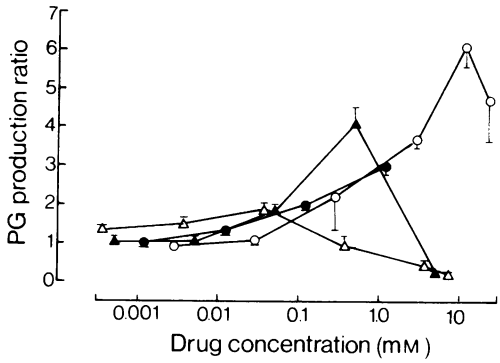

Figure 1 Concentration-response lines for total prostaglandin (PG) production (assayed as prostaglandin E_2) by stimulant drugs in standard test conditions (see Methods section). The prostaglandin production ratio is the ratio of prostaglandin production in the presence of drug to that in its absence. Aloes (●); tyramine (○); capsaicin (△); zingerone (▲). Vertical lines represent standard errors of the mean.

Fundus of rat stomach

To measure the effect of drugs on isolated tissue, a method was adapted from one used previously to study inhibition of prostaglandin synthetase (Bennett, Fox & Stamford, 1973; Collier, 1974). A strip of rat stomach fundus was suspended in oxygenated Krebs solution in an organ bath (10 ml). When the preparation had reached a constant tone, a low concentration of drug was added. The concentration of drug was then doubled and this process continued until tone reached a maximum. The results are expressed as the total concentration to increase tone to 50% maximum (MT_{50}).

Drugs

Table 1 lists the drugs tested and the salts used. Colchicine (Sigma), caffeine citrate (BDH), 3-isobutyl-1-methylxanthine (IBMX; Aldrich), theobromine (Sigma), theophylline (Sigma), capsaicin (Sigma) and *Escherichia coli*, strain 0111 B4, lipopolysaccharide W toxin (Difco) were used. Bile was obtained from the gall bladder of freshly killed guinea-pigs. Quipazine is 1-(2-quinolyl)piperazine (Rodriguez & Pardo, 1971). Solutions were made up in water or, if necessary, in dilute HCl or NaOH and serially diluted in buffer for addition to the incubation mixture. [^{3}H]-prostaglandin

E_2 (160 Ci/mmol) and [^{3}H]-prostaglandin $F_{2\alpha}$ (20 Ci/mmol) (Radiochemical Centre, Amersham), were diluted in a solution of the unlabelled prostaglandin before use. Concentrations of drugs refer to the acid or base.

Sodium arachidonate was prepared by dissolving arachidonic acid (Sigma, grade 1, 99% pure) in ethanol and diluting with 0.2% w/v sodium carbonate. This solution of sodium arachidonate was diluted in an amber glass container with buffer to 200 μg/ml for use.

Drugs were tested at several dilutions to determine the concentration that stimulates total prostaglandin production by 50% (SC_{50}), corresponding to a production ratio of 1.5. Dose-response lines were plotted for drugs in the absence of added hydroquinone or glutathione (standard conditions) that gave a production ratio significantly ($P<0.05$) more than unity. From this line, the SC_{50} value was derived.

Results

Bull seminal vesicle prostaglandin synthetase

Many of the drugs produced a concentration-related increase in total prostaglandin production in the standard test conditions, although some active drugs in high concentrations strongly inhibited prostaglandin production assayed as prostaglandin E_2 (Figure 1). In Table 1, based on dose-response lines of the type shown in Figure 1, drugs are grouped according to probably relevant effect; potency is expressed as the SC_{50} value derived from the rising phase of the dose-response line and effectiveness as the maximal prosta-

Table 1 Potency and effectiveness of test drugs as stimulants of prostaglandin (PG) biosynthesis assayed as prostaglandin E_2

Drug effect	*Drug*	$SC_{50} \pm$ *s.e.*	$S_{max} \pm$ *s.e.*	*Most effective concentration tested*
Emesis	Apomorphine hydrochloride	0.027 ± 0.01	4.13 ± 0.17	0.37
	Codeine phosphate	NS (0.84)	—	—
	Heroin hydrochloride	0.62 ± 0.06	2.06 ± 0.25	2.36
	Morphine sulphate	0.17 ± 0.06	5.43 ± 1.45	3.50
	Naloxone hydrochloride	0.19 ± 0.04	5.23 ± 0.83	2.75
Diarrhoea	Aloes B.P.	9.623 ± 1.67*	2.99 ± 0.13	500*
	Bisacodyl	NS (0.14)	—	—
	Colchicine	6.76 ± 0.25	2.80 ± 1.28	7.5
	Danthron	NS (0.12)	—	—
	Oxyphenisatin acetate	NS (0.16)	—	—
	Phenolphthalein diphosphate	1.85 ± 0.72	1.50 ± 0.14	1.85
Migraine	α-Phenylethylamine hydrochloride	NS (24.8)	—	—
	β-Phenylethylamine hydrochloride	NS (7.96)	—	—
	Tyramine hydrochloride	0.35 ± 0.10	6.08 ± 0.55	11.52
Inhibition of phospho-diesterase	Caffeine citrate	1.35 ± 0.86	2.25 ± 0.19	10.30
	IBMX	1.44 ± 0.76	1.76 ± 0.16	2.25
	Theobromine	Circa 3.6	1.49	3.6
	Theophylline	3.12 ± 2.20	2.09 ± 0.61	11.09
Irritation	Capsaicin	0.006 ± 0.002	1.83 ± 0.22	0.036
	Zingerone	0.041 ± 0.01	4.05 ± 0.45	0.52
	Ethanol	2.5 ± 0.164†	1.50 ± 0.17	2.5†
	Acetaldehyde	NS (910)	—	—
	E. coli toxin	NS (250)*	—	—
	Guinea-pig bile	5.45 ± 2.0†	1.66 ± 0.11	15.0†
	Quipazine maleate	0.07 ± 0.04	1.6 ± 0.08	0.47
Stimulation of PG synthetase	Glutathione	0.021 ± 0.009	9.09 ± 0.77	1.3
	Hydroquinone	0.093 ± 0.044	1.95 ± 0.12	0.33
	Isoprenaline sulphate	0.12 ± 0.024	4.47 ± 0.56	1.35

The SC_{50} is the lowest mM concentration of drug giving a total prostaglandin production that is 50% higher than controls. S_{max} is the prostaglandin production at the most effective stimulatory concentration of drug used, expressed as the ratio of total prostaglandin produced in the presence of drug to that in its absence. NS (0.84) etc., not stimulant at maximal concentration used, 0.84 mM. * μg/ml; † %v/v.

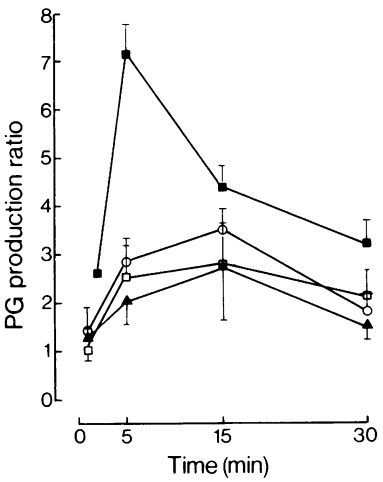

Figure 2 Time-response lines for stimulant drugs in the standard conditions of test. Response is the ratio of total prostaglandin (PG) production reached at a given point in time in incubates containing drug to that in incubates without drug. Apomorphine 0.37 mM (■); colchicine 7.8 mM (□); tyramine 3.6 mM (○); zingerone 0.52 mM (▲). Vertical lines represent standard errors of the means.

glandin production ratio obtained. The slopes of the rising phases of the dose-response lines of all drugs in Table 1 had a value of $P<0.05$, and eleven of these slopes had values of $P<0.01$. Apomorphine and morphine, which have emetic effects, had high activity, and except for codeine their relatives were also active. Activity was shown by aloes, colchicine and phenolphthalein, which have purgative effects, but not by bisacodyl, danthron or oxyphenisatin. Some food substances suspected of causing migraine (Hanington, 1967; Sandler, Youdim & Hanington, 1974), were active (tyramine, present in cheese and theobromine, present in chocolate). The 'hot' flavourings capsaicin, from chilli, and zingerone, from ginger, were also active. Ethanol was active but its metabolite, acetaldehyde, was inactive. The phenylethylamines, in chocolate, were inactive. Quipazine showed high potency but moderate effectiveness. That glutathione or hydroquinone stimulated prostaglandin synthetase indicates the standard test medium contained less than optimal amounts of co-factors.

Figure 2 gives the time-response lines of some active drugs. The onset of stimulation was rapid and the increase of prostaglandin production was roughly linear up to approximately 5 to 15 min, and then fell.

In other experiments, we separated by thin-layer chromatography and estimated separately the prostaglandin E_2- and $F_{2\alpha}$-like material produced in control incubates and in the presence of apomorphine, morphine, naloxone, tyramine or capsaicin. In all incubates, both prostaglandins were regularly detected, prostaglandin E_2 always exceeding $F_{2\alpha}$ by several-fold in amount.

Acetylsalicylate, an inhibitor of prostaglandin synthetase, inhibited the stimulation of total prostaglandin biosynthesis by apomorphine, tyramine, capsaicin, glutathione or hydroquinone (Table 2). The IC_{50} value of acetylsalicylate ranged from 2.0 mM for hydroquinone to 6.4 mM for tyramine.

To explore the mechanism of stimulant action, we tested some of the active drugs in the presence of glutathione or hydroquinone, the co-factors commonly used to stimulate prostaglandin synthetase (Nugteren, Beerthuis & Van Dorp, 1966; Samuelsson, 1967). Hydroquinone somewhat lessened the stimulation of prostaglandin production by two concentrations of aloes, apomorphine, tyramine or zingerone, whereas glutathione markedly enhanced their stimulant action (Table 3). Time-response lines also showed that glutathione strongly enhanced the effect of apomorphine, but hydroquinone did not.

Fundus of rat stomach

Aloes, tyramine, zingerone, ethanol, quipazine, glutathione and hydroquinone were tested for ability to increase the tone of the isolated fundus of rat

Table 2 Concentrations of acetylsalicylate required to inhibit by 50% (IC_{50}) the stimulation of total prostaglandin production by various drugs

Stimulant (mM)	*No. of expts*	*Production ratio in absence of acetylsalicylate*	*IC_{50} of acetylsalicylate (mM)*
Apomorphine (0.037)	4	*2.39*	*2.95*
Apomorphine (0.37)	3	4.09	2.95
Capsaicin (0.0037)	2	1.28	3.69
Capsaicin (0.037)	2	1.70	2.26
Glutathione (0.325)	3	5.09	2.4
Hydroquinone (0.325)	3	1.95	2.0
Tyramine (3.64)	3	4.0	6.4

stomach. Of these, aloes, tyramine, ethanol and quipazine produced a dose-related increase in tone (Table 4). Acetylsalicylate (5.0 mM) abolished the increase produced by each drug at above its MT_{50} concentration, except tyramine, of which a dose lower than the MT_{50} was fully inhibited.

Discussion

Few of the drugs in Table 1 that stimulated prostaglandin biosynthesis appear to have been shown by others to be potent stimulants of this process *in vitro*. After incubation for several days in the presence of 0.1 μg/ml colchicine, cultures of synovial tissue from patients with rheumatoid arthritis produced more than ten times as much prostaglandin as controls (Robinson, Smith, McGuire & Levine, 1975). However, when tested over a 15 min incubation period, colchicine had a relatively low potency and effectiveness (Table 1). The toxic effects of colchicine in man include nausea, emesis, abdominal pain, diarrhoea and a burning sensation of skin and mucous membranes; these appear after a pronounced latency (Woodbury & Fingle, 1975) and this is consistent with the possibility that its action is indirect.

Despite the ability of *Escherichia coli* endotoxin to produce vomiting and diarrhoea *in vivo* (Thomas, 1954; Collier, 1974), it failed to stimulate prostaglandin biosynthesis when incubated for 15 min with BSV homogenate (Table 1). This failure is consistent with the findings that only after a latency of 2 h did *E. coli* endotoxin *in vivo* cause emesis and diarrhoea (Collier, 1974) and that prostaglandin synthesis is stimulated only by toxin administered *in vivo* (Herman & Vane, 1975).

How do drugs stimulate prostaglandin biosynthesis by BSV homogenate? Since the homogenate in the standard test conditions did not appreciably degrade added radio-labelled prostaglandins E_2 or $F_{2\alpha}$, blockade of degradation of synthesized prostaglandins could not explain their increased amounts. It seems

Table 3 Effects of glutathione (GSH) or hydroquinone (HQ), both 0.13 mM, on stimulation of prostaglandin biosynthesis by aloes, apomorphine, tyramine or zingerone in bull seminal vesicle homogenate

		Addition	
Drug	*None*	*GSH*	*HQ*
None	1.01	3.39	2.12
Apomorphine 0.037 mM	1.61	6.10	1.97
Apomorphine 0.37 mM	4.58	9.93	3.83*
None	1.06	4.24	3.14
Aloes 50 μg/ml	1.82	6.72	3.05
Aloes 500 μg/ml	2.53	9.41	1.74*
None	0.87	4.10	2.15
Tyramine 0.36 mM	1.28	6.24	2.17
Tyramine 3.6 mM	3.05	11.94	2.59*
None	1.12	6.2	3.32
Zingerone 0.051 mM	2.15	9.16	4.21
Zingerone 0.51 mM	3.86	9.96	5.50

All results are expressed as μg prostaglandin E_2 equivalents produced. Values are the means of two independent experiments. * Below control levels.

Table 4 Potency of some stimulant drugs in increasing the tone of the rat stomach fundus; and the highest concentration of drug at which 5 mM acetylsalicylate (ASA) abolished the tone increase

Drug	*MT_{50} ± s.e.*	*Max. concn inhibited by ASA*
Aloes B.P.	9.0 ± 1.9 μg/ml	31.8 ± 6.1 μg/ml
Tyramine	0.35 ± 0.02 mM	0.23 ± 0.003 mM
Ethanol	4.2 ± 1.0% v/v	7.0 ± 0.7% v/v
Quipazine	0.017 ± 0.0025 mM	0.03 ± 0 mM

MT_{50} is the total concentration of the test drug to increase tone by 50% of the maximal increase. Each value is the mean of four experiments.

unlikely that the stimulant drugs acted by releasing endogenous arachidonic acid through the activation of phospholipase A, because the medium contains both added arachidonic acid, and EDTA, which inhibits phospholipase A (Roy, 1975).

The stimulant drug might act as a co-factor, supplementing a sub-optimal co-factor concentration in the medium. Lessening by hydroquinone and enhancement by glutathione of the ability of several stimulant drugs to increase prostaglandin biosynthesis (Table 3) fits this view. These considerations lead us to think that the effects observed were mainly due to stimulation of prostaglandin synthetase.

Most drugs showing stimulant activity (Table 1) were phenolic, whereas the only ineffective phenolic drug was danthron. Furthermore, where a pair of drugs differed only in the presence or absence of a phenolic group, such as morphine and codeine, or tyramine and phenylethylamine, only the phenol was active. Comparable results were obtained by Pace-Asciak (1972) with tyramine and phenylethylamine, using rat stomach prostaglandin synthetase. The unexpected activity of heroin may perhaps be explained by some hydrolysis of the acetyl group in the test conditions (heroin is unstable in aqueous solution), and the inactivity of danthron by its low water solubility. We suggest that the stimulant effects on prostaglandin synthesis of certain drugs, described above, depends on the presence of a phenolic group although this group could not be the only determinant of stimulant activity. Potency and effectiveness among phenols would be expected to vary in relation to other aromatic ring substituents of the molecule. Such drugs may thus be thought to play the part of 'phenolic activator' (Smith & Lands, 1971) of prostaglandin synthetase, which may also be played *in vivo* by catecholamines or 5-hydroxyindoles (Sih, Takeguchi & Foss, 1970; Takeguchi, Kohno & Sih, 1971).

It seemed desirable that observations made on a broken cell preparation of bull seminal vesicles should be extended to tissue that was derived from the gastrointestinal tract and contained intact functioning cells. We therefore tested the effects of some active drugs on the resting tone of an isolated strip of the fundus of rat stomach, which is regarded as due to intramural generation of prostaglandins (Eckenfels & Vane, 1972). The test was partly successful, in that four of seven stimulant drugs increased the resting tone of the fundus (Table 4) and, where the fundus responded, the effective concentration was comparable with that in BSV homogenate. The inhibition by acetylsalicylate of a considerable part of the increase of tone suggests that prostaglandin biosynthesis was involved.

That ethanol was effective in both tests is consistent with the recent conclusion of Karppanen & Puurunen (1976) that ethanol inhibits acid gastric secretion in the rat, at least partly through the increased synthesis of prostaglandins. Quipazine, which was active in both tests, has the side-effects of nausea, salivation, emesis, retching, gastric discomfort, abdominal distension, flatulence, colic, diarrhoea, tenesmus and headache (J.E. Villarreal, personal communication).

Our findings point to a hitherto little appreciated facet of drug action: certain drugs stimulate prostaglandin synthetase and this may mediate some of the drugs' effects. Since prostaglandin synthetase occurs in the gut (Pace-Asciak & Wolfe, 1970) and prostaglandin precursors are present in foods, the local concentration of drug might well be enough to stimulate prostaglandin biosynthesis, particularly in the presence of prostaglandin precursors in dietary fats, and thus contribute to symptoms such as dyspepsia, diarrhoea, headache and a sensation of heat in mucous membranes.

We thank Miss J. Copas, Mrs J. Cuthbert, Mr B. Hawkins, Mr I. Richards and Mr C. Shah for technical help.

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(Received January 9, 1976. Revised April 26, 1976.)