Participation of prostaglandin E₂ in the purinergic neurotransmission of gut

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SUMMARY Output of prostaglandin E₂ (PGE₂) from guinea pig ileal strips was detected by radioimmunoassay and the effect of adenosine and adenosine nucleotides on PGE₂ output investigated *in vitro*. Adenosine triphosphate (ATP) and α , β -methylene ATP, as well as adenosine, stimulate the output of PGE₂ in a concentration dependent-manner. Potency of these compounds is in the order of α , β -methylene ATP > ATP > adenosine. The effect of ATP was totally blocked by the cyclooxygenase inhibitor, indomethacin. Actions of these adenylyl compounds were attenuated by apamin, an antagonist of postjunctional P₂-purinoceptor-mediated activity. Effectiveness of these compounds was not modified by 8-phenyltheophylline, a P₁-purinoceptor antagonist, and it was not obtained in the isolated synaptosomal preparations. In addition, effects of adenosine and adenosine nucleotides were augmented only slightly by dipyridamole, a blocker of adenosine uptake. These findings suggest that the formation of PGE₂ is stimulated in muscle cells, through the postjunctional P₂-purinoceptor, taking an active role in the purinergic neurotransmission of guinea pig gut.

Adenosine nucleotides are considered as possible neurotransmitters in purinergic nerves of the gut.^{1,2} Prostaglandins are readily formed by the gut in response to chemical or mechanical stimulation.³ Burnstock proposed that prostaglandins function as mediators of purinergic neurotransmission,⁴ depending on the finding that adenosine triphosphate (ATP) releases prostaglandin like substances from isolated organs.^{5,6} Direct evidence to support the presence of this function in the gut is still missing, however.

The present study was designed to evaluate whether prostaglandin E₂ (PGE₂) was formed by purinergic stimulation in the isolated longitudinal smooth muscle of the guinea pig ileum.

Methods

EXPERIMENTAL PROCEDURES

Guinea pigs of either sex, weighing 300–380 g, were killed by a blow on the head and a segment of ileum (10–15 cm away from the caecum) was removed from each animal. Longitudinal muscle was prepared by

the standard method.⁷ Each strip 4–5 cm in length was placed in an organ tube containing Tyrode solution 2 ml (mmol/l): NaCl 137, KCl 2.7, CaCl₂ 1.8, MgCl₂ 1.05, NaHCO₃ 23.1, NaH₂PO₄ 0.42 and glucose 5.56; pH 7.4 at 37°C and bubbled continuously with 5% CO₂/95% O₂.

After equilibration for 30 minutes with repeated rinsing every 15 minutes, the solution of purine derivatives was added directly to the bathing medium to incubate with the tissue samples for an additional 10 minutes. Blockers were added five minutes before this addition of purine derivatives. Pilot studies indicated that PGE₂ formed in the tube gradually increased with prolongation of incubation time and reached a plateau within 10 minutes. The reaction was stopped by chilling the tube in an ice bath and the tissue was removed immediately. After centrifugation at 11 000 × g (4°C) for 20 minutes, the supernatant was collected for determination of PGE₂. Tissue incubated with the same volume of vehicle was taken as controls.

EXPERIMENT ON THE SYNAPTOSOMAL PREPARATIONS

Synaptosomal fractions of the ileum were prepared exactly as described previously.⁸ The pellet, contain-

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ing isolated nerve varicosities and no identifiable muscle contamination, was gently suspended in Tyrode solution (0.8–1.0 mg protein/ml). The effect of purine derivatives on the synaptosomes was investigated as stated above. After incubation, the synaptosomes were centrifuged at $11\,000\times g$ (4°C) for 30 minutes and the supernatant was collected for PGE_2 assay. Ruptured synaptosomes were also prepared by suspended synaptosomes in Tyrode solution (0.8–1.0 mg protein/ml) and then heating at 100°C for five minutes. The ruptured synaptosomes were incubated with the test compound as for the intact synaptosomal preparations. The values of PGE_2 obtained in the ruptured synaptosomes served as a blank.

ANALYTICAL PROCEDURES

Immunoreactive PGE_2 was measured as previously described in detail,⁹ by a radioimmunological method using commercial assay kits (PGE_2 - ^{125}I RIA-KIT; New Engl Nuc): the detection limit was 0.2–150 pg/tube. The intra- and interassay coefficient of variation were 6% and 9%, respectively. All samples were analysed in triplicate and the values calculated from a log/logit program were expressed as ng/g of tissue wet weight, except the synaptosomal preparations, which were expressed as ng/mg of protein.

STATISTICAL ANALYSIS

The results, after subtracting the value of blanks, were given as mean $\pm$ SEM, and the data analysed by Student's *t* test and Wilcoxon's tests for paired or unpaired variables. The highest *p* value is given and a value less than 0.05 is considered significant.

CHEMICAL COMPOUNDS

Adenosine triphosphate disodium salt (ATP), α , β -methylene adenosine-5-triphosphate lithium salt, adenosine (free base) and dipyridamole crystalline were purchased from Sigma (St Louis, MO 63178, USA) and prepared aqueous stock solutions were used. A stock solution of 10 mmol/l 8-phenyltheophylline (Calbiochem, Los Angeles, USA) was made up in 80% v/v methanol containing 0.2 mol/l NaOH, and aqueous dilutions of this were used. Apamin (Sigma) was dissolved in distilled water and fresh aliquots stored in a freezer were used each day. A stock solution of indomethacin (Sigma) was also prepared with 5 ml ethanol plus 95 ml phosphate buffer (0.1 M; pH=7.2) and aqueous dilutions were used.

Results

EFFECT OF PURINE DERIVATIVES ON THE OUTPUT OF PGE_2

Ileal strips exposed to ATP induced an increase of

Figure 1 Concentration action curves of ATP (solid line) and α , β -methylene ATP (broken line) on the output of prostaglandin E_2 (PGE_2) from guinea pig ileal strips. After subtracting the value from the blank samples, all values are expressed as ng/g of wet tissue weight. Vertical bars indicate the mean $\pm$ SEM from six experiments. In the presence of indomethacin (20 μM), responses to ATP (white circle, solid line) were blocked. **p* < 0.05 from the value of ATP point to point.

PGE_2 output into the incubating medium. This effect of ATP on the output of PGE_2 was achieved in a concentration-dependent manner (Figure). Moreover, α , β -methylene ATP also produced a similar but more marked action than ATP (Figure). Similarly, adenosine also increased the output of PGE_2 in a concentration-dependent fashion ranging from 20–100 μM , with an output of PGE_2 of 19.4 ± 3.1 ng/g (*n*=6) at 100 μM . The maximal value of release of PGE_2 , induced by adenosine, was closer to that of ATP (*p* > 0.05) than of α , β -methylene ATP. The potency of these compounds in stimulating the output of PGE_2 was, in increasing order, α , β -methylene ATP > ATP > adenosine. In the presence of indomethacin (20 μM), however, an inhibitor of cyclooxygenase, the effect of ATP was totally inhibited (Figure). Effect of indomethacin (20 μM) was also obtained from the ileal strips treated with adenosine or α , β -methylene ATP; output of PGE_2 became 0.45 ± 0.27 ng/g (*n*=6) by

50 μM α , β -methylene ATP and 0.24 ± 0.18 ng/g ($n=6$) by 100 μM adenosine. All values were not statistically different from that of blanks ($p>0.05$).

Pretreatment with dipyridamole (1 μM) did not markedly augment the effects of adenosine and adenosine nucleotides (Table). Apamin attenuated the PGE_2 output effect of the adenylyl compounds, in a dose-dependent manner (Table). The inhibitory action of apamin disappeared after washout and no irreversible effect was observed. The effects of adenosine and adenosine nucleotides were not, however, influenced by treatment with 8-phenyltheophylline, even at a higher concentration of 50 μM .

EFFECT OF PURINE DERIVATIVES ON THE SYNAPTOSOMAL PREPARATIONS

In isolated synaptosomal pellets taken from the ileal longitudinal strips of guinea pigs, incubation with adenosine and adenosine nucleotides did not result in an increase of the output of PGE_2 . Tissue samples treated with adenosine (100 μM), ATP (100 μM) and α , β -methylene ATP (50 μM) produced 2.4 ± 0.9 ($n=8$), 1.9 ± 0.7 ($n=8$) and 2.1 ± 0.9 ($n=8$) ng/mg protein of the PGE_2 -immunoreactive response in the incubating medium, respectively. These values are not statistically different from those assayed in the ruptured synaptosomes (1.8 ± 0.7 ng/mg protein, $n=8$, $p>0.05$).

Discussion

We found that PGE_2 output from ileal strips of guinea pigs was stimulated by incubation with the adenosine and adenosine nucleotides; ATP and α , β -methylene ATP. This is consistent with the previous findings that the rebound contraction induced by purinergic stimulation is blocked by indomethacin¹

and that prostaglandins are involved in the contractile response to ATP in the guinea pig digestive tract.⁶ Although several different kinds of prostaglandins have been discovered in the intestine, PGE_2 functions as one of the important substances responsible for the contraction of ileal strips.³ This study was therefore concerned with the formation of PGE_2 only.

ATP is generally believed to be the major purinergic neurotransmitter in the gastrointestinal tract.¹² The purinoceptors in mammalian intestine has been classified into P_1 (adenosine) and P_2 (ATP) subsets.¹⁰ Adenosine acted with greater potency at p_1 -purinoceptor which was further distinguished as two subtypes, A_1 and A_2 .¹¹ Theophylline and other methylxanthines act as the antagonists of P_1 -purinoceptor,^{12,13} present in the prejunctional sites of guinea pig ileum.¹⁴ The effect of adenosine and adenosine nucleotides on PGE_2 -output was not modified by pretreatment with 8-phenyltheophylline, however (Table), a potent P_1 -purinoceptor antagonist.¹³ It is suggested that stimulation of PGE_2 -output by adenylyl compounds is not mediated through the P_1 -purinoceptor sites. This view is further supported by our other findings that ATP, α , β -methylene ATP, and adenosine, did not stimulate the output of PGE_2 from the synaptosomal preparations. In addition, the effect of adenosine and adenosine nucleotides was augmented only slightly by dipyridamole (Table). Dipyridamole is an inhibitor of adenosine uptake¹⁵ and it potentiates the postjunctional action *via* P_1 -purinoceptor.¹⁶ This fact can be used to explain why the post junctional p_2 -action was not markedly enhanced by dipyridamole.

Apamin, a neurotoxic polypeptide, is well known as one of the antagonists of P_2 -purinoceptor.^{17,18} A concentration dependent reduction of the adenosine

Table Effect of drugs on the adenosine nucleotides-induced output of PGE_2 from guinea pig ileal strips

	ATP (10 μM)	α , β -methylene ATP (10 μM)	Adenosine (50 μM)
Control	16.2 ± 0.9	18.7 ± 1.3	14.7 ± 1.8
Dipyridamole			
0.5 μM	15.8 ± 1.1	17.2 ± 0.9	13.8 ± 0.7
1.0 μM	18.1 ± 1.2	20.3 ± 1.4	16.2 ± 1.1
8-Phenyltheophylline			
5 μM	15.4 ± 1.6	17.7 ± 0.9	14.0 ± 1.2
10 μM	16.3 ± 0.8	18.0 ± 1.2	12.9 ± 1.4
50 μM	14.9 ± 1.4	17.2 ± 1.1	13.9 ± 0.9
Apamin			
0.1 μM	$13.1 \pm 0.8^*$	$15.1 \pm 0.7^*$	$11.2 \pm 0.7^*$
0.5 μM	$10.6 \pm 1.0^\dagger$	$11.2 \pm 0.9^\dagger$	$9.3 \pm 0.6^\dagger$
1.0 μM	$7.2 \pm 0.6^\dagger$	$7.4 \pm 0.4^\dagger$	$7.8 \pm 0.9^\dagger$

Control means the value (ng/g of wet tissues) obtained from the treated samples. All values (mean $\pm$ SEM) were taken from six experiments after subtraction from the blank. * $p<0.05$ and $^\dagger p<0.01$ as compared with the control.

nucleotides induced PGE₂ output was observed in the apamin treated tissue samples, suggesting that output of PGE₂ stimulated by adenosine and adenosine nucleotides occurs *via* the postjunctional P₂-purinoceptor. In guinea pig ileum, ATP and adenosine (30–50 μM) depolarised the muscle membrane.¹⁹ This may be correlated to the stimulation of PGE₂ output.

It was previously shown that the major source of PGs in the gut is the muscle cell.²⁰ The present results are consistent with this view. Although we did not detect the activity of cyclooxygenase (the prostaglandin synthetase), the results attained in the direct radioimmunoassay of PGE₂ contents and the blockade of PGE₂ by indomethacin can more than compensate for this fact.

Indomethacin was suggested recently, to have an excess non-specific action on calcium mobilisation, rather than the inhibitory effect on PGs synthetase.²¹ Apamin possesses the ability to block the calcium mediated increase in potassium permeability.²² Therefore, the mechanism of action of adenosine and adenosine nucleotides remains obscure and needs further investigation.

In conclusion, we maintain that PGE₂ is stimulated by adenosine and adenosine nucleotides, and PGs are mediated by purinergic neurotransmissions in the gut of the guinea pig.

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