Protein kinase C inhibits cyclic adenosine monophosphate generation by histamine and truncated glucagon like peptide 1 in the human gastric cancer cell line HGT-1

P McKenna, J M Williams, C P Gespach, P J Hanson

Abstract

The HGT-1 gastric cancer cell line was used to determine the actions of protein kinase C on the stimulation of adenylate cyclase by the human histamine H₂ receptor, and the receptors for gastric inhibitory polypeptide and truncated glucagon like peptide 1 (TGLP-1). Suspensions of HGT-1 cells were preincubated with the activator of protein kinase C, 12-O-tetradecanoylphorbol 13-acetate (TPA, 100 nmol/l), for 10 minutes. The subsequent cyclic adenosine monophosphate (AMP) response to 0.5 mmol/l histamine or 100 nmol/l TGLP-1 was reduced by comparison with control cells preincubated in the absence of TPA. The cyclic AMP response to 100 nmol/l gastric inhibitory polypeptide was enhanced by preincubation with TPA, while the responses to cholera toxin and forskolin were unaffected. Preincubation with pertussis toxin prevented the enhancement of the gastric inhibitory polypeptide response by TPA, suggesting an involvement of an inhibitory guanine nucleotide regulatory subunit of the Gi class, but did not change the inhibition of histamine stimulation. In conclusion, activation of protein kinase C produces a specific inhibition of the effects of histamine and TGLP-1 on adenylate cyclase activity in a human gastric cancer cell line by acting at a site close to their receptors. (Gut 1993; 34: 953-957)

Protein kinase C is the name given to a group of kinases, which when activated by diacylglycerol in the presence of phospholipids such as phosphatidylserine, phosphorylate proteins on serine or threonine. Some forms of protein kinase C require Ca²⁺ for activation.¹ Phorbol esters such as 12-O-tetradecanoylphorbol 13-acetate (TPA) can substitute for diacylglycerol and have been used extensively as tools to investigate actions of protein kinase C.

Acid secretion can be induced in parietal cells by activation of adenylate cyclase by histamine, acting on an H₂ receptor and by β adrenergic agonists. Muscarinic cholinergic agonists and gastrin stimulate acid secretion by activation of polyphosphoinositide breakdown (see² for a review of regulation of acid secretion). Recently, truncated glucagon like peptide 1 (TGLP-1) has been shown to stimulate secretory activity in parietal cells by increasing cyclic adenosine monophosphate (AMP).³ Incubation of parietal cells from rats,⁴ rabbits,⁶ and guinea pigs⁷ with TPA inhibited the stimulation by histamine of

aminopyrine accumulation (an index of acid secretion), but no such effect was found with dog parietal cells.⁸ The site, or sites, at which protein kinase C exerts these inhibitory effects is unresolved. Hatt and Hanson⁹ found that preincubation with TPA inhibited the stimulation by histamine of the cyclic AMP content of rat parietal cells, but this finding was not replicated by Ostrowski and Bomsztyk.⁵

In this work the human gastric cancer cell line HGT-1 has been used to investigate whether protein kinase C modulates signalling by the human histamine H₂ receptor. The HGT-1 cell line was isolated from a human gastric adenocarcinoma,¹⁰ and cannot be considered a complete model for the parietal cell as it does not secrete hydrochloric acid. It does, however, express histamine H₂ receptors, which are pharmacologically indistinguishable from those on human gastric glands.¹¹ It is therefore a useful system to investigate one particular aspect of the control of acid secretion, namely the regulation of histamine H₂ receptor stimulated adenylate cyclase. HGT-1 cells also express receptors for vasoactive intestinal polypeptide, gastric inhibitory polypeptide^{12,13} and for TGLP-1.¹⁴ All of these receptors are coupled to adenylate cyclase in HGT-1 cells.¹¹⁻¹⁴ To enable the identification of the sites of action of protein kinase C, the actions of TPA on the cyclic AMP response to histamine, gastric inhibitory polypeptide, TGLP-1, cholera toxin, and forskolin have been compared.

Methods

REAGENTS

The ¹²⁵I radioimmunoassay kit for cyclic AMP was from NEN, (Stevenage, UK). TGLP-1 (glucagon like peptide 1 (7-36) amide), gastric inhibitory polypeptide, TPA, and pertussis and cholera toxins were obtained from Sigma (Poole, UK). The 4 α -phorbol derivative, 4 α -TPA, was from Scientific Marketing Associates, (London N1, UK).

CULTURE AND INCUBATION OF CELLS

HGT-1 cells were cultured as described previously.¹⁰ Cells were detached from flasks by first washing three times with Ca²⁺- and Mg²⁺-free phosphate buffered saline and then incubating for three minutes at 37°C with the same phosphate buffered saline to which 0.02% (w/v) EDTA had been added. Cells were washed twice

Pharmaceutical Sciences
Institute, Aston
University, Aston
Triangle, Birmingham
B4 7ET
P McKenna
J M Williams
P J Hanson

INSERM U.55, Hospital
Saint-Antoine, 184, rue
du Faubourg Saint
Antoine, 75571 Paris
Cedex 12, France
C P Gespach

Correspondence to:
Dr P J Hanson.

Accepted for publication
27 October 1992

Figure 1: Effect of 12-O-tetradecanoylphorbol 13-acetate (TPA) on the cyclic adenosine monophosphate (AMP) content of suspensions of HGT-1 cells stimulated with 0.5 mmol/l histamine at 37°C. (A) Effect of preincubation with 100 nmol/l TPA for 10 minutes (filled symbols) on the time course of the cyclic AMP response in HGT-1 cells incubated in the presence (■, □) or absence (▲, △) of 0.5 mmol/l histamine. Results are means (SEM) of four experiments. Where no error bars are evident they have been masked by the symbols. Significant effects of TPA determined by ANOVA denoted: * $p < 0.02$; ** $p < 0.01$. (B) Effect of various concentrations of TPA during the preincubation step on the cyclic AMP response of HGT-1 cell suspensions incubated for a further five minutes with 0.5 mmol/l histamine. Results are means (SEM) from four experiments and are expressed as a percentage of the response to 0.5 mmol/l histamine alone which was 13.6 (4.1) pmol cyclic AMP/million cells.

in Eagle's minimum essential medium containing 1 mg/ml bovine serum albumin and 20 mmol/l hydroxyethyl piperazine-ethanesulphonic acid (HEPES) and then finally resuspended at a concentration of 1.5×10^6 cells/ml in the same medium to which 0.1 mmol/l 3-isobutyl-1-methyl xanthine (IBMX) had been added. The airspace above the cells was gassed with 95% $O_2/5\%CO_2$ and the cells were incubated at either 37°C or 20°C. After preincubation for 10 minutes, TPA, or the solvent dimethylsulphoxide (final concentration, 0.2% v/v), was added to the suspensions. Preincubation was usually continued for a further 10 minutes, and then agonists were added to the suspensions and the change in cyclic AMP content recorded at intervals over the following 30 minutes.

Stimulation with cholera toxin involved addi-

Figure 2: Effect of 12-O-tetradecanoylphorbol 13-acetate (TPA) on the cyclic adenosine monophosphate (AMP) content of suspensions of HGT-1 cells stimulated with 0.5 mmol/l histamine at 20°C. (A) Effect of preincubation with 100 nmol/l TPA for 10 minutes (filled symbols) on the time course of the cyclic AMP response in HGT-1 cells incubated in the presence (■, □) or absence (▲, △) of 0.5 mmol/l histamine. Results are means (SEM) of three experiments. Where no error bars are evident they have been masked by the symbols. Significant effects of TPA determined by ANOVA denoted: * $p < 0.02$; ** $p < 0.01$. (B) Effect of various concentrations of TPA during the preincubation step on the cyclic AMP response of HGT-1 cell suspensions incubated for a further five minutes with 0.5 mmol/l histamine, with (□) or without (■) 200 nmol/l staurosporine. Results are means (SEM) from four experiments and are expressed as a percentage of the response to 0.5 mmol/l histamine alone which was respectively 5.5 (0.76) and 8.3 (0.58) pmol cyclic AMP/million cells in the absence and presence of staurosporine.

tion of 15 μ l of toxin dissolved in 5 mmol/l Tris HCl, 174 mmol/l NaCl, 0.3 mmol/l NaN_3 , and 0.1 mmol/l EDTA to each 1.5 ml of suspension to give a final concentration of 500 ng/ml. The cyclic AMP content of the suspensions was measured at one, two, and three hours. Fifteen μ l of vehicle were added to 1.5 ml of control suspensions. Treatment with pertussis toxin was performed on attached cells in culture flasks for two hours before detachment. Vehicle, which was 50% glycerol, 50% 0.05 mol/l phosphate buffer containing 0.5 mol/l NaCl, was present at a dilution of 1:400 in both experimental and control flasks.

ADENYLATE CYCLASE ACTIVITY

Cells were homogenised in a buffer (pH 7.5) containing 10 mmol/l Tris HCl, 1 mmol/l EDTA, 30 mmol/l NaCl, 1 mmol/l dithiothreitol, and 5 μ mol/l phenylmethylsulphonyl-fluoride using a teflon-glass homogeniser. Homogenates were centrifuged at 40000 g for 90 minutes at 4°C, and the pellet was resuspended in homogenisation buffer.¹⁵ Adenylate cyclase in the pellet was determined by adding 15 μ l of homogenate (10 μ g protein) to 235 μ l of incubation buffer to give the following final concentrations in the assay mixture (pH 7.5): 25 mmol/l Tris HCl, 1 mmol/l EGTA, 0.06 mmol/l EDTA 0.06 mmol/l dithiothreitol, 1.8 mmol/l NaCl, 5 mmol/l $MgCl_2$, 1 mmol/l ATP, 20 mmol/l creatine phosphate, 0.1 mg/ml creatine kinase, 0.2 mmol/l IBMX, 1 mg/ml bovine serum albumin, 200 μ g/ml bacitracin, and 200 μ mol/l GTP. Phosphatidylserine (20 μ g/ml) and Ca^{2+} (free concentration, 10^{-5} mol/l) were also present in the assay system. Incubation was for 10 minutes at 30°C.

ASSAY OF CYCLIC AMP

Samples of cell suspension, or of the adenylate cyclase assay system, were added to an equal volume of ice cold ethanol, immediately frozen, and were stored at -20°C. Samples were thawed, vortexed, and then centrifuged at 10000 g for one minute. Portions of the supernatants were evaporated in a vacuum oven at 40°C, dissolved in 0.05 mol/l sodium acetate buffer (pH 6.2), and were analysed for cyclic AMP by using an acetylated [^{125}I] radioimmunoassay procedure (New England Nuclear kit).

STATISTICAL ANALYSIS

Where possible data were subjected to analysis of variance to enable the effects of preincubation with TPA to be evaluated after the removal from consideration of the slight variation in the responsiveness of each cell batch to agonists. Otherwise an appropriate *t* test was used. Data are presented as means (SEM).

Results

EFFECT OF TPA ON STIMULATION OF CYCLIC AMP CONTENT AND ADENYLATE CYCLASE BY HISTAMINE
Stimulation of suspensions of HGT-1 cells with

Figure 3: Effect of preincubation time with 100 nmol/l 12-O-tetradecanoylphorbol 13-acetate (TPA), and of preincubation with 100 μmol/l 1-oleoyl-2-acetyl-glycerol (OAG) for 10 minutes on the stimulation of cyclic adenosine monophosphate (AMP) content above basal by 0.5 mmol/l histamine. Results are means (SEM) from three separate experiments. * $p < 0.01$ for difference from histamine alone by ANOVA and a Newman-Keuls multiple comparison test.

histamine at 37°C produced a rapid, 4.5 fold increase in cyclic AMP content above basal at one minute, which was subsequently attenuated between five and 30 minutes, although remaining above basal (Fig 1A). Preincubation of HGT-1 cells with 100 nmol/l TPA for 10 minutes significantly reduced the initial response to histamine ($p < 0.01$), this effect being most apparent five minutes after the addition of histamine. The effect of preincubation with various concentrations of TPA on the cyclic AMP content five minutes after the addition of 0.5 mmol/l histamine was investigated (Fig 1B). The concentration of TPA which produced half maximal inhibition was 0.15 nmol/l, estimated by using the program FIT.¹⁵

At 20°C, the cyclic AMP response to histamine was much more sustained, with little variation in the cyclic AMP content between one and 15 minute after the addition of 0.5 mmol/l histamine (Fig 2A). Significant effects of preincubation with 100 nmol/l TPA were also evident at this temperature, and the half maximally effective concentration of TPA was estimated to be 4.1 nmol/l. No significant effects of TPA were evident on basal cyclic AMP content after the 10 minute preincubation period, or at the end of the 30 minute incubation (Figs 1 and 2). The inhibitory effect of TPA was the same whether it

was added 30 or 10 minutes before challenge with histamine at 20°C, but TPA was ineffective if added at the same time as histamine (Fig 3). The diacylglycerol analogue, 1-oleoyl-2-acetyl-glycerol, at a concentration at which it inhibited histamine stimulated aminopyrine accumulation in parietal cells,⁴ had a similar inhibitory effect to 100 nmol TPA (Fig 3).

Basal adenylate cyclase activities in a membrane fraction in the absence and presence of 100 nmol/l TPA were 4.2 (0.8) and 4.2 (0.6) pmol/10 minutes per 10⁶ cells respectively. In the presence of histamine (0.5 mmol/l), adenylate cyclase activity was significantly ($p < 0.01$) reduced from a control value of 28 (1.9) to 21 (1.5) by the presence of TPA (100 nmol/l). Data are means (SEM) from four separate experiments.

EFFECT OF STAUROSPORINE AND 4α-TPA ON RESPONSE TO HISTAMINE

Staurosporine is a potent inhibitor of protein kinase C.¹⁶ When added 10 minutes before TPA, 200 nmol/l staurosporine completely abolished the subsequent effects of TPA (Fig 2B). Preincubation with staurosporine alone enhanced the cyclic AMP responses in HGT-1 cells incubated with 0.5 mmol/l histamine for five minutes at 20°C by 36 (7)% (Table I). The presence of 200 nmol/l staurosporine did not alter the time course of the desensitisation that was seen when HGT-1 cells were exposed to histamine at 37°C (data not shown).

The TPA analogue, 4-αTPA, does not activate protein kinase C.¹⁷ The cyclic AMP response to incubation with 0.5 mmol/l histamine for five minutes at 20°C, after preincubation with 4-αTPA for 10 minutes, was 102 (12)% of control cells preincubated with vehicle (mean (SEM) of three separate experiments).

EFFECTS OF TPA ON THE CYCLIC AMP RESPONSE TO TGLP-1, GASTRIC INHIBITORY POLYPEPTIDE, FORSKOLIN, AND CHOLERA TOXIN

At maximally effective concentrations of peptides (100 nmol/l),¹²⁻¹⁴ preincubation with 100 nmol/l TPA reduced the cyclic AMP response to TGLP-1 and enhanced the response to gastric inhibitory polypeptide. There was no effect on the response to 5 μmol/l forskolin (Figs 4A, B and 5A). At 37°C, 100 nmol/l TPA did not alter the effect of cholera toxin on cyclic AMP content (Fig 5B). These experiments had to be performed at 37°C because at 20°C cholera toxin did not stimulate cyclic AMP content.

Preincubation with staurosporine abolished the stimulatory effect of 100 nmol/l TPA on the response to 100 nmol/l gastric inhibitory polypeptide and the inhibitory effect of TPA on the response to TGLP-1 (Table I). In the absence of TPA, staurosporine enhanced the response to gastric inhibitory polypeptide by 49 (14)% (Table I).

EFFECT ON PREINCUBATION WITH PERTUSSIS TOXIN

Preincubation of layers of attached HGT-1 cells

TABLE I Effect of preincubation with staurosporine on the stimulation of cyclic adenosine monophosphate (AMP) content by histamine, truncated glucagon like peptide 1 (TGLP-1), and gastric inhibitory polypeptide (GIP) in the absence and presence of 12-O-tetradecanoylphorbol 13-acetate (TPA)

Agonist	Staurosporine (200 nmol/l)	Increase in cyclic AMP (pmol/million cells) above basal	Change in cyclic AMP (pmol/million cells) induced by 100 nmol/l TPA
Histamine (0.5 mmol/l)	—	5.09 (0.5)	—2.17 (0.4)
	+	6.94 (0.9)*	—0.16 (0.3)**
TGLP-1 (100 nmol/l)	—	2.15 (0.3)	—1.12 (0.1)
	+	2.48 (0.4)	—0.15 (0.08)*
GIP (100 nmol/l)	—	2.06 (0.6)	1.24 (0.2)
	+	2.96 (0.8)*	—0.28 (0.05)*

Results are means (SEM) from 3–4 cell batches. Staurosporine was added 20 minutes and TPA 10 minutes before agonist. Challenge with agonist was for five minutes (histamine and GIP) and 15 minutes (TGLP-1) at 20°C. * $p < 0.05$, ** $p < 0.01$ for effect of staurosporine by paired *t* test. Staurosporine did not affect the basal cyclic AMP content of the cells.

Figure 4: Effect of 100 nmol/l 12-O-tetradecanoylphorbol 13-acetate (TPA) on the cyclic adenosine monophosphate (AMP) content of suspensions of HGT-1 cells incubated at 20°C in 100 nmol/l truncated glucagon like peptide 1 (TGLP-1) (A) and 100 nmol/l gastric inhibitory polypeptide (GIP) (B). Results in A and B are from three separate experiments in each case and are presented as means (SEM). Where no error bars are evident they have been masked by the symbols. (■, □) agonist present, (▲, △) agonist absent. Open symbols, TPA absent, closed symbols, TPA present. Effects of TPA have been analysed by ANOVA and are denoted: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

in culture flasks in the presence of 500 ng/ml of pertussis toxin for two hours before detachment prevented the effects of TPA on the response to gastric inhibitory polypeptide. No such effect of pertussis toxin on the inhibitory action of TPA was observed when histamine was used to stimulate the cyclic AMP content of HGT-1 cells (Table II). The basal contents of cyclic AMP in the cell suspensions were 3.9 (0.4) and 3.8 (0.7) pmol/million cells after pretreatment with vehicle or pertussis toxin respectively (means (SEM) from three separate experiments).

Discussion

The main finding of this work is that the effects of the human histamine H_2 receptor on adenylate cyclase are inhibited by protein kinase C and that the site of action of protein kinase C is close to the receptor. New information is also provided on the modulation of the activity of the gastric inhibitory polypeptide and TGLP-1 receptors in HGT-1 cells.

The cyclic AMP content of the cell suspension rose to a peak one minute after the addition of histamine at 37°C and then fell. The fall in cyclic AMP means that a cyclic AMP phosphodiesterase must have been active despite the presence of IBMX (0.1 mM). Indeed not all

cyclic AMP phosphodiesterases are inhibited by this compound.¹⁸ This decline in cyclic AMP content has been found previously in HGT-1 cells stimulated with histamine at 37°C, and can be explained by a decrease in adenylate cyclase activity associated with desensitization of the histamine receptor.^{11,19} Such desensitization is inhibited at 20°C,^{11,19} and this temperature was preferred for most experiments since it enabled effects of activation of protein kinase C to be assessed against a relatively stable control value.

It is necessary to establish that the effects of TPA on the cyclic AMP content of HGT-1 cells were mediated through activation of protein kinase C. The half maximally effective concentrations of TPA were in the low nanomolar region or below, which indicates specificity, as does the lack of effect of the inactive isomer 4 α -TPA. Furthermore, the inhibitor of protein kinase C, staurosporine, completely prevented the effects of TPA on stimulation of the cyclic AMP content by histamine, TGLP-1, and gastric inhibitory polypeptide. Staurosporine stimulated the response to both histamine and gastric inhibitory polypeptide in the absence of TPA. This action cannot be interpreted as an inhibition by staurosporine of negative modulation of the histamine H_2 receptor by a basal activity of protein kinase C because similar effects were found with gastric inhibitory polypeptide, the action of which was not inhibited by activation of protein kinase C. Finally 1-oleoyl-2-acetyl-glycerol, a diacylglycerol analogue which activates protein kinase C in intact cells,¹ inhibited histamine stimulation of cyclic AMP content in HGT-1 cells at a concentration at which it inhibited histamine stimulated aminopyrine accumulation in parietal cells.⁴

The cyclic AMP content of cells can potentially be regulated at many sites.¹⁸ A stimulation of the breakdown of cyclic AMP by the activation of a cyclic AMP phosphodiesterase is unlikely to be responsible for the effects seen here. Such an action would have been expected to lower the cyclic AMP content irrespective of the agonist used to stimulate the cells. The effects of TPA on intact HGT-1 cells are therefore likely to be mediated by effects on the activity of the adenylate cyclase system, and this is supported by the direct effect of TPA on histamine stimulated adenylate cyclase activity in the isolated membrane fraction. Protein kinase C immunoreactivity has been detected in this fraction by protein blotting (J M Williams and P J Hanson, unpublished data).

Forskolin produces a direct activation of the catalytic subunit of adenylate cyclase, while cholera toxin causes a permanent activation of the guanine nucleotide regulatory subunit, Gs. The lack of effect of preincubation with TPA on the responses to forskolin and cholera toxin suggests that the actions of TPA on the responses to histamine, TGLP-1, and gastric inhibitory polypeptide are not mediated at the catalytic subunit or at the level of the interaction between Gs and the catalytic subunit. The prevention by pertussis toxin of the stimulatory effect of TPA on the action of gastric inhibitory polypeptide suggests an involvement of a guanine nucleotide binding protein of the class designated Gi in this

Figure 5: Lack of effect of preincubation of HGT-1 cell suspensions with 100 nmol/l 12-O-tetradecanoylphorbol 13-acetate (TPA) on the subsequent stimulation of the cyclic adenosine monophosphate (AMP) content of a suspension of HGT-1 cells by 5 μ mol/l forskolin (A) and 500 ng/ml cholera toxin at 37°C (B). Results are means (SEM) of three separate experiments. (■, □) agonist present, (▲, △) agonist absent. Open symbols, TPA absent, closed symbols, TPA present.

TABLE II Effect of preincubation with pertussis toxin on the cyclic adenosine monophosphate (AMP) content of a suspension of HGT-1 cells stimulated with histamine or gastric inhibitory polypeptide (GIP) in the presence and absence of 12-O-tetradecanoylphorbol 13-acetate (TPA)

Pertussis toxin (ng/ml)	Increase in cyclic AMP (pmol/million cells) above basal				Effect of TPA (% control)	
	Histamine (0.5 mM)		GIP (100 nM)		Histamine	GIP
	Control	100 nM TPA	Control	100 nM TPA		
–	6.21 (0.29)	3.76 (0.39)*	1.70 (0.21)	3.42 (0.31)**	61 (5)	204 (17)
500	6.84 (0.26)	4.62 (0.56)*	2.46 (0.40)	2.88 (0.53)	67 (7)	115 (6)

Attached cells in culture flasks were treated with pertussis toxin for two hours at 37°C or with vehicle. Cells were then detached and measurements of the response to agonists in the presence and absence of TPA were made using cell suspensions as described in methods. Results are means (SEM) of three separate experiments using histamine and of four using GIP. Significant effects of TPA on the response to agonist (by paired *t* test) are denoted as follows: **p* < 0.05; ***p* < 0.01.

action of TPA. Protein kinase C phosphorylates Gi and prevents its inhibitory action on the catalytic subunit.²⁰ Pertussis toxin pretreatment also inactivates Gi and therefore a reduced or negligible effect of activation of protein kinase C is seen under these circumstances.¹⁸ The actions of the gastric inhibitory polypeptide receptor on adenylate cyclase seems to be under the control of an activated form of Gi in HGT-1 cells.

A generalised effect of TPA on receptor-Gs coupling mediated by a phosphorylation of Gs seems an unlikely explanation of the effects of protein kinase C on the actions of histamine and TGLP-1. The heterotrimeric Gs protein does not seem to be a substrate for protein kinase C,¹⁸ and treatment of cells with pertussis toxin to remove the influence of Gi did not unmask an inhibitory effect of protein kinase C on the response to gastric inhibitory polypeptide. The sites of action for protein kinase C are likely to be close to the receptors for histamine and TGLP-1. Since pretreatment with TPA did not completely destroy histamine stimulated adenylate cyclase activity, it seems possible that the effect of phosphorylation by protein kinase C is to reduce the efficiency of coupling between the receptor and Gs rather than to abolish it completely. The C-terminal cytosolic portion of the human histamine H₂ receptor²¹ exhibits several sites at which serines are flanked by basic residues and could therefore be a substrate for protein kinase C.²² It has also been suggested that protein kinase C uncouples a receptor for glucagon-29 from Gs by phosphorylating the receptor.¹⁸ The receptor for TGLP-1 in the rat gastric mucosa seems to be different from that for glucagon-29,²³ but they may be part of a closely related family. The present new finding of an inhibitory effect of protein kinase C on the stimulation of adenylate cyclase by TGLP-1 is thus reasonable.

Activation of protein kinase C may have a role in the short term regulation of acid secretion, as in the inhibitory action of high concentrations of carbachol on histamine stimulated secretory activity in guinea pig parietal cells.²⁵ Alternatively, protein kinase C could regulate the turnover or insertion of the histamine H₂ receptor into the membrane and be involved with the effects of long term exposure to gastrin on the response to histamine.²⁶ Investigation of this 'crosstalk' between receptors may prove difficult in HGT-1 cells because muscarinic cholinergic and gastrin receptors have not been detected (McKenna *et al*, unpublished data), although it may be possible to induce cholinergic receptors

by culturing the cells with butyrate (J P Bali, personal communication).

In conclusion, this work shows negative modulation by protein kinase C of the stimulation of adenylate cyclase by the human histamine H₂ receptor. This finding could have implications for the regulation of gastric acid secretion in normal and pathological states in man.

This work was supported by a grant from the Medical Research Council. JMW is in receipt of a Studentship from SERC.

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