Involvement of calcium-sensitive phospholipid-dependent protein kinase in control of acid secretion by isolated rat parietal cells

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The relative potency with which phorbol esters inhibited histamine-stimulated aminopyrine accumulation (an index of acid secretion) paralleled that which has been established for the activation of purified protein kinase C. The inhibitory effect of 1-oleoyl-2-acetyl-glycerol on aminopyrine accumulation stimulated by various secretagogues was similar to that of 12-*O*-tetradecanoylphorbol 13-acetate. Protein kinase C activity was present in a parietal-cell-enriched fraction. In conclusion, protein kinase C could be involved in mechanisms regulating gastric acid secretion.

INTRODUCTION

The phorbol ester TPA (32 nM) substantially inhibited the accumulation of aminopyrine in isolated rat parietal cells that had been stimulated with histamine or dibutyryl cyclic AMP (Anderson & Hanson, 1984). Aminopyrine is a weak base which becomes trapped in acidic spaces within the parietal cells, and its accumulation is used as an index of acid-secretory activity (Soll, 1980). TPA is an activator of the Ca^{2+} -sensitive phospholipid-dependent protein kinase (protein kinase C) (Castagna *et al.*, 1982). The aim of the present work was to establish whether the effect of TPA on aminopyrine accumulated was mediated by direct activation of parietal-cell protein kinase C, for this would suggest an involvement of the enzyme in intracellular mechanisms regulating gastric acid secretion.

EXPERIMENTAL

Animals

Male Wistar rats (200–300 g body wt.) were obtained from Banting and Kingman, Hull, U.K., and were fed on Heygates' breeding diet, supplied by Pilsbury, Birmingham, U.K.

Reagents

Secretagogues, phorbol esters, histone H1 (type V-S), phosphatidylserine, leupeptin, pepstatin A, Percoll and Eagle's minimum essential medium with Earle's salts were obtained from Sigma. OAG was bought from Molecular Probes, Junction City, OR, U.S.A. [1,2- ^3H]Poly(ethylene glycol) (M_r 4000) and [dimethylamine- ^{14}C]aminopyrine were supplied by New England Nuclear, and [γ - ^{32}P]ATP (sp. radioactivity 23–43 Ci/mmol) was obtained from Amersham International. Other reagents were purchased from BDH.

Preparation and purification of parietal cells

Cells were prepared from the fundic portion of the stomach of fed rats, and were purified by centrifugation in Percoll, as described previously by Shaw *et al.* (1985).

Measurement of accumulation of aminopyrine

Methodology. Aminopyrine accumulation by parietal cells was measured as described by Anderson & Hanson (1984), except that the concentration of bovine serum albumin was decreased from the usual 30 mg/ml to 1 mg/ml in the experiments involving OAG, as this agent was found to be more active under such conditions. The effects of phorbol esters were the same at either albumin concentration. Both phorbol esters and OAG were dissolved in dimethyl sulphoxide, the final concentration of which was made 0.01% (v/v) in all vials, including the controls. Crude cell fractions containing 20% parietal cells could be used in these experiments, since aminopyrine is accumulated only within the parietal cells (Sonnenberg *et al.*, 1979).

Analysis and presentation of results. The aminopyrine accumulation ratio (the ratio of the concentration of aminopyrine in the cell to that in the medium) was calculated as described by Berglin *et al.* (1976). The computer program FIT (Barlow, 1983) was used to analyse the relationship between the concentration of phorbol ester or OAG and the effect on aminopyrine accumulation.

Assay of protein kinase C activity

Cells were homogenized by sonication (MSE Soniprep, 6×5 s, 10 μm amplitude) in buffer [40 mM-Tris/HCl (pH 7.4)/2 mM-EDTA/50 mM-2-mercaptoethanol/10 mM-benzamide/leupeptin (1 $\mu\text{g}/\text{ml}$)/pepstatin (1 $\mu\text{g}/\text{ml}$)] at 4 °C, and the homogenate was centrifuged at 100 000 *g* for 1 h at 4 °C. Protein kinase C was assayed in the supernatant as described by Lord & Ashcroft (1984). Phosphatidylserine (20 $\mu\text{g}/\text{ml}$), TPA (20 ng/ml) and sufficient calcium to give a free Ca^{2+} concentration of 50 μM [calculated by computer program (Storer & Cornish-Bowden, 1976)] were added when required. Kinase activity in stomach-cell fractions was linearly related to the protein concentration in the assay mixture, but, by contrast with spleen extracts, phosphorylation of histone was not linear over the 2 min measurement period, and therefore activity may have been under-

Abbreviations used: TPA, 12-*O*-tetradecanoylphorbol 13-acetate; OAG, 1-oleoyl-2-acetyl-glycerol; protein kinase C, Ca^{2+} -sensitive phospholipid-dependent protein kinase.

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estimated. Protein was determined by the method of Lowry *et al.* (1951).

RESULTS AND DISCUSSION

Relationship of structure of phorbol esters to activity

The half-maximally effective concentration of TPA required to inhibit histamine-stimulated aminopyrine accumulation was 3.3 nM (Fig. 1), which is close to the value, 2.5 nM, required for the half-maximal activation of partially purified protein kinase C (Arcoleo & Weinstein, 1985). The potency of 4 β -phorbol 12,13-didecanoate was 21 times lower than that of TPA, and 4 α -phorbol 12,13-didecanoate was ineffective over the concentration range studied (Fig. 1). At a concentration of 27 nM, the phorbol esters 4 β -phorbol 12-monomyristate and 4 β -phorbol 13-monoacetate, and 4 β -phorbol itself, did not affect histamine-stimulated aminopyrine accumulation, and at this concentration none of the phorbol esters mentioned above, including TPA, affected basal aminopyrine accumulation. In conclusion, the effect of TPA is a specific one, and the relative potency of the phorbol esters suggests an involvement of protein kinase C in their

Fig. 1. Effect of the concentration of phorbol esters on inhibition of aminopyrine accumulation by parietal cells stimulated with histamine (0.5 mM) and 3-isobutyl-1-methylxanthine (0.1 mM)

Results are presented as means $\pm$ S.E.M., for at least four separate batches of cells: $\blacklozenge$, TPA; $\blacktriangle$, 4 β -phorbol 12,13-didecanoate; $\bullet$, 4 α -phorbol 12,13-didecanoate. The concentrations of phorbol esters having half-maximal effect were 3.3 nM and 69.5 nM for TPA and 4 β -phorbol 12,13-didecanoate respectively. Maximal inhibition was 77.9% for TPA and 72.5% for 4 β -phorbol 12,13-didecanoate. The positions of the lines and the values of the above constants were calculated by computer program.

Fig. 2. Effect of the concentration of OAG on inhibition of aminopyrine accumulation by parietal cells stimulated with histamine (0.5 mM) and 3-isobutyl-1-methylxanthine (0.1 mM)

Results are presented as means $\pm$ S.E.M. for four cell batches. The position of the line was calculated by computer program, and the concentration of OAG having a half-maximal effect was 57.6 μ M, with the maximal inhibition being 83.2%.

action on aminopyrine accumulation, for it parallels that found for activation of purified protein kinase C (Castagna *et al.*, 1982).

Effects of OAG

The natural activators of protein kinase C are probably diacylglycerols substituted with long-chain fatty acids, and an analogue, OAG, is capable of activating protein kinase C when added to intact cells (Kaibuchi *et al.*, 1982). OAG inhibited histamine-stimulated aminopyrine accumulation by parietal cells (Fig. 2), and the maximal effect (83% inhibition) was similar to that obtained with TPA (78% inhibition; Fig. 1). OAG (150 μ M) had a similar effect whether aminopyrine accumulation was stimulated by histamine (69.4 $\pm$ 5.7% inhibition; n = 5) or dibutyryl cyclic AMP (69.1 $\pm$ 7.4% inhibition; n = 4), but it was less effective (P < 0.025, by analysis of variance) against carbachol-stimulated secretion (39.8 $\pm$ 8.8% inhibition; n = 4). These results parallel those obtained previously by using a high concentration of TPA close to that which has a maximal effect on histamine-stimulated acid secretion (Anderson & Hanson, 1984).

TPA acts directly on parietal cells

Since a completely pure preparation of parietal cells was not available, it was necessary to consider whether another cell type could be mediating the effect of TPA by itself releasing an inhibitor, which then acted on the parietal cell. However, data for the effect of phorbol esters on histamine-stimulated aminopyrine accumulation (Fig.

Fig. 3. Lack of relationship between total cell concentration and the inhibitory effect of 32 nM-TPA on aminopyrine accumulation by parietal cells stimulated with histamine (0.5 mM) and 3-isobutyl-1-methylxanthine (0.1 mM)

The correlation coefficient was not significantly different from zero, and therefore no line has been drawn. Results are for 12 different batches of cells.

1) fitted closely to the classical dose-response model (Barlow, 1983), and there was no relationship between cell concentration and the action of TPA (Fig. 3). Neither of these findings is readily compatible with the effect of TPA on parietal cells being mediated through a second cell type. In addition, the inhibitory effect of TPA on histamine-stimulated aminopyrine accumulation was similar in crude and purified preparations of parietal cells (Anderson & Hanson, 1984).

Presence of protein kinase C in parietal cells

The incorporation of ³²P into histone H1 was significantly increased (paired *t* test, $P < 0.025$, $n = 5$ cell batches) when phosphatidylserine (20 µg/ml) and TPA (20 ng/ml) were added to the basic assay system plus 50 µM-Ca²⁺. This increase, $1.09 (\pm 0.25) \times 10^5$ c.p.m./2 min per mg of protein, was taken to represent activity of protein kinase C and was obtained by using five cell fractions containing $75 \pm 3\%$ parietal cells (about 89% of the aggregated cell volume occupied by the larger parietal cells). The corresponding result for four cell fractions containing $24 \pm 2\%$ parietal cells (occupying about 46% of the aggregated cell volume) was $1.26 (\pm 0.51) \times 10^5$ c.p.m./2 min per mg. The activity in the fractions enriched with parietal cells was therefore unlikely to have resulted solely from contamination with other cell types, for if this had been the case then a substantially lower activity of protein kinase C would have been expected in the fractions enriched with parietal cells by comparison with the unenriched fractions. Protein kinase C is therefore probably present in parietal cells, and indeed, given the very wide tissue distribution of the enzyme (Kuo *et al.*, 1980), its absence would have been somewhat surprising. The activity of protein kinase C in the fractions enriched with parietal cells was 12% of that found in the spleen, which has a very high activity of the enzyme (Kuo *et al.*, 1980).

General discussion and conclusion

Stimulation of acid secretion by isolated parietal cells results in the generation of areas of low pH within the tubulovesicles and secretory canaliculi, and it is within

these regions that aminopyrine accumulates (Soll, 1980). It is unlikely that phorbol esters affect aminopyrine accumulation so as to prevent its acting as a measure of the sequestration of acid within parietal cells, for only those phorbol esters that activate protein kinase C inhibit aminopyrine accumulation. The most reasonable conclusion is that activation of protein kinase C, by either certain phorbol esters or OAG, decreases the accumulation of acid within stimulated parietal cells, and that protein kinase C is therefore probably involved in intracellular mechanisms regulating gastric acid secretion.

If accumulation of acid is decreased, either by an inhibition of secretion across the membrane of the secretory canaliculus or by an enhanced back-flux of protons across this membrane, then the action of protein kinase C on net acid secretion would seem to be inhibitory. Stimulation of the parietal-cell muscarinic cholinergic receptor is likely both to increase the cytosolic Ca²⁺ concentration and to activate protein kinase C (Berridge, 1984), and there is a synergistic interaction between the secretagogues histamine and carbachol (Soll, 1982). One way of reconciling the possibility of an inhibitory action of protein kinase C on histamine-stimulated acid secretion, and the above synergism between histamine and carbachol, is to suggest that, although the positive modulator promoting the potentiation between histamine and carbachol is unidentified, if this interaction were also subject to negative modulation at any stage during the induction or cessation of acid secretion then such a process might involve protein kinase C.

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