Inhibitory effect of 12-*O*-tetradecanoylphorbol 13-acetate on acid secretion by rat stomach in vivo

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The effect of 12-*O*-tetradecanoylphorbol 13-acetate (TPA) on acid secretion by rat stomach in vivo has been investigated to establish whether a previously determined inhibitory effect of TPA on aminopyrine accumulation by isolated parietal cells genuinely reflected a reduced acid secretion. Perfusion of 1 μM TPA through the lumen of the stomach had little effect on basal or carbachol-stimulated secretion, but inhibited secretion induced by histamine or pentagastrin. 4α-Phorbol 12,13-didecanoate did not affect pentagastrin-stimulated secretion. It is concluded that TPA exerts a specific inhibitory effect on acid secretion in vivo, which parallels that found on aminopyrine accumulation in isolated parietal cells.

Gastric acid secretion *Phorbol ester* *(Stomach)* *Protein kinase C*

1. INTRODUCTION

The phorbol ester TPA (32 nM) inhibited aminopyrine accumulation in rat parietal cells that had been stimulated with histamine or dibutyryl cyclic AMP, but had no effect on resting cells or on those stimulated with carbachol [1]. The effect of TPA on histamine-stimulated aminopyrine accumulation probably resulted from activation of parietal cell protein kinase C [22].

Aminopyrine is a weak base which becomes trapped within acidic regions in parietal cells, and the extent of its accumulation is dependent on the pH in the intracellular secretory canaliculi [3]. Thus, the inhibition of aminopyrine accumulation by TPA could represent a reduction in net acid secretion or alternatively an effect on the retention of acid within the cell. To differentiate between these two possibilities it is necessary to investigate the effect of TPA in a situation in which acid secretion can be measured directly in the steady state. The effect of perfusion of TPA through the stomach lumen in vivo on acid secretion has therefore been investigated.

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Abbreviations: TPA, 12-*O*-tetradecanoylphorbol 13-acetate; α-PDD, 4α-phorbol 12,13-didecanoate

2. MATERIALS AND METHODS

2.1. *Reagents*

Phorbol esters, histamine diphosphate and carbachol were obtained from Sigma. Pentagastrin injection (Peptavlon) was purchased from ICI.

2.2. *Animals*

Male Wistar rats of 250–350 g body wt were obtained from Banting & Kingman (Hull, England) and were fed on Heygates' breeding diet supplied by Pilsbury (Birmingham, England).

2.3. *Measurement of acid secretion*

Rats were deprived of food overnight and anaesthetized by an intramuscular injection of sodium pentobarbitone (Sagatal; 60 mg/kg body wt). Anaesthesia was maintained by hourly injec-

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tions of sodium pentobarbitone at a dose of 20 mg/kg body wt. The jugular vein was cannulated and the appropriate infusion begun at a rate of 3.7 μl/min. The oesophagus was cannulated close to the stomach, and the oesophagus and both trunks of the vagus nerve were then severed just proximal to the cannula. Vagotomy was performed so that investigation of the effect of TPA on acid secretion induced by infusion of various secretagogues was not complicated by the presence of background vagal activity [4]. The stomach was flushed out with saline (9 g NaCl/l) at 37°C, and after the insertion of a pyloric cannula, was perfused with the same saline at a rate of 1 ml/min.

The acid secreted into 5 min fractions of perfusate was measured by titration with 0.02 M NaOH using phenolphthalein as indicator. The time taken for secretion to stabilize varied between animals and with the secretagogue but usually a plateau was reached within 1 h. Secretion was recorded for a further 30 min after stabilization (control period), then perfusion of saline containing phorbol ester was initiated and secretion recorded for a further 40 min (experimental period).

2.4. *Analysis of results*

A two-way analysis of variance was used to investigate the effect of time on secretion. In no case did secretion during the control period show a significant dependence on time. The mean value for the control period was compared with in-

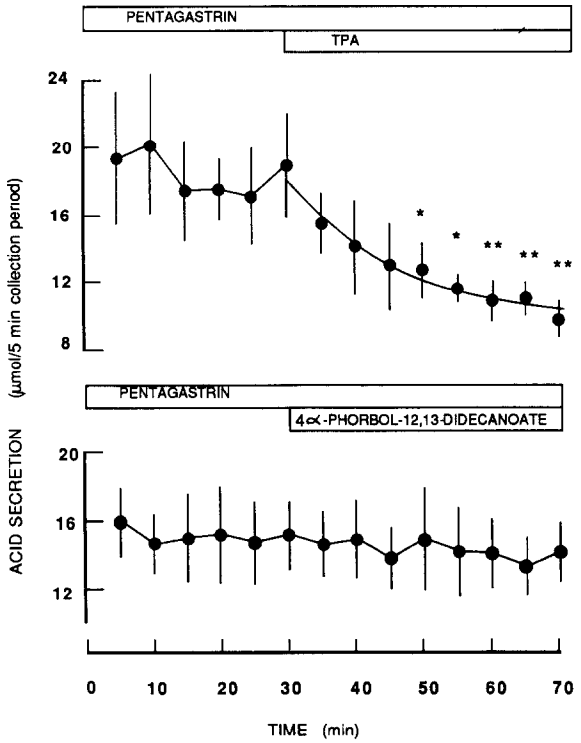

Fig.1. Effect of the presence of TPA (1 μM) and α-PDD (1 μM) in the luminal perfusate on gastric acid secretion in vivo in rats infused intravenously with pentagastrin (1.6 μg/min per kg body wt). Results are means ± SE for 5 experiments with TPA and 4 experiments with α-PDD. Asterisks indicate a significant difference from the mean value for secretion during the control period: * $p < 0.05$, ** $p < 0.01$.

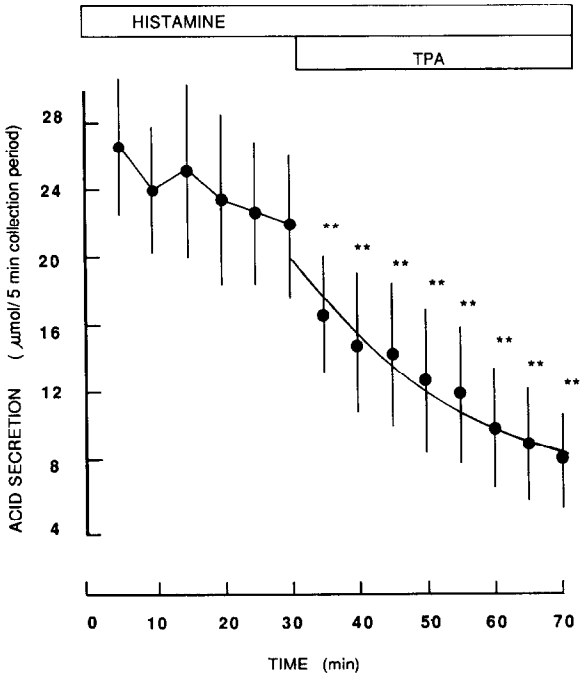

Fig.2. Effect of the presence of TPA (1 μM) in the luminal perfusate on gastric acid secretion in vivo in rats infused intravenously with histamine phosphate (30 μg/min per kg body wt). Results are means ± SE for 5 experiments. Asterisks indicate a significant difference from the mean value for secretion during the control period: ** $p < 0.01$. Note that in the statistical treatment the analysis of variance separates the contribution of animal variation from that due to perfusion time, but that the effect of animal variation still contributes to the size of the SE shown in the figure.

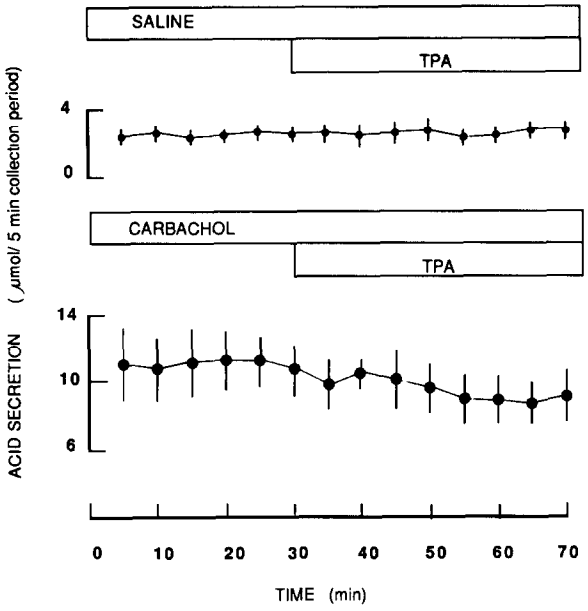

Fig.3. Lack of effect of the presence of TPA (1 μM) in the luminal perfusate on gastric acid secretion in vivo in rats infused intravenously with saline (9 g NaCl/l at 3.7 μl/min) or with carbachol (0.1 μg/min per kg body wt). Results are means ± SE for 4 experiments with saline and 5 experiments with carbachol.

dividual values for the experimental period by using Dunnett's test [5].

3. RESULTS

TPA (1 μM) significantly inhibited pentagastrin-stimulated secretion, but α-PDD (1 μM), which is not an activator of protein kinase C [6], was ineffective (fig.1). TPA also inhibited histamine-stimulated secretion (fig.2). The relationship of both pentagastrin- and histamine-stimulated secretion to the time after the initiation of perfusion with TPA could be fitted to an asymptotic regression [7] using the computer program ASYMPTOTE [8] with values for the coefficient of multiple correlation of 0.99 and 0.97, respectively. The calculated limit values for secretion were 9.49 μmol H^+/5 min (representing 48% inhibition by comparison with the mean of the control period) for the pentagastrin data, and 5.95 μmol H^+/5 min (75% inhibition) for the histamine data. The time dependency of the inhibitory effect of TPA is probably a consequence of restrictions on the access of TPA to parietal cells located deep in the gastric glands. TPA had no significant effect on basal gastric acid secretion or on secretion induced by carbachol (fig.3).

4. DISCUSSION

The two points which require discussion are firstly whether the inhibitory effect of TPA perfused through the gastric lumen in vivo is a specific one, and not simply the result of generalized tissue damage, and secondly whether the effect of TPA in vivo is similar to that observed on aminopyrine accumulation in isolated parietal cells in vitro [1,2].

If TPA were acting in a non-specific manner then a similar degree of inhibition of acid secretion might have been expected whatever the secretagogue. In fact, while TPA inhibited histamine and pentagastrin-stimulated secretion, there was no significant effect on secretion induced by carbachol. Also TPA still inhibited histamine-stimulated secretion when infusion of histamine was such that secretion during the control period was similar to that obtained with carbachol (Shaw, G.P. and Hanson, P.J., unpublished). An agent which was causing generalized tissue malfunction might have been expected to reduce basal acid secretion, but TPA did not do so. Finally, α-PDD, which is structurally quite similar to TPA but does not activate protein kinase C [6], had no effect on pentagastrin-stimulated secretion, yet if the phorbol esters had been acting non-specifically then α-PDD might well have mimicked the effect of TPA. It therefore seems reasonable to conclude that the inhibitory effect of TPA found in this work was a specific one, probably mediated by activation of protein kinase C.

In isolated parietal cells TPA was a much more potent and efficacious inhibitor of aminopyrine accumulation stimulated by histamine than by carbachol, and it was ineffective against basal aminopyrine accumulation [1]. These results are similar to the present findings in vivo, for no action of TPA against basal acid secretion or that stimulated by carbachol was found, but TPA was inhibitory when histamine was the secretagogue.

In conclusion, TPA perfused through the gastric lumen in vivo can inhibit acid secretion in a specific fashion, and it seems reasonable to assume

that the effects of TPA on aminopyrine accumulation in isolated parietal cells [1,2] genuinely represented an inhibition of secretion.

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