Loubatieres, A., Alric, R., Mariani, M. M., Chapal, J. (1972) J. Pharmacol. (Paris) 3: 103–108

Malaisse, W. J., Pipeleers, D. G., Mahy, M. (1973) Diabetologia 9: 1–5

Moore, R. A., Seki, T., Ohsumi, S. (1977) Curr. Med. Res. Opin. 5: Suppl. 25–32

Sadee, W., Segal, S., Finn, C. (1973) J. Pharmacokinet. Biopharm. 1: 295–305

J. Pharm. Pharmacol. 1981, 33: 737–738
Communicated April 14, 1981

0022–3573/81/110737–02 $02.50/0

Inhibition of histamine-induced acid secretion in rat isolated gastric mucosa by esters of phorbol and 12-deoxyphorbol

JACALYN B. PEARCE, A. W. BAIRD, ELIZABETH M. WILLIAMSON, F. J. EVANS*, *Departments of Pharmacology and Pharmacognosy*, The School of Pharmacy, University of London, 29–39, Brunswick Square, London, WC1N 1AX, U.K.*

Tigliane esters, isolated from plants of the family Euphorbiaceae (Evans & Soper 1978) are biologically active and have been shown to be potent skin irritants (Schmidt & Evans 1980) and to cause a two-stage aggregation of human platelets (Westwick et al 1981). In this communication we describe the antisecretory activity of four tigliane derivatives on the rat isolated gastric mucosa (Main & Pearce 1978).

Method

Immature rats of either sex (80 to 100 g, home bred from the Olac strain), allowed free access to food and water, were anaesthetized with pentobarbitone (60 mg kg^{-1} s.c.) and the stomach exteriorized. The muscle layer overlying the non-antral glandular region was separated from the mucosa by blistering (Main & Pearce 1978). The muscle sheet was pulled back to expose the mucosa; the stomach was then removed and the rat killed. Two 1 cm^2 pieces of mucosa from the stomach were placed in organ baths containing 35 ml of a buffered Krebs' solution at 37 °C (serosal solution [mM]; NaCl 110·0, KCl 5·0, $CaCl_2.6H_2O$ 3·6, $MgCl_2.6H_2O$ 1·2, $NaHCO_3$ 26·0, glucose 16·7), gassed with a 95% O_2 and 5% CO_2 mixture, which bathed the serosal surface of the preparation. The mucosal surface was superfused at 0·5 ml min^{-1} with an unbuffered solution (mucosal solution [mM]; NaCl 136·0, KCl 5·0, $CaCl_2.6H_2O$ 3·6, $MgCl_2.6H_2O$ 1·2, glucose 16·7) gassed with O_2. Acid output was recorded via a dual micro-electrode in the mucosal solution, connected to an antilog unit and a potentiometric pen recorder.

Experimental design. Paired mucosae, from a single stomach, were allocated to treatment groups. The secretagogue, histamine or pentagastrin, was added to all mucosae 120 min into the experiment, and two further responses were obtained at 210 and 300 min. For each response, the secretagogue was added to the serosal solution and left in contact for 30 min; consecutive responses were separated by a 60 min washout period. One mucosa from each pair, the untreated control, was used to monitor time-dependent changes in response to the secretagogue. A test compound was added to the second tissue at 180 min, 30 min before the second response, and left in contact for 60 min.

The acid secretory response is calculated as the increase in output at the peak of the response (P) over the preceding basal rate (B; i.e. P–B). The effect of the test compound was assessed by comparing the magnitude of the response in the treated mucosa with that in the paired control. Data were analysed by the Wilcoxon matched Pairs test (1 tailed; $P \leq 0.05$ was considered to be significant).

Drugs. Solutions of pentobarbitone (Nembutal, Abbott), histamine acid phosphate (BDH) and pentagastrin (Peptavlon, ICI) were prepared in 0·15 M NaCl solution. Phorbol, isolated from the seed oil of *Croton tiglium*, was dissolved in ethanol. 12-Deoxyphorbol phenylacetate (12-DOPPA) and 12-deoxyphorbol phenylacetate-20-acetate (12-DOPPAA), isolated from the latex of *Euphorbia Poisonii Pax* (Schmidt & Evans 1979), were dissolved in acetone, as was 12-*O*-tetradecanoyl phorbol acetate (TPA, Sigma).

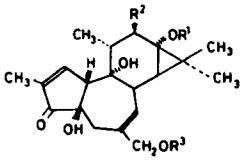
Results

In mucosae treated with phorbol (4×10^{-6} M, n = 6, first histogram in Fig. 2), the response to histamine (5×10^{-5} M) was slightly, but not significantly, reduced in the presence of the test compound; the third response was similar to that initially obtained in these mucosae. The same pattern of responses was observed in the untreated control preparations; histamine-induced secretion was reduced at the second response but subsequently increased. Between paired test and control mucosae, there were no significant differences in the secretory responses to histamine.

As shown in the second histogram, TPA (10^{-7} M, n = 11) produced a small but significant inhibition of histamine-induced secretion. In preparations treated with TPA consecutive responses to histamine were similar in magnitude. However, the responses in control mucosae increased steadily throughout the experiment. There was a significant reduction in secretion, relative to the controls, in the presence of TPA ($P < 0.001$) and in the response following its removal ($P < 0.001$).

Similarly, in experiments with 12-DOPPA (3×10^{-7} M, n = 6, third histogram) responses to histamine were significantly reduced in the presence of the ester ($P < 0.05$) and at the subsequent response ($P < 0.05$), following its removal. Inhibition of histamine-induced secretion by 12-DOPPAA (3×10^{-7} M, n = 6, fourth histogram) was more marked. Although the response in the presence of

* Correspondence.



	R^1	R^2	R^3
12-deoxyphorbol-phenyl acetate (12-DOPPA)	OC.CH_2-⬡	H	H
12-deoxyphorbol-phenyl acetate-20-acetate (12-DOPPAA)	OC.CH_2-⬡	H	OC.CH_3
Tetradecanoyl-phorbol-acetate (TPA)	OC.CH_3	OC.$(CH_2)_{12}CH_3$	H
Phorbol	H	OH	H

FIG. 1. Structural formulae of the tumour-promoting tigliane esters 12-DOPPA, 12-DOPPAA and TPA, and phorbol.

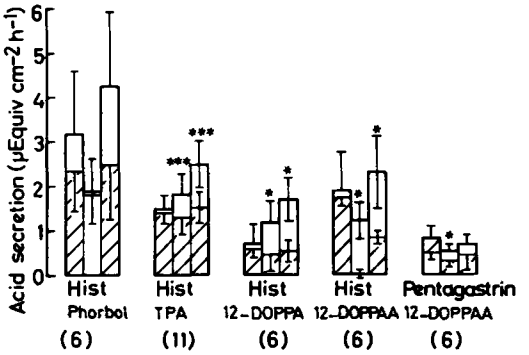


FIG. 2. Acid secretory responses to histamine (5×10^{-5} M, 30 min) or pentagastrin ($1{\cdot}8 \times 10^{-8}$ M, 30 min). Each column represents the mean ± s.e.m. of n observations. Hatched columns indicate responses in preparations treated with phorbol (4×10^{-6} M), TPA (2×10^{-7} M), 12-DOPPA or 12-DOPPAA (both 3×10^{-7} M), added 30 min before the second response. Open columns indicate responses in the untreated control preparations. Significance of data: Wilcoxon Matched Pairs test; * $P < 0{\cdot}05$, *** $P < 0{\cdot}001$.

12-DOPPAA was abolished ($P < 0{\cdot}05$), the next response demonstrated some degree of recovery; however, secretion was still significantly lower than in control mucosae ($P < 0{\cdot}05$).

In contrast to the abolition of the response to histamine, secretion induced by pentagastrin ($1{\cdot}8 \times 10^{-8}$ M, n = 6) in the presence of 12-DOPPAA (3×10^{-7} M) was significantly ($P < 0{\cdot}05$) but only partially reduced; at the next period of contact, responses in test and control mucosae were not significantly different.

Discussion

The use of an isolated gastric mucosal preparation allows the direct actions of drugs on acid secretion to be measured without interference from indirect influences such as changes in nervous activity or mucosal blood flow.

Phorbol (4×10^{-6} M), the parent alcohol of the tigliane esters, did not inhibit histamine-induced secretion while its ester TPA (2×10^{-7} M) produced only a partial reduction (by 30%). In contrast, 12-DOPPA and 12-DOPPAA (3×10^{-7} M) demonstrated marked inhibition of responses to histamine (by 70 and 95% respectively). 12-DOPPAA was less effective against pentagastrin (reduced by 50%).

The lack of activity of phorbol was expected since this compound does not exhibit the biological activities of the esterified forms in either in vitro (Soper & Evans 1977) or in vivo systems (Schmidt & Evans 1979). TPA, a potent tumour-promoting agent (Driedger & Blumberg 1980), was only weakly active in this system. However, 12-DOPPA and 12-DOPPAA, which lack appreciable tumour-promoting activity (Driedger & Blumberg 1980), were potent inhibitors of histamine-induced secretion.

The mechanism of action of 12-deoxyphorbol esters on the rat gastric mucosa is not known. They do not appear to be non-specific tissue poisons since their effects are reversible, and the most potent compound 12-DOPPAA, at a concentration which abolished the response to histamine, was only partially effective against another secretagogue pentagastrin.

REFERENCES

Driedger, P. E., Blumberg, P. M. (1980) Cancer Res. 40: 339–346

Evans, F. J., Soper, C. J. (1978) Lloydia 41: 193–233

Main, I. H. M., Pearce, J. B. (1978) J. Pharmacol. Methods 1: 27–38

Schmidt, R. J., Evans, F. J. (1979) Acta Pharmacol. Toxicol. 45: 181–191

Schmidt, R. J., Evans, F. J. (1980) Arch. Toxicol. 44: 279–289

Soper, C. J., Evans, F. J. (1977) Cancer Res. 37: 2487–2491

Westwick, J., Williamson, E. M., Evans, F. J. (1981) Thrombosis Res. 20: 683–692