kinase C (Nishizuka, 1984), while IP₃ seems to be implicated in the mobilization of Ca²⁺ from intracellular pools (Streb et al., 1983) and the triggering of intracellular responses mediated by Ca²⁺. We have now used isolated rat enterocytes to study whether such a mechanism operates in the control of intestinal secretion.

Cells were obtained from rat small intestine as described by Watford et al. (1979). The method includes washing the intestine with a Ca²⁺-free Krebs-Ringer buffer and filling the intestine with a Krebs buffer containing 5 mM-EDTA and incubating it at 37°C for 15 min. After this period cells are separated from the epithelium and after washing, they are resuspended in the appropriate buffer.

Cells were resuspended in a medium designed to resemble the intracellular medium and containing controlled concentrations of Ca²⁺. These cells were unable to accumulate α-methyl glucoside above the medium concentrations. Moreover, the cells accumulated ⁴⁵Ca in an ATP-dependent process. The fact that 100% of the accumulated ⁴⁵Ca was released by addition of the calcium ionophore A23187 (5 μM) indicates that the cation was contained in an intracellular membrane limited compartment. It therefore appears that during their preparation, the cells have become permeable to Ca²⁺ (pancreatic acinar cells are also Ca²⁺-permeable when washed in a Ca²⁺-free media; Streb et al., 1983). When the cells were resuspended in a medium containing 10-20 μM free Ca²⁺, the uptake was partially inhibited by 1 mM N-sodium orthovanadate and also by the presence of oligomycin (5 μg/ml) and antimycin (6 μg/ml), suggesting that both a mitochondrion and a nonmitochondrial (possibly endoplasmic reticulum) compartments were responsible for sequestering the cation. When the concentration of free Ca²⁺ was lower than 1 μM the accumulation was inhibited up to 90% by vanadate while oligomycin and antimycin exerted a small effect (about 5% inhibition). Fig. 1 shows that when IP₃ was added to a concentration of 6.25 μM after enterocytes had accumulated ⁴⁵Ca, nearly 50% of the accumulated ⁴⁵Ca was released. The extent of the effect IP₃ was concentration-dependent for concentrations lower than 10 μM. In some experiments the release phase was immediately followed by a second phase in which Ca²⁺ was accumulated again while in others (as the one shown) the released Ca²⁺ was not accumulated again. We do not know the reason for this difference but it might be related to differences in IP₃ metabolism.

The results presented, taken together with the observation made that enterocytes contain a Ca²⁺-activated, phospholipid-dependent protein kinase (G. Velasco & P. S. Lazo, unpublished work), lend novel support to the hypothesis that Ca²⁺-dependent secretagogues act by stimulating a membrane-bound phospholipase and triggering thereafter the phosphatidylinositol-phosphate kinase C transduction mechanism.

We thank the British Council and the M.E.C. (Spain) for the granting of an Accion Integrada and the Medical Research Council (U.K.) and the Fondo de Investigaciones Sanitarias (Spain) for financial support.


Received 9 June 1986

Action of protein kinase C on gastric acid secretion

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The phorbol ester, 12-O-tetradecanoylphorbol 13-acetate (TPA), inhibited the histamine-stimulated accumulation of the weak base aminopyrine in isolated rat parietal cells (Anderson & Hanson, 1984) probably by activating protein kinase C (Ca²⁺-sensitive phospholipid-dependent protein kinase) (Anderson & Hanson, 1985). Aminopyrine accumulation is a consequence of the sequestration of acid in the secretory canaliculi of parietal cells. The inhibition of this process by TPA could derive from an increased dissipation of acid from the secretory canaliculi, either into the extracellular medium or back into the cytosol, or from a reduction in the secretion of acid into the canaliculi.

In isolated vesicles containing the H⁺/K⁺-ATPase provision of K⁺ at the luminal (internal) face is sufficient to initiate ATP hydrolysis, but inhibition of ATP hydrolysis by vanadate is associated with insulin-stimulated vesicle membrane potential. After addition of insulin, the vesicle membrane potential was increased (Fig. 1B), but ATP hydrolysis was inhibited by vanadate (Fig. 1C). These results suggest that insulin increases vesicle membrane potential by stimulating ATP hydrolysis. In contrast, the vesicle membrane potential was not increased by insulin when ATP was not provided (Fig. 1D). These results indicate that ATP hydrolysis is required for insulin to increase vesicle membrane potential.
stimulate the pump (Sachs et al., 1976), and in intact cells a high extracellular K⁺ concentration can also stimulate aminopyrine accumulation possibly by a similar direct activation of the H⁺/K⁺-ATPase. The action of TPA on cells stimulated by high extracellular K⁺ has been investigated to help establish the mechanism by which protein kinase C influences aminopyrine accumulation.

Parietal cells were prepared from the fundic region of rat stomachs by pronase digestion and intermittent Ca²⁺-chelation (Shaw et al., 1985). Cells were incubated in a Krebs-Ringer bicarbonate medium (Shaw et al., 1985) modified by elevation of [K⁺] to 100 mM with a compensatory reduction in [Na⁺]. Glucose (5 mM), isoleucine (0.1 mM), bovine serum albumin (1 mg/ml), [¹⁴C]aminopyrine (0.1 μCi/ml, 0.9 μM) and [¹⁴C]poly(ethylene glycol) (0.4 μCi/ml) were also present. Aminopyrine accumulation was determined as described previously (Shaw et al., 1985).

The stimulation of aminopyrine accumulation by 100 mM extracellular K⁺ varied with time. The presence of 10 nM-TPA resulted in an aminopyrine accumulation at 30 min which was 133% of the control (P < 0.01 by paired t-test) (Fig. 1). By contrast 4α-phorbol 12,13-didecanoate (10 nM), which does not activate protein kinase C, produced an aminopyrine accumulation which was (mean ± s.E.M., cell batches in parentheses) 97 ± 6% (4) of the control (100 mM-K⁺ alone) at 30 min. The percentage of parietal cells excluding Trypan Blue was 88 ± 3 (6) after 30 min in the presence of 100 mM-K⁺ and 84 ± 2 (6) in the presence of 100 mM-K⁺ and 10 nM-TPA (no significant effect of TPA by paired t-test). Cells incubated for 30 min in a normal Krebs-Ringer bicarbonate medium containing 149 mM-Na⁺ and 4.5 mM-K⁺ exhibited a significantly higher (P < 0.025) exclusion of Trypan Blue 94 ± 1 (11) by comparison with the cells incubated in the high-K⁺ medium (see above).

The elevation of K⁺-stimulated aminopyrine accumulation by TPA at 30 min was still significant in the presence of cimetidine to block the histamine H₁ receptor or of atropine to block the muscarinic cholinergic receptor [% stimulation by 10 nM-TPA: 100 mM-K⁺ alone, 39 ± 2 (16); 100 mM-K⁺ + 0.1 mM-cimetidine, 36 ± 11 (5); 100 mM-K⁺ + 10 μM-atropine, 27 ± 6 (4)]. Aminopyrine accumulation in the presence of 100 mM-K⁺ was not reduced by cimetidine or atropine, but 10 mM-NSA did lower it from 90 ± 3 (4) to 3 ± 0.6 (4) (P < 0.001).

The partially transient stimulation of aminopyrine accumulation by high K⁺ may be because the H⁺/K⁺-ATPase pump is only active initially after elevation of K⁺ (Hersey et al., 1981), but high K⁺ may also cause deterioration of the cells for there is swelling (Gibert & Hersey, 1982), and an increased proportion of cells become permeable to Trypan Blue by comparison with incubation in normal medium (see above). The most important feature of the above results is that 10 nM-TPA did not inhibit aminopyrine accumulation stimulated by high extracellular K⁺. This finding strongly suggests that the inhibitory effect of TPA on histamine-stimulated aminopyrine accumulation did not involve an enhanced dissipation of acid in the secretory canaliculi, for otherwise TPA would have resembled the 'uncoupler' NaSCN and prevented K⁺-stimulated aminopyrine accumulation. Furthermore, the absence of an inhibition of K⁺-stimulated secretion by TPA suggests that any inhibitory site of action of TPA lies proximal to the H⁺/K⁺-ATPase pump. Finally, the enhancement of K⁺-stimulated aminopyrine accumulation by TPA at 30 min was a specific effect, which was probably not mediated by the action of histamine or of acetylcholine on the parietal cell. The cause is uncertain, but both stimulatory and inhibitory effects of TPA in the same cell-type have been noted previously (Sagi-Eisenberg et al., 1985).

Supported by grants from the M.R.C. (U.K.) and the Wellcome Trust.

Anderson, N. G. & Hanson, P. J. (1985) Biochem. J. 232, 609-611

Received 9 June 1986

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