*Biochimica et Biophysica Acta* 937 (1988) 328–334
Elsevier

BBA 73853

# $Na^+$-dependent elevation of the acidic cell surface pH (microclimate pH) of rat jejunal villus cells induced by cyclic nucleotides and phorbol ester: possible mediators of the regulation of the $Na^+/H^+$ antiporter

Tadahito Shimada and Takeshi Hoshi

*Department of Physiology, Faculty of Medicine, University of Tokyo, Tokyo (Japan)*

(Received 26 August 1987)

Key words: pH, cell surface; Sodium ion/proton antiport; cAMP; cGMP; Phorbol ester; (Rat jejunum)

**The effects of cyclic nucleotides and phorbol ester on the acidic cell surface pH of rat jejunal villi were studied by using single-barrelled pH-sensitive microelectrodes. Addition of dibutyryl cAMP (1 mM) to the mucosal bathing solution caused an elevation of the cell surface pH from 6.19 ± 0.04 ($n$ = 12 measurements from three animals) to 6.53 ± 0.03 (12) in the presence of $Na^+$ in the medium. However, dibutyryl cAMP had no significant effect in the absence of $Na^+$ and presence of 1 mM amiloride. Dibutyryl cGMP (1 mM) also had an $Na^+$-dependent inhibitory effect on the cell surface pH. A phorbol ester, phorbol 12-myristate 13-acetate, caused an elevation of the cell surface pH only in the presence of $Na^+$ from 6.14 ± 0.07 (12) to 6.46 ± 0.08 (12). Phorbol and phorbol 13-acetate, which do not stimulate protein kinase C, were without significant effects. These results suggest that increased levels of the intracellular cyclic nucleotides and activation of protein kinase C raise the acidic cell surface pH by inhibiting the activity of the brush-border $Na^+/H^+$ antiporter in the rat jejunal villus cells.**

## Introduction

Lucas et al. [1] first demonstrated the presence of an acid microclimate layer (microclimate pH) in the close vicinity of the cell surface of rat jejunum in vitro. Subsequently, Lucas' group and other investigators disclosed important properties of this pH layer [2–8]. From these studies, it has become clear that $H^+$ secretion by the intestinal epithelial cells and the presence of the surface mucus are of fundamental importance in the formation and maintenance of the acid microclimate, although there are many other factors affecting it.

Among the mechanisms of $H^+$ secretion by the intestinal epithelial cells, $Na^+/H^+$ antiport has been shown to be most important. The brush-border membrane of enterocytes possesses $Na^+/H^+$ antiporters [9–11], and this antiport mechanism functions to regulate intracellular pH [12]. In regard to the microclimate pH, Lucas et al. [2,4] first showed that it is sensitive to the presence of $Na^+$ in the luminal medium. We confirmed the role of the $Na^+/H^+$ antiporter in the formation of the acid microclimate [8]. Accordingly, it is expected that the activity level of the $Na^+/H^+$ antiporter is reflected in the acidity of the microclimate, so that one can examine factors modulating the activity of the $Na^+/H^+$ antiporter by measuring the microclimate pH. In the present study, we focussed on the regulation of the $Na^+/H^+$ antiporter by possible intracellular mediators,

Abbreviation: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Correspondence (present address): T. Shimada, Second Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Tokyo 113, Japan.

and the effects of cyclic nucleotides and phorbol ester on the acidic cell surface pH were investigated.

## Method

*Preparations and pH measurements*

Experimental procedures were almost identical to those described previously [8]. In short, male Wistar rats (weighing 200–250 g) were anesthetized by intraperitoneal injection of urethane (1 g/kg). The proximal part of the jejunum was excised, opened along the mesenteric border, and fixed on a small Lucite chamber with the mucosal side upwards. The preparations were perfused with a standard or a test solution saturated with 100% $O_2$ at a rate of 10 ml/min. The temperature of the perfusion solution was kept at 37 °C. All experiments were performed within 30 min after the excision. Within this period of time, the tissue viability seemed to be preserved, as described previously [8].

pH measurements were performed with single-barrelled liquid-ion-exchanger-type pH-sensitive microelectrodes which had a tip diameter of around 1 μm. A mixture of 10% tri-*n*-dodecylamine, 89.3% *O*-nitrophenyl-*n*-octyl ether and 0.7% sodium tetraphenylborate [13] was used as an $H^+$-ligand. Fabrication and properties of the pH-microelectrodes were as described previously [8]. Calibration was made using a 140 mM NaCl solution which was buffered with 10 mM Hepes-Tris to pH 5.5–7.5. The average slope of the pH-microelectrodes in the present study was 61.7 $\pm$ 0.48 ($n = 37$) mV/pH in the pH range of 5.5–7.5 at 37 °C. The electrodes were connected to the input of an electrometer (FD-223, WP Instruments, New Haven, CT). As a reference electrode, a polyethylene bridge filled with 2% agar in 1 M KCl was used to connect the bathing solution to a 1 M KCl solution where an Ag/AgCl wire was immersed. pH measurements were performed in an earthed metal cage. A well-shielded wire was employed for the input circuit, and all pieces of equipment, including the micromanipulators and stereomicroscope, were grounded.

The pH-microelectrode was positioned at an angle of 53° to the tissue, and the tip of the electrode was advanced gradually by a micromanipulator (Narishige). The lowest values of pH recorded just before the cell membrane penetration at the villus tip region were compared. Such lowest values of pH are referred to as the 'cell surface pH' in the present study. The excised tissues were perfused with a solution which contained one of the test substances for 5 min before pH measurements. Control tissues were perfused with a standard solution for the same period of time before pH measurements. Cell surface pH was measured at several points in one tissue, and three tissue samples from three animals were used to study the effects of one test substance.

The standard perfusion solution used in the present study had the following composition (in mM): NaCl, 135; KCl, 3; $CaCl_2$, 1.8; $MgCl_2$, 1.0; D-mannitol, 20; and Hepes-Tris, 2 (pH 7.30). NaCl was replaced by equimolar choline chloride for the $Na^+$-free condition. Finally, the pH of all solutions was carefully readjusted to 7.30 with tetramethylammonium hydroxide at 37 °C.

*Chemicals and statistics*

Dibutyryl cAMP, prostaglandin $E_2$, dibutyryl cGMP, phorbol 12-myristate 13-acetate, phorbol, phorbol 13-acetate and calcium ionophore A23187 were obtained from Sigma. Theophylline was obtained from Wako (Tokyo). Amiloride was a kind gift from Merck Sharp & Dohme. All other chemicals were of reagent grade purity. All data are presented as means $\pm$ S.E. Statistical significance of differences between the mean values was evaluated by Student's *t*-test.

## Results

First, the effect of $Na^+$ replacement on the cell surface pH was reinvestigated in the present preparations. As seen in Table I, the replacement of $Na^+$ with choline caused a significant elevation of the cell surface pH, confirming the previous observations [8]. As it was considered possible that the $Na^+$ concentration in the unstirred layer including intermicrovillus spaces might not be rapidly equalized to the bulk phase [14], the effect of amiloride was tested in the absence of $Na^+$ from the perfusion solution. Addition of 1 mM amiloride to the choline-substituted medium caused further elevation of the cell surface pH.

TABLE I

EFFECTS OF $Na^+$ REPLACEMENT AND AMILORIDE ON THE CELL SURFACE pH OF RAT JEJUNAL VILLUS CELLS

Cell surface pH was measured in the $Na^+$-containing standard medium (control) (135 mM NaCl, 3 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 20 mM D-mannitol, 2 mM Hepes-Tris, pH 7.30), choline medium (NaCl was replaced by choline chloride), and choline medium which also contained 1 mM amiloride. Data are expressed as means ± S.E. Figures in parentheses are the number of observations.

| | Cell surface pH |
|---|---|
| Control | 6.10 ± 0.03 (11) |
| Choline medium | 6.43 ± 0.05 (13) ($P < 0.01$) |
| Choline medium plus amiloride (1 mM) | 6.63 ± 0.02 (13) ($P < 0.01$) |

The pH values were 6.10 ± 0.03 ($n = 11$) in the control condition ($Na^+$-containing medium), 6.43 ± 0.05 (13) in the choline medium and 6.63 ± 0.02 (13) in the choline medium which also contained 1 mM amiloride. Therefore, in the subsequent experiments, choline-substituted medium which contained 1 mM amiloride was used as the $Na^+$-free solution.

Table II shows the effects of 1 mM dibutyryl cAMP and dibutyryl cGMP on the cell surface pH in the presence and absence of $Na^+$ in the medium. Both dibutyryl cAMP and dibutyryl cGMP raised the cell surface pH in the presence of $Na^+$. In contrast, they did not cause any significant changes in the cell surface pH in the $Na^+$-free medium. In these $Na^+$-free experiments, $Na^+$ concentration was not exactly zero, since the sodium salt of dibutyryl cAMP or cGMP was used. Therefore, 1 mM NaCl was also added to the choline-substituted solution in control observations. However, because of the presence of amiloride, the activity of the $Na^+/H^+$ antiporter is considered to be sufficiently suppressed.

Table III shows the effects of 10 mM theophylline, a nonspecific inhibitor of phosphodiesterase, on the cell surface pH. When theophylline was added to the perfusion solution, equimolar D-mannitol was omitted from the solution. Theophylline is known to increase the levels of both cAMP and cGMP in the samll intestine [15]. As seen in Table III, theophylline raised the cell surface pH significantly when the medium contained $Na^+$. However, the same dose of theophylline had no significant effect in the choline medium containing 1

TABLE II

EFFECTS OF DIBUTYRYL cAMP AND DIBUTYRYL cGMP ON THE CELL SURFACE pH OF RAT JEJUNAL VILLUS CELLS IN THE PRESENCE AND ABSENCE OF $Na^+$ IN THE PERFUSION SOLUTION

$+Na^+$ indicates the measurements in the $Na^+$-containing standard medium (135 mM NaCl, 3 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 20 mM D-mannitol, 2 mM Hepes-Tris, pH 7.30). $-Na^+$ indicates the measurements in the $Na^+$-free medium (NaCl was replaced by choline chloride). $Na^+$-free medium also contained 1 mM amiloride. Data are expressed as means ± S.E. Figures in parentheses are the number of observations. n.s. indicates statistical insignificance.

| | Cell surface pH | |
|---|---|---|
| | $+Na^+$ | $-Na^+$ |
| Control | 6.19 ± 0.04 (12) | 6.79 ± 0.02 (12) |
| Dibutyryl cAMP (1 mM) | 6.53 ± 0.03 (12) ($P < 0.01$) | 6.81 ± 0.03 (12) (n.s.) |
| Control | 6.13 ± 0.03 (12) | 6.73 ± 0.04 (12) |
| Dibutyryl cGMP (1 mM) | 6.43 ± 0.03 (12) ($P < 0.01$) | 6.79 ± 0.03 (12) (n.s.) |

TABLE III

EFFECTS OF THEOPHYLLINE AND PROSTAGLANDIN $E_2$ ON THE CELL SURFACE pH OF RAT JEJUNAL VILLUS CELLS IN THE PRESENCE AND ABSENCE OF $Na^+$ IN THE PERFUSION SOLUTION

$+Na^+$ indicates the measurements in the $Na^+$-containing standard medium (135 mM NaCl, 3 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 20 mM D-mannitol, 2 mM Hepes-Tris, pH 7.30). $-Na^+$ indicates the measurements in the $Na^+$-free medium (NaCl was replaced by choline chloride). $Na^+$-free medium also contained 1 mM amiloride. Data are expressed as means ± S.E. Figures in parentheses are the number of observations. n.s. indicates statistical insigificance.

| | Cell surface pH | |
|---|---|---|
| | $+Na^+$ | $-Na^+$ |
| Control | 6.15 ± 0.03 (12) | 6.67 ± 0.05 (12) |
| Theophylline (10 mM) | 6.66 ± 0.03 (12) ($P < 0.01$) | 6.73 ± 0.03 (12) (n.s.) |
| Control | 6.09 ± 0.04 (12) | 6.72 ± 0.02 (12) |
| Prostaglandin $E_2$ ($10^{-6}$ M) | 6.45 ± 0.03 (12) ($P < 0.01$) | 6.73 ± 0.05 (12) (n.s.) |

mM amiloride. Prostaglandin $E_2$ is also known to increase intracellular cAMP levels by activating adenylate cyclase in the small intestine [16–18]. Table III also shows the effects of $10^{-6}$ M prostaglandin $E_2$ both in the presence and absence of $Na^+$. Prostaglandin $E_2$ was dissolved in ethanol for the stock solution, and the final concentration of ethanol in the test medium was 0.1%. Prostaglandin $E_2$ had significant inhibitory effects on the cell surface pH only in the presence of $Na^+$.

Phorbol esters are reported to be potent activators of protein kinase C [19]. The effects of $10^{-6}$ M phorbol 12-myristate 13-acetate on the cell surface pH are shown in Table IV. Phorbol 12-myristate 13-acetate was dissolved in dimethyl sulphoxide (DMSO), and the final concentration of DMSO in the test medium was 0.1%. As seen in Table IV, phorbol 12-myristate 13-acetate also had a significant inhibitory effect on the cell surface pH only in the presence of $Na^+$. To examine whether or not the effect of phorbol 12-myristate 13-acetate is specific with regard to stimulation of protein kinase C, phorbol and phorbol 13-acetate, which are known to have no stimulatory effects on protein kinase C [19], were tested. Phorbol and phorbol 13-acetate were dissolved in DMSO, and the final concentration of DMSO in the medium was 0.1%. Table V shows the effects of these substances in the presence of $Na^+$. Apparently, they had no significant effects on the cell surface pH.

In the next series of experiments, the effects of Ca ionophore A23187 ($2 \cdot 10^{-6}$ M) was examined. A23187 was dissolved in ethanol, and the final concentration of ethanol in the test medium was 0.1%. As shown in Table VI, A23187 had no significant effects on the cell surface pH, regardless of whether or not $Na^+$ was present in the medium.

In an additional experiment, we examined whether the choline medium containing 1 mM

TABLE IV

EFFECTS OF PHORBOL 12-MYRISTATE 13-ACETATE ON THE CELL SURFACE pH OF RAT JEJUNAL VILLUS CELLS IN THE PRESENCE AND ABSENCE OF $Na^+$ IN THE PERFUSION SOLUTION

$+Na^+$ indicates the measurements in the $Na^+$-containing standard medium (135 mM NaCl, 3 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 20 mM D-mannitol, 2 mM Hepes-Tris, pH 7.30). $-Na^+$ indicates the measurements in the $Na^+$-free medium (NaCl was replaced by choline chloride). $Na^+$-free medium also contained 1 mM amiloride. Data are expressed as means ± S.E. Figures in parentheses are the number of observations. n.s. indicates statistical insignificance.

| | Cell surface pH | |
|---|---|---|
| | $+Na^+$ | $-Na^+$ |
| Control | 6.14 ± 0.07 (12) | 6.65 ± 0.03 (12) |
| Phorbol 12-myristate 13-acetate ($10^{-6}$ M) | 6.46 ± 0.08 (12) ($P < 0.01$) | 6.73 ± 0.04 (12) (n.s.) |

TABLE V

EFFECTS OF PHORBOL AND PHORBOL 13-ACETATE ON THE CELL SURFACE pH OF RAT JEJUNAL VILLUS CELLS

Cell surface pH was measured in the presence of $Na^+$ in the perfusion solution (135 mM NaCl, 3 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 20 mM D-mannitol, 2 mM Hepes-Tris, pH 7.30). Data are expressed as means ± S.E. Figures in parentheses are the number of observations. n.s. indicates statistical insignificance.

| | Cell surface pH |
|---|---|
| Control | 6.18 ± 0.03 (12) |
| Phorbol ($10^{-6}$ M) | 6.20 ± 0.03 (12) (n.s.) |
| Phorbol 13-acetate ($10^{-6}$ M) | 6.23 ± 0.02 (12) (n.s.) |

TABLE VI

EFFECTS OF A23187 ON THE CELL SURFACE pH OF RAT JEJUNAL VILLUS CELLS IN THE PRESENCE AND ABSENCE OF $Na^+$ IN THE PERFUSION SOLUTION

$+Na^+$ indicates the measurements in the $Na^+$-containing standard medium (135 mM NaCl, 3 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 20 mM D-mannitol, 2 mM Hepes-Tris, pH 7.30). $-Na^+$ indicates the measurements in the $Na^+$-free medium (NaCl was replaced by choline chloride). $Na^+$-free medium also contained 1 mM amiloride. Data are expressed as means ± S.E. Figures in parentheses are the number of observations. n.s. indicates statistical insignificance.

| | Cell surface pH | |
|---|---|---|
| | $+Na^+$ | $-Na^+$ |
| Control | 6.15 ± 0.02 (12) | 6.77 ± 0.02 (12) |
| A23187 ($2 \cdot 10^{-6}$ M) | 6.21 ± 0.01 (12) ($P < 0.01$) | 6.77 ± 0.02 (12) (n.s.) |

amiloride affected tissue viability. The cell surface pH was first measured in the choline medium containing 1 mM amiloride; thereafter the medium was changed to the $Na^+$-containing standard solution followed by the cell surface pH measurement. Almost complete restoration of the acidic cell surface pH was seen from an elevated pH of $6.60 \pm 0.02$ (12) to $6.14 \pm 0.02$ (12). This indicates that the viability of tissues was well-preserved during incubation in the choline medium containing 1 mM amiloride.

## Discussion

The results of the present study show that not only cyclic nucleotides but also phorbol ester cause an elevation of the microclimate pH (cell surface pH) in rat jejunum in the presence of $Na^+$ in the perfusion solution. In the absence of $Na^+$ and presence of 1 mM amiloride, these substances did not show any significant effects on the cell surface pH. Such $Na^+$-dependent inhibition of the acidic microclimate pH is most likely to occur via the inhibition of $Na^+/H^+$ antiporter activity in the brush-border membrane.

Lucas [20] first demonstrated that mucosal acidification by the rat jejunum is significantly inhibited by aminophylline. Lucas and Blair [2] subsequently showed directly that the microclimate pH became less acidic in the presence of 10 mM aminophylline. Lucas [21] also showed the inhibitory effects of dibutyryl cAMP and cAMP on the microclimate pH and suggested the presence of some linkage between $H^+$ secretion and the intracellular cAMP level.

cAMP is a putative intracellular messenger which alters ion transport in the small intestine [22,23]. An increase in intracellular cAMP increases electrogenic $Cl^-$ secretion and inhibits the linked $Na^+$ and $Cl^-$ absorptive process [22,23]. For the villus cells, the latter effect is important, since electrogenic $Cl^-$ secretion is localized to the crypt cells [24]. This linked $Na^+$ and $Cl^-$ absorptive process is now explained by a combination of $Na^+/H^+$ antiport and $Cl^-/OH^-(HCO_3^-)$ antiport [23]. Therefore, the inhibitory effects of cAMP on the linked $Na^+$ and $Cl^-$ absorption may be due to the inhibition of these antiporters. Recently, Semrad and Chang [25] showed directly the inhibitory effects of cAMP on the $Na^+/H^+$ antiporter in chicken enterocytes.

In the present study, the measured cell surface pH was not modified by the $Cl^-/OH^-(HCO_3^-)$ antiporter, because the $Cl^-/OH^-(HCO_3^-)$ antiporter has not been detected in the brush-border membrane of rat jejunum [11]. Our previous study [8] also showed no significant effect of $Cl^-$ replacement on the microclimate pH. Therefore, the present results can be taken as evidence for the inhibition of the $Na^+/H^+$ antiporter by cAMP in the mammalian small intestine.

Reuss and Petersen [26] demonstrated the inhibitory effect of cAMP on the $Na^+/H^+$ antiporter in *Necturus* gall gladder epithelium. Petersen et al. [27] also demonstrated a similar inhibitory effect of cAMP on the $Na^+/H^+$ antiporter in guinea pig gall bladder epithelium. Kahn et al. [28] showed this inhibitory effect of cAMP in rabbit proximal tubules. These findings, together with the present results, indicate that $Na^+/H^+$ antiporters located in the cell membranes of epithelial tissues may be inhibited by an increased level of intracellular cAMP.

Another cyclic nucleotide, cGMP, is also a putative intracellular messenger which can affect ion transport in the intestine, although little is known about the detailed mechanism of cGMP-related regulation as compared to that of cAMP [23]. An increase in cGMP causes inhibition of the linked $Na^+$ and $Cl^-$ absorptive process, as in the case of cAMP [15,29,30]. These effects of cGMP indicate the possibility that cGMP also inhibits $Na^+/H^+$ antiporter activity in the intestine. However, there are no further data available concerning the effects of cGMP on the $Na^+/H^+$ antiporter in the intestine and other tissues. The present results provide evidence that cGMP also inhibits the $Na^+/H^+$ antiporter, at least in the rat jejunum.

Recently, protein kinase C has been recognized as an important regulator of intestinal ion transport [31]. Phorbol esters, which stimulate protein kinase C [19], are useful tools in the study of the role of protein kinase C in the regulation of intestinal transport. Phorbol esters have been reported to activate the $Na^+/H^+$ antiporter in various cell types, such as human fibroblast, HeLa cell, neuroblastoma [32], rat myoblast [33], rat

thymocytes [34], porcine neutrophils [35] and human neutrophils [36]. In contrast to these cell types, Ahn et al. [37] reported that phorbol ester inhibits the $Na^+/H^+$ antiporter in rabbit proximal colon. Donowitz et al. [38] also showed inhibition of $Na^+$ and $Cl^-$ absorption by phorbol ester in rat colon. The present results clearly show that, in the small intestine, the $Na^+/H^+$ antiporter is inhibited by the stimulation of protein kinase C. It is very interesting that phorbol esters have opposite effects on the $Na^+/H^+$ antiporter activity in intestinal epithelia compared to other tissues. There may be different regulation mechanisms for $Na^+/H^+$ antiport among tissues. The exact reason for this difference is not clear, and further studies are needed.

$Ca^{2+}$ is another important intracellular messenger for ion transport in the intestine [23]. Increase in the cytosol $Ca^{2+}$ concentration itself is reported to inhibit the linked $Na^+$ and $Cl^-$ absorptive process in the small intestine [39,40]. Semrad and Chang [25] reported that in chicken enterocytes, the inhibitory effects of cAMP on the $Na^+/H^+$ antiporter is mediated by $Ca^{2+}$. Therefore, it should be examined whether an increase in the cytosolic $Ca^{2+}$ level affects $Na^+/H^+$ antiporter activity. In the present study, A23187 did not affect the cell surface pH, even in the presence of $Na^+$ in the perfusion solution. This seems to indicate that $Ca^{2+}$ does not affect $Na^+/H^+$ antiporter activity directly, at least in the rat jejunal villus cells. However, further studies are needed because we did not produce direct evidence that A23187 really raised the intracellular $Ca^{2+}$ concentration in the present preparation.

Regulation of the $Na^+/H^+$ antiporter in the small intestinal villus cells is important in the control of intracellular pH and the control of $Na^+$ absorption. The results of the present study indicate that cAMP, cGMP, and protein kinase C are important intracellular mediators for the regulation of the $Na^+/H^+$ antiporter in the rat jejunal villus cells. Regulation of the $Na^+/H^+$ antiporter is directly reflected in the regulation of acidic microclimate pH. Therefore, the transport of weak acids or bases, or $H^+$-coupled transport of dipeptides [41–43], which are sensitive to pH or transmembrane pH gradients, could be regulated indirectly by these intracellular messengers.

## References


1 Lucas, M.L., Shineider, W., Haberich, F.J. and Blair, J.A. (1975) Proc. R. Soc. Lond. B 192, 39–48.
2 Lucas, M.L. and Blair, J.A. (1978) Proc. R. Soc. Lond. A 200, 27–41.
3 Lucas, M.L., Cooper, B.T., Lei, F.H., Johnson, I.T., Holmes, G.K.T., Blair, J.A. and Cook, W.T. (1978) Gut 19, 735–742.
4 Lucas, M.L., Lei, F.H. and Blair, J.A. (1980) Pflügers Arch. 385, 137–142.
5 Lucas, M.L. (1983) Gut 24, 734–739.
6 Daniel, H., Neugebauer, B., Kratz, A. and Rehner, G. (1985) Am. J. Physiol. 248, G293–G298.
7 Shiau, Y.F., Fernandez, P., Jackson, M.J. and MacMonagle, S. (1985) Am. J. Physiol. 248, G608–G617.
8 Shimada, T. (1987) J. Physiol. (Lond.) 392, 113–127.
9 Murer, H., Hopfer, U. and Kinne, R. (1976) Biochem. J. 154, 597–604.
10 Gunther, R.D. and Wright, E.M. (1983) J. Membr. Biol. 74, 85–94.
11 Cassano, G., Stieger, B. and Murer, H. (1984) Pflügers Arch. 400, 309–317.
12 Shimada, T. and Hoshi, T. (1987) Biochim. Biophys. Acta 901, 265–272.
13 Ammann, D., Lanter, F., Steiner, R.A., Schulthess, P., Shijo, Y. and Simon, W. (1981) Anal. Chem. 53, 2267–2269.
14 Lucas, M.L. and Cannon, M.J. (1983) Biochim. Biophys. Acta 730, 41–48.
15 Field, M., Graf, L.H., Laird, W.J. and Smith, P.L. (1978) Proc. Natl. Acad. Sci. USA 75, 2800–2804.
16 Kimberg, D.V., Field, M., Johnson, J., Henderson, A. and Gershon, E. (1971) J. Clin. Invest. 50, 1218–1230.
17 Matuchansky, C. and Bernier, J.-J. (1973) Gastroenterology 64, 1111–1118.
18 Bukhave, K. and Rask-Madsen, J. (1980) Gastroenterology 78, 32–42.
19 Castagna, M., Takai, Y., Kaibuchi, K., Sano, K., Kikkawa, U. and Nishizuka, Y. (1982) J. Biol. Chem. 257, 7847–7851.
20 Lucas, M.L. (1976) J. Physiol. (Lond.) 257, 645–662.
21 Lucas, M.L. (1984) in Pharmacology of Intestinal Permeation, Vol. 2 (Csáky, T.Z., ed.), pp. 119–163, Springer-Verlag, Berlin.
22 Field, M. (1981) in Physiology of the Gastrointestinal Tract, 1st Edn. (Johnson, L.R., ed.), pp. 963–982, Raven Press, New York.
23 Donowitz, M. and Welsh, M.J. (1987) in Physiology of the gastrointestinal Tract, 2nd Edn. (Johnson, L.R., ed.), pp. 1351–1388, Raven Press, New York.
24 Welsh, M.J., Smith, P.L., Fromm, M. and Frizzell, R.A. (1982) Science 218, 1219–1221.
25 Semrad, C.E. and Chang, E.B. (1987) Am. J. Physiol. 252, C315–C322.
26 Reuss, L. and Petersen, K.U. (1985) J. Gen. Physiol. 85, 409–429.
27 Petersen, K.U., Wehner, F. and Winterhager, J.M. (1985) Pflügers Arch. 405, S115–S120.
28 Kahn, A.M., Dulson, G.M., Hise, M.K., Bennett, S.C. and Weinman, E.J. (1985) Am. J. Physiol. 248, F212–F218.

29 Rao, M.C., Guandalini, S., Laird, W.J. and Field, M. (1979) Infect. Immun. 26, 875–878.
30 Rao, M.C., Orellana, S.A., Field, M., Robertson, D.C. and Giannella, R.A. (1981) Infect. Immun. 33, 165–170.
31 Fondacaro, J. (1986) Am. J. Physiol. 250, G1–G8.
32 Moolenaar, W.H., Tertoolen, L.G.J. and De Laat, S.W. (1984) Nature 312, 371–374.
33 Vigne, P., Frelin, C. and Lazdunski, M. (1985) J. Biol. Chem. 260, 8008–8013.
34 Grinstein, S., Cohen, S., Goetz, J.D., Rothstein, A. and Gelfand, E.W. (1985) Proc. Natl. Acad. Sci. USA 82, 1429–1433.
35 Grinstein, S., Elder, B. and Furuya, W. (1985) Am. J. Physiol. 248, C379–C386.
36 Grinstein, S. and Furuya, W. (1986) Am. J. Physiol. 251, C55–C65.
37 Ahn, J., Chang, E.B. and Field, M. (1985) Am. J. Physiol. 249, C527–C530
38 Donowitz, M., Cheng, H.Y. and Sharp, G.W.G. (1986) Am. J. Physiol. 251, G509–G517.
39 Bolton, J.E. and Field, M. (1977) J. Membr. Biol. 35, 159–173.
40 Donowitz, M. (1983) Am. J. Physiol. 245, G165–G177.
41 Ganapathy, V., Burckhardt, G. and Leibach, F.H. (1984) J. Biol. Chem. 259, 8954–8959.
42 Ganapathy, V. and Leibach, F.H. (1983) J. Biol. Chem. 258, 14189–14192.
43 Takuwa, N., Shimada, T., Matsumoto, H., Himukai, M. and Hoshi, T. (1985) Jpn. J. Physiol. 35, 629–642.