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OSMOTIC STABILIZERS DIFFERENTIALLY INHIBIT PERMEABILITY ALTERATIONS INDUCED IN VERO CELLS BY *CLOSTRIDIUM PERFRINGENS* ENTEROTOXIN

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Using a sensitive Vero (African green monkey kidney) cell model system, studies were performed to further investigate whether *Clostridium perfringens* enterotoxin acts via disruption of the colloid-osmotic equilibrium of sensitive cells. Enterotoxin was shown to cause a rapid loss of intracellular $^{86}Rb^+$ (M_r approx. 100) with time- and dose-dependent kinetics. The enterotoxin-induced release of intracellular $^{86}Rb^+$ preceded the loss of two larger labels, ^{51}Cr label (M_r approx. 3500) and ^{3}H-labeled nucleotides (M_r less than 1000). The osmotic stabilizers, sucrose and poly(ethylene glycol), differentially inhibited enterotoxin-induced larger label loss versus $^{86}Rb^+$ loss. Further, enterotoxin was shown to cause a rapid influx of $^{24}Na^+$ that was not significantly inhibited by osmotic stabilizers. Additional studies demonstrated that lysosomotropic agents were not protective against characteristic enterotoxin-induced membrane permeability alterations or morphological damage. Taken collectively, these results are consistent with an action for enterotoxin which involves a disruption of the osmotic equilibrium.

Introduction

It has been proposed [1–4] that the mechanism of action of *Clostridium perfringens* enterotoxin involves a direct membrane interaction which induces structural and functional changes in the plasma membrane of sensitive cells. The enterotoxin caused rapid membrane bleb formation in cultured Vero (African green monkey kidney) cells [2,5] and intestinal epithelial cells [1]. Additionally, the enterotoxin has been shown to rapidly induce permeability alterations in Vero cells [3,6] and hepatocytes [7].

Preliminary studies [4,5] suggest that the membrane action of *C. perfringens* enterotoxin involves a disruption of the colloid-osmotic (Donnan) equilibrium of the cell. Osmotic stabilizers * such as sucrose, poly(ethylene glycol), Dextran and bovine serum albumin were protective against enterotoxin-induced changes in morphology [4,5] and some enterotoxin-induced plasma membrane permeability alterations [4]. This protection was apparently not from binding inhibition.

The present studies investigate further the osmotic-disruption hypothesis for the action of *C. perfringens* enterotoxin. Additionally, studies were performed to determine the effects of lysosomotropic agents on the expression of the biological activity of enterotoxin.

* Osmotic stabilizers are agents which do not penetrate the plasma membrane of eukaryotic cells, but inhibit cytolytic toxins by decreasing colloidal-osmotic swelling [8].

Materials and Methods

Enterotoxin

C. perfringens enterotoxin (M_r 35 000) was prepared as described [9] and the biological activity determined in plating efficiency units * [10]. The specific activity of the enterotoxin preparations varied from 400 000 to 800 000 plating efficiency units/mg protein. Enterotoxin was dissolved in double-distilled water and 60-μl aliquots were stored frozen at −20°C until used. Aliquots were used within 2 weeks of their preparation. Enterotoxin preparations had no detectable RNAase, proteinase or phospholipase activity [11].

Culture of Vero cells

Vero cells were grown in 150-cm^2 tissue culture flasks (Corning) with Medium 199 supplemented with 5% newborn-calf serum (Flow Laboratories) and 0.075% $NaHCO_3$. When monolayers reached confluency, they were removed by trypsinization with 0.25% trypsin in Ca^{2+}-free and Mg^{2+}-free Hanks' balanced salts solution. Unless otherwise specified, cells were inoculated into 16-mm wells in tissue culture cluster dishes (Costar). The seeding density was $5 \cdot 10^4$ cells in 3 ml of medium per well. The cluster dishes were incubated at 37°C in a humidified atmosphere containing 5% CO_2 in air. After 3 days, fresh medium was added. After 6 days, each well contained a confluent monolayer with $(6–8) \cdot 10^5$ cells.

Nucleotide labeling of Vero cells

Cells were nucleotide-labeled by methods previously reported [3,4]. Confluent 6-day-old monolayers were washed with Medium 199 without serum and labeled by the addition of 2 ml Medium 199 containing 2 μCi [^{3}H]uridine (42 mCi/mmol, New England Nuclear). After 2 h, the labeling medium was removed. Previous studies [3] have shown that cells labeled by this method contain approx. 70% of the total cytoplasmic label in a nucleotide form.

* A plating efficiency unit is defined as that amount of enterotoxin that caused a 25% inhibition of the plating of 200 cells inoculated into 100 μl of medium in a microwell culture system.

^{51}Cr labeling of Vero cells

Cells were labeled with ^{51}Cr by methods previously reported [3]. Confluent monolayers were rinsed with Hanks' balanced salt solution and incubated for 1 h with 1 ml Hanks' balanced salt solution containing $Na_2{}^{51}CrO_4$ (219 mCi/mg, New England Nuclear) at a concentration of 50 μCi/ml.

^{86}Rb labeling of Vero cells

Confluent monolayers were washed with Medium 199 without serum and incubated for 2 h with 2 ml Medium 199 containing 4 μCi ^{86}RbCl (1.4 mCi/mg, New England Nuclear).

Treatment of [^{3}H]nucleotide-labeled, ^{51}Cr-labeled, or ^{86}Rb-labeled cultures with enterotoxin

Medium 199 used for washing cells and for enterotoxin treatment of Vero cells did not contain serum.

Determination of the effects of osmotic stabilizers on label release. Unless otherwise specified, [^{3}H]nucleotide-, ^{51}Cr- or ^{86}Rb-labeled cultures were incubated with 1500 plating efficiency units of enterotoxin diluted in 2 ml of the specified osmotic test medium (Medium 199, Medium 199 containing 0.3 M sucrose or Medium 199 containing 25% poly(ethylene glycol)). After the desired incubation period, the culture supernatant was gently removed, vortex-mixed, and centrifuged at 4°C for 3 min in an Eppendorf microcentrifuge. Radioactivity was measured in the supernatant.

Determination of the effects of lysosomotropic agents on enterotoxin-induced nucleotide label release. [^{3}H]Nucleotide-labeled cultures were preincubated for 30 or 60 min with Medium 199 without serum, containing chloroquine or NH_4Cl at specified concentrations. Following this preincubation, the preincubation medium was removed, the cultures were washed once with Medium 199 without serum, and each culture was incubated with 1500 plating efficiency units of enterotoxin diluted in 2 ml of the appropriate test medium (Medium 199 without serum containing either chloroquine (Sigma) or NH_4Cl at the same concentration used for preincubation). After the desired incubation period, the culture supernatant was gently removed, vortex-mixed, and centrifuged at 4°C for 3 min in an Eppendorf microcentrifuge. Radioactivity was measured in the supernatant.

Determination of percent of maximal release

Released radioactivity was calculated as described by Thelestam and Möllby [12]:

$$\%\text{ of maximal release} = \frac{\text{toxin-induced release} - \text{spontaneous release}}{\text{maximal release} - \text{spontaneous release}} \times 100$$

Spontaneous release after 30 min at 37°C was less than 5% for [^{3}H]nucleotide label and 10% for ^{51}Cr label. Spontaneous release of ^{86}Rb label after 15 min at 37°C was approx. 30%. As described previously [3,4], the maximal release of cytoplasmic label was determined after cell-membrane rupture by the addition of 1 ml of 1 M citric acid and 1 ml of a 0.5% saponin buffer per well. Maximal release was $(1-2)\cdot 10^5$ cpm/culture for nucleotide label, $(3-5)\cdot 10^5$ cpm/culture for ^{51}Cr label, and $5\cdot 10^3$ cpm/culture for ^{86}Rb label.

Estimate of size of ^{86}Rb-labeled material

Cell lysates and released material from ^{86}Rb-labeled cultures were chromatographed on a Bio-Gel P-2 (Bio-Rad Laboratories) column. Bio-Gel P-2 was equilibrated in Tris-buffered saline which contained 0.02 M Tris-HCl and 0.15 M NaCl (pH 7.0). The column was 1.5 × 30 cm and the flow rate was 3 ml/h per cm^2. Fractions (1.5 ml) were collected and radioactivity in a 1.0 ml sample was determined from each fraction.

Determination of the effects of enterotoxin on $^{24}Na^+$ uptake

Confluent 6-day-old cultures were washed with 1 ml Medium 199 without serum (uptake medium). $^{24}Na^+$ uptake was then measured by the addition to each monolayer of 2 ml warm uptake medium (with or without 0.3 M sucrose, as specified) containing 1250 plating efficiency units of enterotoxin and 2 μCi of $^{24}Na^+$ (more than 4 Ci/g, Breazeale Nuclear Reactor, Pennsylvania State University). Incubation was continued at 37°C for 5 min and then $^{24}Na^+$ uptake was terminated by the rapid washing of each monolayer with ice-cold Hanks' balanced salt solution. The washing procedure was then quickly repeated twice to remove extracellular counts completely. After washing, cells were then lysed with 1 ml of 1 M NaOH, and 0.6 ml were removed for radioactivity determination and 0.3 ml were removed for protein determination by the method of Lowry et al. [13]. Bovine serum albumin was used as the standard for protein determinations.

Measurement of radioactivity

Samples (0.2 ml) from [^{3}H]nucleotide-labeled cultures were counted in a Searle Mark III liquid-scintillation spectrophotometer. ^{51}Cr samples (1.6 ml), $^{86}Rb^+$ samples (1.6 ml) and $^{24}Na^+$ samples (0.6 ml) were counted in a Packard gamma spectrophotometer.

Results

If the action of *C. perfringens* enterotoxin involves disruption of the colloid-osmotic equilibrium, as previously suggested [4,5], it should be possible to detect rapid loss of an intracellular ion such as K^+. To determine the effects of enterotoxin on K^+ efflux, $^{86}Rb^+$ has been utilized in the present studies as a radiotracer for K^+ fluxes [14,15], since it has a longer half-life than $^{42}K^+$ and preliminary studies (not shown) have confirmed the appropriateness of $^{86}Rb^+$ as a K^+ tracer. Fig. 1 demonstrates that enterotoxin causes

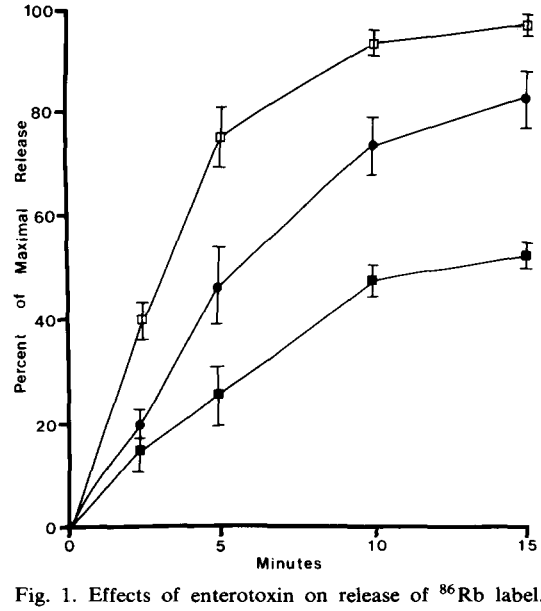

Fig. 1. Effects of enterotoxin on release of ^{86}Rb label. The effects of 2.5 μg enterotoxin/ml (□——□). 1.25 μg enterotoxin/ml (●——●) and 0.625 μg enterotoxin/ml (■——■) are shown.

a rapid loss of $^{86}Rb^+$ from Vero cells in a dose- and time-dependent manner. With 1500 plating efficiency units of enterotoxin per culture, nearly all intracellular $^{86}Rb^+$ was lost within 10 min of enterotoxin treatment. It is important to note that characteristic enterotoxin-induced morphological alterations [2,4] were not observed until approx. 15 min of enterotoxin treatment with 1500 plating efficiency units per culture.

To confirm that the intracellular ^{86}Rb label was not complexing with larger cellular material, gel chromatography on Bio-Gel P-2 was performed (Fig. 2). Since ^{86}Rb-labeled cell lysate co-elutes with [$^{86}Rb^+$]Cl, these studies demonstrate that intracellular ^{86}Rb label retains a similar molecular size to [$^{86}Rb^+$]Cl. Additionally, it was observed (data not shown), that following enterotoxin treatment (1500 plating efficiency units per culture for 15 min), released ^{86}Rb label eluted from the P-2 column with an elution profile similar to [$^{86}Rb^+$]Cl or ^{86}Rb-labeled cell lysate. Taken collectively, these results indicate that enterotoxin is inducing loss of $^{86}Rb^+$ as a small, noncomplexed ion.

Previous enterotoxin studies [3,4] have described radiolabeling conditions which resulted in Vero cells containing radiolabels of defined molecular sizes. For the present report, enterotoxin-induced permeability alterations were studied by comparing the effects of enterotoxin on loss of two previously defined [3] larger labels, ^{51}Cr label (M_r approx. 3500) and [^{3}H]nucleotide label (M_r less than 1000), with enterotoxin-induced $^{86}Rb^+$ (M_r approx. 100) leakage. Fig. 3 clearly shows that the effect of enterotoxin on membrane permeability alterations was size-dependent, confirming previous studies [3]. Further, Fig. 3 demonstrates that extensive enterotoxin-induced loss of intracellular ions, such as K^+, can be detected prior to significant larger molecule loss. These observations would be expected if the enterotoxin is osmotically-active.

To investigate further the action of enterotoxin, comparative studies were performed to examine the protection offered by osmotic stabilizers, such as sucrose, against enterotoxin-induced permeability alterations for markers of several defined molecular sizes. Table I shows that sucrose was highly protective against enterotoxin-induced [^{3}H]nucleotide loss, as previously reported [4], and ^{51}Cr label loss. This demonstrates that the inhibitory action of sucrose on enterotoxin-induced permeability alterations is not limited to [^{3}H]nucleotides. Additionally, poly(ethylene glycol) was highly protective against the enterotoxin-induced

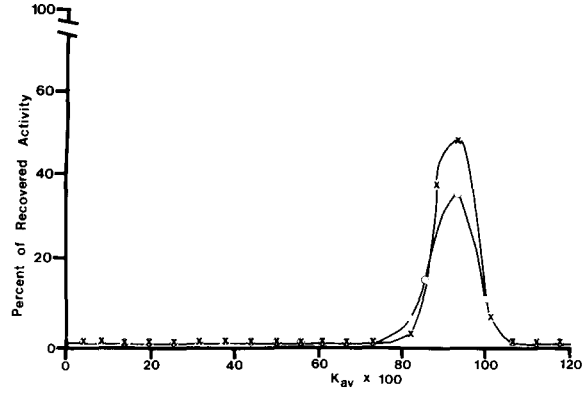

Fig. 2. Gel chromatography on Bio-Gel P-2 of cell lysates (○——○) and [$^{86}Rb^+$]Cl (×——×). Following chromatography, radioactivity in each fraction was measured and background activity was subtracted from each value. Data are expressed as percentage of total recovered activity.

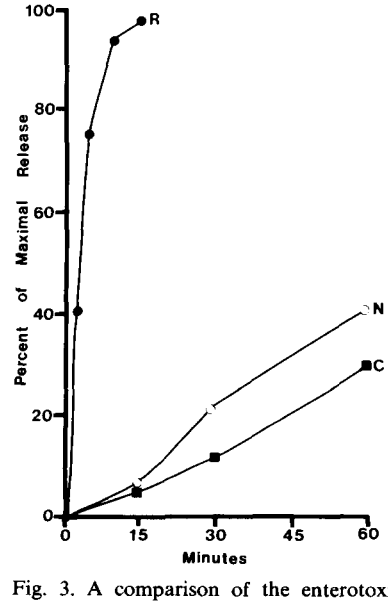

Fig. 3. A comparison of the enterotoxin-induced release of materials of different molecular sizes. The release of various markers after treatment with 1500 plating efficiency units of enterotoxin is shown. The results show the release of $^{86}Rb^+$ (●——●), [^{3}H]nucleotides (○——○) and ^{51}Cr label (■——■).

TABLE I

PROTECTIVE EFFECTS OF OSMOTIC STABILIZERS ON ENTEROTOXIN-INDUCED PERMEABILITY ALTERATIONS

Each Vero cell culture was radiolabeled to contain either intracellular $^{86}Rb^+$, ^{51}Cr label or [^{3}H]nucleotides. The cultures were then treated with 1500 plating efficiency units of enterotoxin in the specified (osmotic) test medium. Following incubation, released radiolabel was determined in culture supernatants. Morphological alterations induced by enterotoxin were observable after approx. 12–15 min. Values are given as mean ± S.E. ($n = 6$). PEG, poly(ethylene glycol); n.d., not determined.

Incubation time (min)	Enterotoxin-induced percent of maximal release [a]				
	Medium 199	Medium 199 containing 0.3 M sucrose	Inhibition (%) [b]	Medium 199 containing 25% PEG	Inhibition (%)
^{86}Rb label release [c]					
5	75.1 ± 6.7	63.4 ± 7.9	15.6	78.8 ± 5.6	0
15	96.9 ± 1.8	82.1 ± 5.1	14.7	84.1 ± 5.4	13.2
[^{3}H]Nucleotide label release					
15	3.8 ± 1.4	0.9 ± 0.5	76	n.d.	
30	23.3 ± 2.4	4.8 ± 1.1	79.4	6.1 ± 1.2	73.9
60	41.1 ± 1.6	10.1 ± 3.0	75.5	12.4 ± 0.3	70.0
^{51}Cr label release					
15	3.7 ± 0.8	1.8 ± 0.5	51.4	n.d.	
30	11.8 ± 0.7	5.5 ± 0.9	53.4	n.d.	
60	29.1 ± 2.4	13.9 ± 0.3	52.3	n.d.	

[a] Calculated as described previously [12].

[b] Percent inhibition as compared to maximal release in control Medium 199 (without osmotic stabilizer).

[c] Size of labels were $^{86}Rb^+$ (M_r approx. 100), [^{3}H]nucleotides (M_r less than 1000) and ^{51}Cr label (M_r approx. 3500). See text.

loss of [^{3}H]nucleotides, as previously reported [4], confirming that the inhibitory action of osmotic stabilizers against the enterotoxin-induced loss of larger molecules was not limited to sucrose. The effects of poly(ethylene glycol) on enterotoxin-induced ^{51}Cr label loss were not determined due to high variability in background ^{51}Cr label loss in 25% poly(ethylene glycol).

Importantly, Table I also demonstrates that sucrose and poly(ethylene glycol) had only limited protective effects against enterotoxin-induced $^{86}Rb^+$ loss. For example, after 15 min of treatment with 1500 plating efficiency units of enterotoxin, Medium 199 containing 0.3 M sucrose inhibited $^{86}Rb^+$ loss by only approx. 15%, although similar conditions showed that ^{51}Cr label and [^{3}H]nucleotide label loss were inhibited by 51 and 76%, respectively. Therefore, sucrose appears to differentially inhibit the enterotoxin-induced leakage of larger molecules with little protective effect on intracellular ion loss. This observation is consistent with an osmotic-disruption action for enterotoxin.

Since results presented in Fig. 1 indicate that enterotoxin induced an apparent rapid K^+ efflux,

TABLE II

EFFECTS OF ENTEROTOXIN ON $^{24}Na^+$ UPTAKE IN VERO CELLS

Uptake of $^{24}Na^+$ in Medium 199 or Medium 199 containing 0.3 M sucrose was determined after a 5 min incubation. 1250 plating efficiency units of enterotoxin were added to each culture simultaneously with $^{24}Na^+$ containing medium. Results are expressed as cpm/μg protein. Values are given as mean ± S.E. ($n = 8$). PEU, plating efficiency units.

Incubation medium	$^{24}Na^+$ Uptake (cpm/μg protein)
Medium 199	1.23 ± 0.11
+ 1250 PEU enterotoxin	2.37 ± 0.25
+ 0.3 M sucrose	1.33 ± 0.12
+ 0.3 M sucrose + 1250 PEU enterotoxin	2.15 ± 0.30

studies were made to determine whether enterotoxin also causes alterations in Na^+ influx, as would be expected [8,16] by an osmotically-active agent. Table II demonstrates that enterotoxin caused a 2-fold increase in $^{24}Na^+$ influx into treated Vero cells. Further, Table II also shows that addition of sucrose to Medium 199 was not significantly protective against enterotoxin-induced alterations in $^{24}Na^+$ influx.

To further investigate whether enterotoxin has a plasma membrane, as opposed to cytoplasmic, site of action [1–4], studies were performed to determine the effects of lysosomotropic agents on the action of enterotoxin. Using cell culture model systems, lysosomotropic agents have been shown [17–19] to significantly inhibit the action of diphtheria toxin by prevention of the acidification of endocytic vesicles. Table III demonstrates that lysosomotropic drugs are not protective against enterotoxin. Despite 60 min preincubation with chloroquine or NH_4Cl prior to enterotoxin addition, lysosomotropic agents were unable to prevent the enterotoxin-induced loss of [^{3}H]nucleotides. Similar results (not shown) were obtained with 30 min preincubation prior to enterotoxin addition. Additionally, lysosomotropic agents did not prevent formation of morphological alterations characteristic [2,4,5] of enterotoxin treatment of Vero cells.

TABLE III

STUDIES TO DETERMINE THE EFFECTS OF LYSOSOMOTROPIC AGENTS ON ENTEROTOXIN-INDUCED PERMEABILITY ALTERATIONS

The preincubation medium for all experiments was Medium 199 without serum. [^{3}H]Nucleotide-labeled cultures were preincubated for 60 min in preincubation medium containing lysosomotropic agents (as specified), washed and treated with 1500 plating efficiency units of enterotoxin dissolved in the appropriate preincubation medium. Enterotoxin-induced morphological alterations were observed after approx. 12–15 min. Values represent maximal enterotoxin-induced release of [^{3}H]nucleotides and are given as mean ± S.E. ($n = 4$).

Preincubation medium	Percent of maximal release
Medium 199	32.0 ± 2.2
+ 0.01 M chloroquine	34.4 ± 3.5
+ 0.1 M chloroquine	30.0 ± 0.8
+ 10 mM NH_4Cl	29.8 ± 3.9
+ 100 mM NH_4Cl	42.8 ± 2.4

Discussion

Results presented in this report strongly support a previous proposal [4] that the primary action of *C. perfringens* enterotoxin involves the disruption of the osmotic barrier of the cell. The kinetics of enterotoxin-induced $^{86}Rb^+$ versus larger molecule loss (in the presence or absence of osmotic stabilizers) are similar to previous studies with the osmotically active agents staphylococcal α toxin [20] and complement [16]. However, enterotoxin-induced permeability alterations are quite distinguishable from permeability alterations induced in erythrocytes by streptolysin O, a membrane-active bacterial toxin shown [14] not to possess an osmotic-disruption action.

This report also strongly suggests that enterotoxin treatment rapidly lowers intracellular K^+ levels. K^+ depletion may directly explain some of the previously noted [21] biological effects of *C. perfringens* enterotoxin, including the inhibitory effects of enterotoxin on DNA and protein synthesis [2]. K^+ depletion of mammalian cells has been shown [22,23] to cause inhibition of DNA and protein synthesis.

Besides enterotoxin-induced alterations in $^{86}Rb^+$ efflux, this report also demonstrates that *C. perfringens* enterotoxin rapidly increased $^{24}Na^+$ influx into Vero cells. Increased Na^+ influx could explain previous observations [7] describing a rapid enterotoxin-induced rise in intracellular Na^+ levels. In addition to K^+ and Na^+ transport alterations, there may be a Ca^{2+} involvement in the action of *C. perfringens* enterotoxin [5,24]. Further comparative ion flux studies are essential for clarification of the relationship between enterotoxin and membrane permeability alterations.

Fig. 3 clearly demonstrates that $^{86}Rb^+$ loss was detectable within 2.5 min of enterotoxin treatment. McDonel [25] and Tolleshaug et al. [26] have shown that enterotoxin binding occurs rapidly, with kinetics very similar to our studies of enterotoxin-induced $^{86}Rb^+$ loss. Taken collectively, these observations strongly suggest that the biological action of enterotoxin occurs simultaneoulsy with, or immediately following, binding. Studies

by Matsuda and Sugimoto [5], and McDonel and Demers [27], are most consistent with a two-step process: (1) binding, rapidly followed by (2) expression of biological activity.

Previous studies [1–6,26,28] have suggested that the site of action of *C. perfringens* enterotoxin is the plasma membrane. However, these studies might lack sufficient sensitivity and/or be subject to misinterpretation if only a small amount of intracellular enterotoxin was required to kill a cell. Importantly, a single intracellular fragment of diptheria toxin fragment A has been shown [29] to be lethal for a mammalian cell.

Table III shows that lysosomotropic agents did not neutralize *C. perfringens* enterotoxin-induced morphological or permeability alterations. These results indicate differences between *C. perfringens* enterotoxin versus diptheria toxin and *Clostridium difficile* cytotoxin. Both diptheria toxin and *C. difficile* cytotoxin are intracellularly active bacterial toxins whose biological activities are inhibited by lysosomotropic agents [17–19,29]. This inhibition is believed to be at least partially due to prevention of the acidification of endocytic vesicles during toxin internalization. While the lysosomotropic agent studies do not eliminate the possibility of *C. perfringens* enterotoxin internalization, these results are consistent with a plasma membrane action for this enterotoxin.

Lastly, the present study provides additional indirect support suggesting that the action of *C. perfringens* enterotoxin does not involve internalization by endocytosis. As discussed, enterotoxin biological activity occurs simultaneously with, or soon after, binding. Internalization of enterotoxin via endocytosis would be expected to take several minutes [19,30]. Further, Larkin et al. recently reported [32] that depletion of intracellular K^+ arrests receptor-mediated endocytosis in fibroblasts. Since enterotoxin induces a significant rapid K^+ (Rb^+) loss and requires specific receptors for expression of biological action [6], it could be expected that endocytosis in enterotoxin-treated Vero cells would be similarly arrested.

In summary, this study provides support for a previously proposed [4] model for the action of *C. perfringens* enterotoxin which suggested that this enterotoxin acts via a direct membrane interaction leading to rapid ion flux changes immediately following binding. The extent of these enterotoxin-induced ion flux changes are probably sufficient to directly impair some physiological processes while also causing further progressive membrane permeability alterations. With time, vital processes are shut down due to changes in levels of intracellular ions and precursors, resulting in cell death.

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References

1 McDonel, J.L., Chang, L.W., Pounds, J.G. and Duncan, D.L. (1978) Lab. Invest. 39, 210–218
2 McClane, B.A. and McDonel, J.L. (1979) J. Cell Physiol. 99, 191–200
3 McClane, B.A. and McDonel, J.L. (1980) Biochim. Biophys. Acta 600, 974–985
4 McClane, B.A. and McDonel, J.L. (1981) Biochim. Biophys. Acta 641, 401–109
5 Matsuda, M. and Sugimoto, N. (1979) Biochem. Biophys. Res. Commun. 91, 629–636
6 McDonel, J.L. and McClane, B.A. (1979) Biochem. Biophys. Res. Commun. 87, 497–504
7 Giger, O. and Pariza, M.W. (1980) Biochim. Biophys. Acta 595, 264–276
8 Cooper, L.A., Madoff, M.A. and Weinstein, L. (1964) J. Bacteriol. 87, 136–144
9 Granum, P.E. and Whitaker, J.R. (1980) Appl. Environ. Microbiol. 39, 1120–1122
10 McDonel, J.L. and McClane, B.A. (1981) J. Clin. Microbiol. 13, 940–946
11 Möllby, R., Nord, C.E. and Wadstrom, T. (1973) Toxicon 11, 139–147
12 Thelestam, M. and Möllby, R. (1975) Infect. Immun. 12, 225–232
13 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
14 Duncan, J.L. (1974) Infect. Immun. 9, 1022–1027
15 Rindler, M.J., McRoberts, J.A. and Saier, M.H. (1982) J. Biol. Chem. 257, 2254–2259
16 Green, H., Barrow, P. and Goldberg, B. (1959) J. Exp. Med. 110, 699–713
17 Draper, R.K. and Simon, M.I. (1980) J. Cell Biol. 87, 849–854
18 Sandvig, K. and Olsnes, S. (1980) J. Cell Biol. 87, 828–832
19 Marnell, M.H., Shia, S.P., Stookey, M. and Draper, R.K. (1984) Infect. Immun. 44, 145–150

20 Madoff, M.A., Cooper, L.Z. and Weinstein, L. (1964) J. Bacteriol. 87, 145–149
21 McDonel, J.L. (1980) Pharmac. Ther. 10, 617–655
22 Ledbetter, M.L.S. and Lubin, M. (1977) Exp. Cell Res. 105, 223–236
23 Lopez-Rivas, A., Adelberg, E.A. and Rosengurt, E. (1982) Proc. Natl. Acad. Sci. USA 79, 6275–6279
24 Dasgupta, B.R. and Pariza, M.W. (1982) Infect. Immun. 38, 592–597
25 McDonel, J.L. (1980) Biochemistry 21, 4801–4807
26 Tolleshaug, H., Skjelkvale, R. and Berg, T. (1982) Infect. Immun. 37, 486–491
27 McDonel, J.L. and Demers, G.W. (1982) J. Infect. Dis. 145, 490–494
28 Petrie, H.T., McDonel, J.L. and Schlegel, R.A. (1982) Cell Biol. Int. Rep. 6, 705–711
29 Florin, I. and Thelestam, M. (1983) Biochim. Biophys. Acta 763, 383–392
30 Yamaizumi, M., Makada, E., Uchida, T. and Okada, Y. (1978) Cell 15, 245–250
31 Brown, M.S., Anderson, R.G.W. and Goldstein, J.L. (1983) Cell 32, 663–667
32 Larkin, J.M., Brown, M.S., Goldstein, J.L. and Anderson, R.G.W. (1983) Cell 33, 273–285