Corticostatic peptides cause nifedipine-sensitive volume reduction in jejunal villus enterocytes

(permeability/Ca^{2+} channels/ω-conotoxin/osmolarity/defensins)

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ABSTRACT We studied cell-volume changes caused by adding corticostatin (CS) or defensin-like peptides to villus enterocytes isolated in suspension from guinea pig jejunum. Guinea pig CS (10^{-9} M) added to villus cells in Na^+-containing medium reduced volume, but immediate cell swelling was caused by 10^{-6} M guinea pig CS. In Na^+-free N-methyl-D-glucamine-containing medium 10^{-9} M guinea pig CS accelerated the initial rate of shrinkage compared with cells in N-methyl-D-glucamine-containing medium alone as well as causing greater cell shrinkage. Guinea pig CS-stimulated cell shrinkage was prevented by a Ca^{2+}-channel blocker—5 μM nifedipine, by chelation of extracellular Ca^{2+} with 100 μM EGTA, or by ω-conotoxin (10^{-9} M). The Ca^{2+} ionophore A23187 (2.5 μM) reduced volume when added to villus cells in N-methyl-D-glucamine-containing medium; this action was prevented by EGTA, or quinine—an inhibitor of K^+ conductance, or 9-anthracenecarboxylic acid—a Cl^- channel blocker, suggesting that the volume reduction occurred because K^+ and Cl^- conductances were activated. Guinea pig CS-stimulated volume reduction was also prevented by 100 μM quinine or 9-anthracenecarboxylic acid. We conclude that jejunal villus enterocytes possess a Ca^{2+}-activated Cl^- conductance and a K^+ conductance that need not be stretch-activated. Corticostatic peptides cause volume reduction in villus cells by activating L-type Ca^{2+} channels; other defensin-like peptides were without effect.

Corticostatins (CS) and defensins are a family of structurally related cationic peptides recently purified from phagocytic cells of rabbit, human, guinea pig, and rat origin (1–6, §). These 29–34 amino acid peptides contain a consensus distribution of six cysteine residues aligned in a highly conserved fashion (see Table 1). Members of the CS/defensin family are antimicrobial and thought to participate in the non-oxygen-dependent killing of phagocytosed bacteria (8). Other family members are corticostatic in that they inhibit the steroidogenic activity of corticotropin (ACTH), apparently by competing for binding of ACTH to its receptor (2, 9). Recently an abundant mRNA from mouse jejunum and ileum coding for a 6000-M_r peptide was predicted to contain a carboxyl-terminal sequence of 32 residues bearing the CS/defensin consensus of six cysteine residues (10). The mRNA encoding this peptide, termed cryptidin, was localized to the lower crypt epithelium of adult mouse jejunum (11). Localization of Cl^- secretion to the crypt epithelia of the small intestine (12) prompted us to assess the effect of CS and defensin peptides on ion transport in villus enterocytes.

Intestinal epithelial cells use ion transport, with Na^+, K^+, and Cl^- fluxes predominating, both to maintain and to regulate their volume (13, 14). Changes in cell volume therefore indicate changes in cation and anion transport pathways. Homogeneous populations of viable mature villus enterocytes can be isolated in suspension (15, 16), and their volume can be accurately determined by using an electronic cell-sizing technique (14). We used this technique to measure volume changes that occur after the addition of CS or other defensin peptides to villus cells in suspension and report a biological activity that is restricted to CS peptides.

MATERIALS AND METHODS

Villus-Cell Isolation. Described in detail elsewhere (14), our procedure involved placing segments of adult guinea pig jejunum over metal spiral rods and vibrating them in Ca^{2+}-free phosphate-buffered physiologically balanced salt solution. Isolated cells were collected by centrifugation at 50 × g for 5 min and resuspended at 0.8–1.5 × 10^6 cells per ml in RPM1 1640 medium (without HCO_3^-) containing bovine serum albumin at 1 mg/ml and 20 mM NaHepes, pH 7.3, at 37°C. Viability, assessed by trypan blue exclusion, was 85%, 3 hr after suspension in medium. After cell separation, the remaining jejunal tissue consisted of intact villus cores and crypt epithelia but no remaining villus enterocytes.

Electronic Cell Sizing. Cell volume was measured by using a Coulter Counter (model ZM) with a Coulter Channelyzer (c-256) as described (14). Cell volume electronically determined over a range of tonicities correlated positively ($r = 0.967$) with direct measurements of cell water (14). Relative cell volume was determined as the ratio of cell volume under study conditions to the volume under basal conditions in an isotonic medium immediately before challenge. Initial rates of volume reduction were calculated by comparing relative cell volumes at 30 s, 2 min, and 3 min and expressing the result as % per min.

Purification of Human, Rat, and Guinea Pig Corticostatins and Related Peptides. Human CS (HP-4) and the structurally related defensin peptide HP-1 were purified from extracts of human peritoneal exudate exactly as described (4). Members of the rat CS/defensin family were purified by reversed-phase HPLC from extracts of bone marrow obtained from rat femurs[§]. Five rat peptides were identified and sequenced. Some of these peptides have also been purified and characterized by others from extracts of rat peritoneal neutrophils (6). Full details of the purification and characterization of these five rat peptides will be reported elsewhere (D.B.,

Abbreviations: CS, corticostatin; RVD, regulatory volume decrease; NMDG, N-methyl-D-glucamine; 9-AC, 9-anthracenecarboxylic acid; ACTH, corticotropin.
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[§]Belcourt, D., Bateman, A., Singh, A., Lazure, C., Bennett, H. P. J. & Solomon, S., 72nd Annual Meeting of the Endocrine Society, June 20–23, 1990, Atlanta, GA, p. 276 (abstr.).

A.B., A. Singh, C. Lazure, S.S., and H.P.J.B., unpublished work). A single representative of the CS/defensin family was purified from marrow of guinea pig femurs by using reversed-phase HPLC techniques. The sequence was determined and found identical to that previously reported for guinea pig defensin (GPNP-1) purified from peritoneal neutrophils (5). Details of the purification of guinea pig CS will be reported elsewhere (J.H., D.B., A.B., C. Lazure, H.P.J.B., and S.S., unpublished work).

For assessing volume changes, guinea pig CS was used, and in the text CS generally refers to guinea pig CS. The amino acid sequences of members of the corticostatin/defensin family used in this study appear in Table 1. Corticostatic activity of the various CSs was determined exactly as described (2). Concentrations of CS peptide necessary to inhibit steroidogenesis by 50% in isolated rat adrenal cells in response to ACTH at 150 pg/ml were as follows: synthetic rabbit, 25 nM; guinea pig, 400 nM; rat, 50 nM; and human, 700 nM.

Solutions and Pharmacological Agents. For cell-volume experiments the buffer contained 140 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM D-glucose, and 10 mM Hepes (pH 7.3). Na^+-free medium was prepared by replacing NaCl isotonically with N-methyl-D-glucamine ($NMDG^+$). Hepes-buffered medium was 295 mOsM/kg. 9-Anthracene-carboxylic acid (9-AC) (100 mM) was prepared in dimethyl sulfoxide. Corticostatic and defensin peptides were solubilized in 0.1 M HCl containing defatted bovine serum albumin at 50 μg/ml and were added to cells from 10 μM stocks. Nifedipine and ω-conotoxin were added to cells from stock solutions (10 mM and 10 μM, respectively). Synthetic rabbit CS was a gift from American Peptide, Santa Clara, CA. Quinine and nifedipine were purchased from Sigma; N-methyl-D-glucamine and 9-AC were from Aldrich; ω-conotoxin was from Calbiochem; RPMI 1640 medium was purchased from GIBCO.

Statistics. Data are reported as means ± SEM of four to eight experiments done in duplicate. Differences in means were determined by using Student's t test (paired or unpaired as appropriate). In studies where multiple means were compared, analysis of variance (ANOVA) was used for groups of unequal sizes.

RESULTS

Effect of Corticostatin on Villus-Cell Volume in Na^+ or Na^+-Free Medium. Ehrlich ascites cells (17) and villus enterocytes (18) when hypotonically swollen subsequently shrink by exercising a regulatory volume decrease (RVD). This cell shrinkage in hypotonic medium is due to the stimulation of separate Ca^{2+}-activated K^+ and Cl^- conductances (18, 19). Suspensions of either cell type in Na^+-free medium hyperpolarizes the membrane. Membrane hyperpolarization allows the ionophore gramicidin to accelerate RVD (17, 18) by enhancing the K^+ efflux rate in addition to increasing the driving force for Cl^- exit. Consequently, it is possible to determine the activation of different ion conductances that subsequently change volume, depending on rate and extent of the volume changes in Na^+-containing com-

pared with Na^+-free medium. For example, a hormonal or physiological challenge that stimulated the loss of both a cation and Cl^- from a villus cell should cause a slower and smaller volume change in Na^+-containing medium compared with that in Na^+-free medium because the latter optimized Cl^- efflux. Therefore, we compared the effect of corticostatic or defensin peptides on villus-cell volume in Na^+-containing and Na^+-free mediums. The mean basal volume of guinea pig villus enterocytes suspended in isotonic Na^+-containing medium was 1390 ± 190 fl ($n = 75$). Within 30 s of CS addition to a final concentration of 10^{-9} M, cells had begun to shrink and by 5 min had shrunk to a relative volume (0.95 ± 0.01) that did not change for the remainder of the experiment (Fig. 1A). The addition of CS to 10^{-11} M had no effect on cell volume. Addition of 10^{-6} M CS caused cell swelling within 10 min; the relative volume of cells was 1.05 ± 0.02. After 20-min incubation, the mean relative volume of cells in 10^{-6} M CS was greater than those in 10^{-11} M CS (1.05 ± 0.02 vs. 0.99 ± 0.01, $P < 0.05$); the final relative volume of cells was smaller after 20 min in 10^{-9} M CS compared with 10^{-11} M CS (0.94 ± 0.01 vs. 0.99 ± 0.01, $P < 0.05$).

The effect of 10^{-9} M CS on isosmotic villus-cell volume was determined in medium where Na^+ was isotonically replaced by $NMDG^+$ medium. When suspended in $NMDG^+$ medium, the villus cells shrank (Fig. 1B) and within 5 min reached a relative volume (0.91 ± 0.01) that did not change for the rest of the experiment. Such a volume change would be expected from Na^+-absorbing epithelia since Na^+ influx has been prevented, while Na^+ efflux together with some anion leak proceeds. In 10^{-9} M CS the initial rate of shrinkage was significantly greater than in its absence (8.5 ± 2.4% per cell per min vs. 3.3 ± 0.3% per cell per min, $P < 0.05$), and the final relative volume of cells at 20 min was less than that of cells in $NMDG^+$ medium alone (0.81 ± 0.01 vs. 0.90 ± 0.01, $P < 0.001$). In 10^{-9} M CS the initial rate of shrinkage in $NMDG^+$ medium was faster compared with Na^+ medium (8.5 ± 2.4% per cell per min vs. 2.0 ± 0.4% cell per min, $P < 0.001$): the final relative volume of cells was less in $NMDG^+$ medium compared with Na^+ medium (0.81 ± 0.01 vs. 0.94 ± 0.01, $P < 0.001$). We also determined the effect of the Ca^{2+} channel blocker nifedipine on CS-stimulated shrinkage (Fig. 1B). Nifedipine alone had no effect on cell volume (data not shown). Nifedipine (5 μM) was added simultaneously to cells with 10^{-9} M CS. There was no difference in the rate of volume change or the final relative volumes of cells in 5 μM nifedipine/10^{-9} M CS compared with cells in $NMDG^+$ medium without additions. The final relative volume of cells exposed to nifedipine/CS was larger than that of cells treated with CS alone (0.90 ± 0.01 vs. 0.81 ± 0.01, $P < 0.001$).

CS-Stimulated Shrinkage Is ω-Conotoxin Sensitive. The requirement of extracellular Ca^{2+} for CS-stimulated volume reduction was confirmed with data from additional experiments (Fig. 2). When 10^{-9} M CS was added to cells in nominally Ca^{2+}-free $NMDG^+$ medium containing EGTA (100 μM), CS-stimulated volume reduction was prevented. Final relative volume of cells in EGTA/CS was greater compared with CS alone (0.90 ± 0.01 vs. 0.81 ± 0.01, $P < 0.001$). Addition of a potent Ca^{2+} channel blocker, ω-conotoxin (10^{-9} M), also prevented CS-stimulated shrinkage. Cells that were challenged with CS (10^{-9} M) were smaller within 2 min compared with ω-conotoxin/CS (relative volume 0.88 ± 0.01 vs. 0.94 ± 0.01, $P < 0.001$). Final relative volume of cells challenged with CS/ω-conotoxin was greater compared with cells receiving CS alone (0.92 ± 0.01 vs. 0.81 ± 0.01, $P < 0.001$). ω-Conotoxin (10^{-9} M) itself had no effect on cell volume during the experiment (data not shown).

Structure–Activity Relationships. We determined whether synthetic rabbit CS or CS isolated from other species affected cell volume and compared this response with other defensin-like peptides isolated from the same species (Table 2).

Table 1. Amino acid sequences of rabbit, rat, human, and guinea pig CSs and related peptides

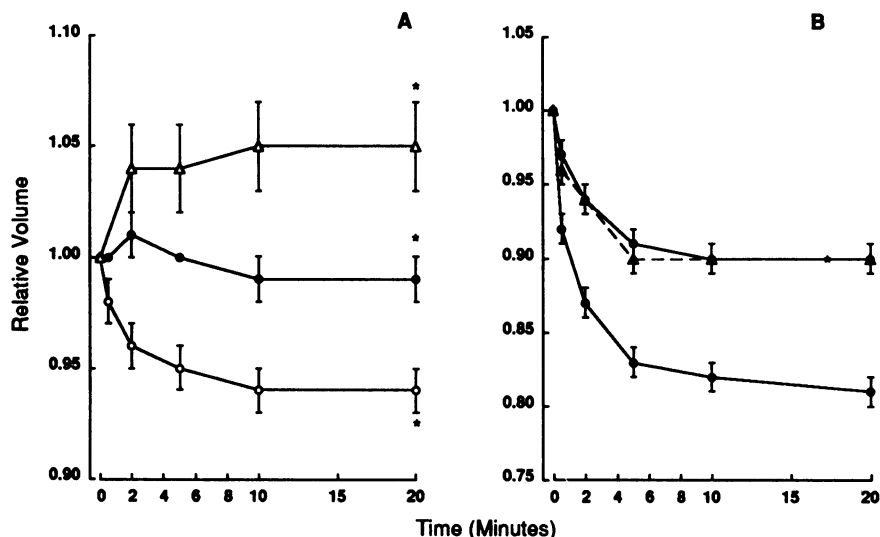| Rabbit	CS-1	GICACRRRFCPNSERFSGYCRVNGARYVRCCSRR
Rat	R-2	VTCSCRTSSCRFGERLSGACRLNGRIYRLCC
Rat	R-4	VTCYCRRTRCGFRERLSGACGYRGRIYRLCCRR
Human	HP-1	ACYCRIPACIAGERRYGTCIYQGRLWAFCC
Human	HP-4	VCSCRLVFCRRTELRVGNCLIGGVSFTYCCTRV
Guinea pig	CS	RRCICTTRTCRFPYRRLGTCIFQNRVYTFCC

Single-letter system is used.

Fig. 1. Effect of guinea pig CS on villus enterocyte volume in Na^+-containing medium (A) or Na^+-free ($NMDG^+$) medium (B). (A) ○, 10^{-9} M CS, n = 5; ●, 10^{-11} M CS, n = 4; △, 10^{-6} M CS, n = 4; *, $P < 0.05$. (B) ●, 10^{-9} M CS, n = 6; ▲, 5 μM nifedipine/10^{-9} M CS, n = 5; ○, $NMDG^+$, no additions, n = 6; *, $P < 0.001$. Volume measured electronically is expressed relative to isosmotic control.

Synthetic rabbit CS caused greater shrinkage ($P < 0.001$) compared with control ($NMDG^+$ medium). Human CS (HP-4) caused more shrinkage ($P < 0.001$) in comparison with a human defensin (HP-1). Rat CS (R-4) caused more shrinkage ($P < 0.02$) compared with a rat defensin (R-2). No differences in cell shrinkage occurred with guinea pig CS (Figs. 1B and 2), synthetic rabbit CS, human CS, or rat CS. Volume reductions from synthetic rabbit CS, human CS, and rat CS were each prevented by 5 μM nifedipine (data not

Fig. 2. Effect of extracellular Ca^{2+} chelation or ω-conotoxin on CS-stimulated volume reduction in Na^+-free ($NMDG^+$) medium. ●, 10^{-9} M CS, n = 7; ○, Ca^{2+}-free medium with 100 μM EGTA/10^{-9} M CS, n = 6; ▲, 10^{-9} M ω-conotoxin/10^{-9} M CS, n = 6. *, $P < 0.001$. Volume measured electronically is expressed relative to isosmotic control.

shown). ACTH (10^{-9} M) had no effect on the amount of cell shrinkage seen when cells were suspended in $NMDG^+$ medium. Neither HP-1 nor R-2, the human and rat defensins, altered cell volume when compared with controls.

Effect of Ca^{2+} Ionophore on Villus-Cell Volume Under Isosmotic Conditions. The ω-conotoxin and nifedipine sensitivity of CS-stimulated shrinkage in the absence of Na^+ suggested that when extracellular Ca^{2+} was mobilized intracellularly, it activated ionic conductances that shrunk cells. To determine which conductances were activated when intracellular Ca^{2+} increased from Ca^{2+} channel opening, we examined the effect of calcium ionophore A23187 on villus-cell volume in $NMDG^+$ medium (Fig. 3A). Addition of A23187 alone (2.5 μM) accelerated the initial rate of shrinkage compared with A23187 added to cells in nominally Ca^{2+}-free medium/100 μM EGTA (7.6 ± 0.8% per min per cell vs. 3.6 ± 0.6% per min per cell, $P < 0.001$). Final relative volume of cells in EGTA/A23187 was greater compared with cells in Ca^{2+}-containing medium/A23187 (0.90 ± 0.01 vs. 0.78 ± 0.01, $P < 0.001$). Because cell shrinkage is due to salt loss with osmotically obliged water (17, 18), we determined the effect of an inhibitor of Cl^- conductance, 9-AC, on the A23187-induced shrinkage. 9-AC (300 μM) added to cells together with A23187 attenuated shrinkage. Final relative volume of cells in 9-AC/A23187 was greater compared with A23187 alone (0.88 ± 0.02 vs. 0.78 ± 0.01, $P < 0.01$).

Fig. 3B shows that K^+ conductance had been activated by A23187. The rationale for this experiment was that the inhibition of volume changes by drugs specific for K^+ conductance may be bypassed with the ionophore gramicidin (20,
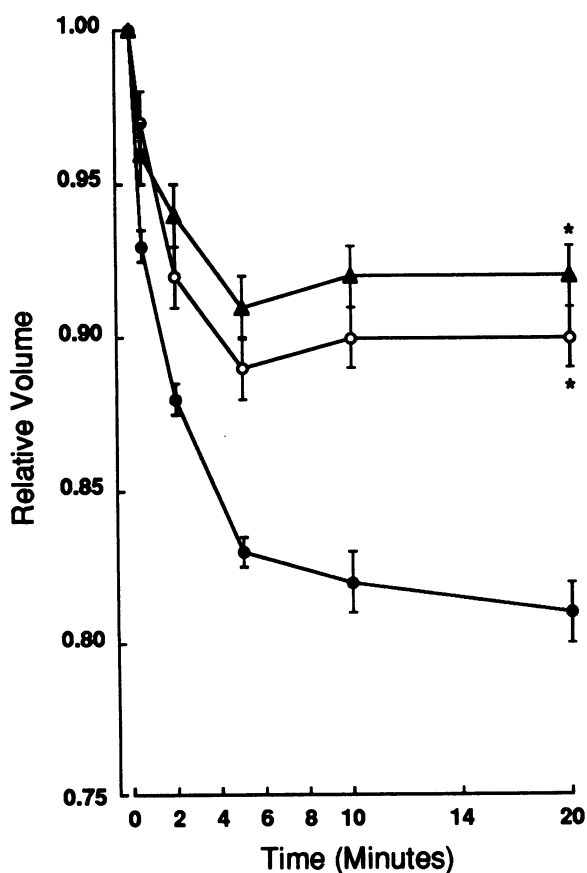
Table 2. Effect of members of the CS/defensin family of peptides on villus-cell volume

Addition	Cell shrinkage at 20 min, %	P
Control ($NMDG^+$; n = 9)	10.4 ± 0.9	
Rabbit CS (10^{-9} M; n = 6)	17.8 ± 1.3	<0.01
HP-1 (10^{-9} M; n = 5)	11.4 ± 0.6	NS*
HP-4 (10^{-9} M; n = 6)	18.3 ± 1.2	<0.01†
R-2 (10^{-9} M; n = 6)	12.3 ± 1.9	NS*
R-4 (10^{-9} M; n = 6)	18.7 ± 1.3	<0.05‡
ACTH (10^{-9} M; n = 5)	10.0 ± 0.3	NS

Peptides were added to cells in Na^+-free medium. Volume was assessed electronically and expressed relative to isotonic control. Analysis of variances (ANOVA) was used to compare groups of unequal sizes. NS, not significant.
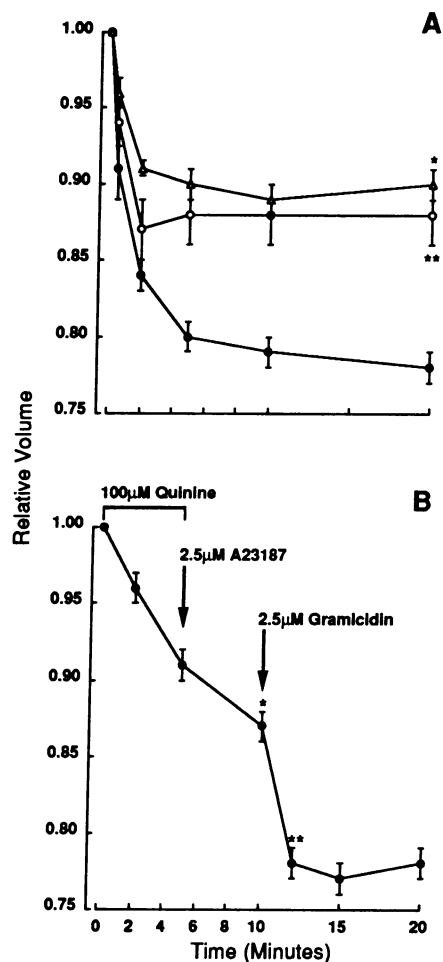*Compared with control.
†Compared with HP-1.
‡Compared with R-2.

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FIG. 3. Effect of Ca^{2+}-ionophore A23187 on villus enterocyte volume in Na^+-free ($NMDG^+$) medium. (*A*) ●, 2.5 μM A23187, n = 8; ○, 300 μM 9-AC/2.5 μM A23187, n = 7; △, Ca^{2+}-free medium with 100 μM EGTA/2.5 μM A23187, n = 7. *, P < 0.001; **, P < 0.01. (*B*) Gramicidin bypass of quinine inhibition of A23187-stimulated shrinkage. n = 8. *, Not different from 5-min relative volume. **, P < 0.001. Volume measured electronically is expressed relative to isosmotic control.

21). An inhibitor of Ca^{2+}-activated K^+ conductance, quinine (100 μM), was added to villus cells suspended in $NMDG^+$ medium. The cells lost some volume, but the loss was consistent with the volume reduction that occurred after these cells were suspended in $NMDG^+$ medium (Fig. 1*B*). A23187 was added, and no significant volume reduction occurred over 5 min (relative volume 0.87 ± 0.01 vs. 0.91 ± 0.01, not significant). At 10 min, 5 μM gramicidin was added, and 2 min later relative volume of the cells was less compared with the volume before gramicidin (0.78 ± 0.01 vs. 0.87 ± 0.01, P < 0.001). These data are consistent with the interpretation that quinine has inhibited a K^+ conductance activated by A23187 because gramicidin allowed volume changes to proceed. Together, the data in Fig. 3 suggest that when extracellular Ca^{2+} is mobilized, K^+ and Cl^- conductances are activated, which reduce volume.

In additional experiments we assessed the effect of the K^+ conductance inhibitor quinine and the Cl^- conductance inhibitor 9-AC on the CS-stimulated shrinkage in Na^+-free medium. Suspension in Na^+-free medium reduced volume (amount of cell shrinkage at 20 min) by 10.2 ± 1.5%. Alone, CS (10^{-9} M) reduced volume 19.7 ± 1.5%. With quinine (100 μM) CS shrinkage was inhibited (13.0 ± 0.6 vs. 19.7 ± 1.5%, P < 0.02, n = 4). Volume reduction stimulated by CS was also inhibited by 300 μM 9-AC (10.7 ± 1.8% vs. 19.7 ± 1.5%, P

< 0.02, n = 4). These results suggest that CS causes cell shrinkage by first activating a Ca^{2+} channel; the putative increase in intracellular Ca^{2+} subsequently activated K^+ and Cl^- conductances. Loss of these ions together with osmotically obliged water then reduced volume.

DISCUSSION

CS at a concentration 5-fold less than the minimum effective concentration of CS found to inhibit ACTH-stimulated corticosterone production from rat adrenal cells (2) caused guinea pig jejunal villus cells to shrink under isosmotic conditions. The volume response of cells to CS addition in Na^+-containing medium was dose-responsive. The highest concentration tested, 10^{-6} M, generated cell swelling consistent with ion permeabilization seen when the cation ionophore gramicidin was added to hypotonically swollen lymphocytes (20) or when ion efflux was prevented, but ion influx continued by ouabain addition to villus cells under isotonic conditions (14). Antimicrobial defensin peptides can induce ion-permeable channels in lipid bilayers at 10^{-5} M (22) in accord with an interpretation of our data that comparable concentrations of CS permeabilize enterocyte membranes to ions.

Our experiments in Na^+-free medium showed that CS (10^{-9} M) activated a Ca^{2+} channel because the volume reduction stimulated by CS was prevented in Ca^{2+}-free medium and by two different classes of Ca^{2+} channel blockers, the dihydropyridine nifedipine (23) and ω-conotoxin (24), a peptide isolated from gastropod venom. Voltage-gated L-type Ca^{2+} channels are inhibited by these blockers (25). Hyperpolarizing the membrane potential by suspending cells in Na^+-free medium provided conditions that allow gramicidin to accelerate RVD in hypotonically swollen Ehrlich ascites cells (17) and villus enterocytes (18), which in both cell types was due to stimulation of Ca^{2+}-activated K^+ and Cl^- conductances (19). Therefore, we speculated that if a physiological stimulus worked through Ca^{2+}—either by mobilizing extracellular Ca^{2+} or liberating intracellular Ca^{2+}— providing the stimulus to cells in Na^+-free medium should cause accelerated rate of shrinkage and greater cell shrinkage (from K^+ and Cl^- efflux) compared with the same stimulus to cells in Na^+-containing medium. We found that CS addition to cells under hyperpolarizing conditions both accelerated the rate of shrinkage and caused greater cell shrinkage compared with CS added to cells in Na^+ medium. It is difficult to reconcile the sensitivity of the CS effect on cell volume to the two different classes of Ca^{2+} channel blockers if CS were forming Ca^{2+} channels itself. Indeed, as the channel-forming activity of other cysteine-rich defensin peptides requires 10^3-10^4 more protein (22), that CS was selectively permeabilizing the enterocyte to Ca^{2+} was unlikely. Together these results suggest that the pivotal event for the CS effect on villus-cell volume is activation of a voltage-gated L-type Ca^{2+} channel; subsequent to Ca^{2+} influx, ion conductances are activated, allowing volume to be reduced. Although we recognize that nifedipine inhibition is not pathognomonic for L-type Ca^{2+} channels, the best interpretation of the data favors this conclusion.

Addition of Ca^{2+} ionophore A23187 to the villus cells in Na^+-free medium caused a rapid volume reduction that could be prevented by either extracellular Ca^{2+} chelation or 9-AC, an inhibitor of Cl^- conductance. The 9-anthracene derivative inhibits (*i*) Cl^- secretion from the thick ascending limb of the loop of Henle in rabbit (26) and rabbit colonic crypts (27) and (*ii*) cAMP-stimulated ^{36}Cl efflux and hypotonic RVD in guinea pig villus enterocytes (18, 28). The anthracene sensitivity of the Ca^{2+} ionophore-stimulated volume reduction is consistent with activation of a Cl^- conductance. Recently, Ca^{2+} ionophore-stimulated Cl^- conductance was described

in T-84 cells, which is physically distinct from the cAMP-activated Cl^- conductance in the same cells (29). Our data suggest that villus enterocytes also have a Ca^{2+}-activated Cl^- conductance in addition to cAMP-sensitive and volume-activated Cl^- conductances (18, 28).

The gramicidin bypass experiment of the quinine inhibition of Ca^{2+} ionophore-stimulated volume reduction suggests that a K^+ conductance was stimulated by the Ca^{2+} ionophore because the volume reduction proceeded after gramicidin addition. If quinine had been inhibiting Cl^- conductance or KCl cotransport, gramicidin, by providing addition K^+ conductance, would not have further reduced volume. Comparable results have been described by using Ehrlich ascites cells (17, 19). Our results with Ca^{2+} ionophore are consistent with villus-cell volume reduction being due to activation by Ca^{2+} of both K^+ and Cl^- conductances. That the volume reduction occurred under isosmotic conditions suggests the K^+ conductance is not stretch activated, unlike other epithelia (30, 31). Neither quinine nor 9-AC affects villus-cell basal volume. The sensitivity of the CS-stimulated volume reduction to both quinine and 9-AC is consistent with activation of K^+ and Cl^- conductances, comparable with the Ca^{2+} ionophore-stimulated volume reduction. Both the ionophore and CS cause influx of extracellular Ca^{2+} that activates ion efflux in these cells.

Although rabbit CS (CS-1 in Table 1) has been reported to have the most potent corticostatic activity (2, 4, 9, §), there was no difference between the extracellular Ca^{2+}-requiring volume reduction elicited by rabbit, rat, guinea pig, or human CSs. In spite of considerable variability in the corticostatic potency of these peptides, clearly all were equally effective in activating an L-type Ca^{2+} channel in villus enterocytes. However, defensin-like peptides tested that lacked corticostatic activity (2, 4, 9, §) had no effect on villus-cell volume. Consequently, another biological activity of corticostatic peptides is their ability to activate nifedipine-sensitive L-type Ca^{2+} channels. It is not known whether ACTH-responsive adrenal cells exercise volume reduction that is nifedipine sensitive when challenged with corticostatic peptides. Such a response would implicate electrolyte flux in steroid secretion, comparable to recent examples of peptide exocytosis that is both Ca^{2+} dependent and osmotically sensitive (7). The small intestine is not steroidogenic, and ACTH had no effect on the volume of villus enterocytes. We speculate that in villus enterocytes CS peptides recognized either a site on a Ca^{2+} channel or an unidentified receptor that is coupled to a Ca^{2+} channel. Binding to this site could then activate the channel.

It is apparent from our data that future research should be addressed to understanding how corticostatic peptides inhibit ACTH action while also activating Ca^{2+} channels that might increase adrenal activity.

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