

Cl⁻ Secretion in a Model Intestinal Epithelium Induced by a Neutrophil-derived Secretagogue

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Abstract

A secreted product of activated neutrophils, NDS (neutrophil-derived secretagogue), elicits a short circuit current (Isc) in epithelial monolayers derived from the human intestinal cell line T84 (*J. Clin. Invest.* 1991. 87:1474–1477). Here, we identify and characterize the source of this Isc and examine associated signaling pathways. ¹²⁵I efflux studies suggested that NDS activates an anion conductive channel. Bidirectional ²²Na ³⁶Cl flux studies showed that electrogenic Cl⁻ secretion fully accounts for the NDS-induced Isc response. NDS behaved in many respects as a cAMP-mediated secretagogue: NDS did not further increase maximal cAMP-induced Cl⁻ secretion; NDS potentiated Ca²⁺-mediated Cl secretion; and NDS elicited measurable ¹²⁵I but not ⁸⁶Rb effluxes. However, NDS did not elicit a detectable rise in intracellular cAMP. Such data suggest that NDS may elicit Cl⁻ secretion by effecting distal events in the cAMP-mediated pathway. Data derived from cell volume assays of isolated guinea pig intestinal crypt cells indicated that NDS also directly elicits Cl⁻ secretion from natural intestinal epithelia. Additionally, since NDS activity is released from PMN by stimuli normally present in the colonic lumen, since NDS is active when applied apically to this model intestinal epithelium, and since the NDS-elicited Isc response is indicative of electrogenic chloride secretion, we speculate NDS may contribute to the secretory diarrhea encountered in many patients with inflammatory intestinal disease. (*J. Clin. Invest.* 1992. 89:1938–1944.) Key words: inflammation • intestinal disease • second messengers

Introduction

Many diseases of the human intestine are, in the active phase, histologically characterized by infiltration of the crypt epithelium by PMN (1–3). PMN subsequently collect in the crypt lumen to form “crypt abscesses.” Since the colonic lumen normally contains bacterial derived products such as endotoxin (4) and *N*-formylated peptides (5) in concentrations known to trigger both PMN respiratory burst activity (6) and degranulation

(7), the PMN that collect in crypt lumens are likely to be activated. As a result of such activation, PMN produce a plethora of products ranging from oxidants to proteases (7). A few of the PMN-derived products, in isolated purified form, have been shown to affect transport characteristics of native or model intestinal epithelia (8, 9). However, it is less clear which of these products might be of potential clinical significance, since concentrations of these products in crypt abscesses are unknown, and doses required to obtain a secretory response may not be physiological. Furthermore, PMN products often display interactive effects which would not be predicted by the effects of the individual products.

Recently, we modeled the intestinal crypt abscess (10) by placing purified human PMN over T84 monolayers, a model human intestinal cell line with crypt-like phenotype (11) and function (12, 13). When PMN and epithelial cells were combined, using cell ratios known to occur in crypt abscesses, no substantive effects were noted on epithelial function. In contrast, when PMN were subsequently exposed to activating conditions similar to those naturally present in the lumen of the human colon, T84 cells responded with electrogenic ion transport, as indicated by stimulation of a short-circuit current (Isc).¹ This PMN-elicited Isc presumably represents Cl⁻ secretion, the transport event that serves as the basis for secretory diarrhea (14). This novel neutrophil-derived secretagogue (NDS) activity did not require PMN-epithelial contact, and was present in the cell-free supernatant derived from activated PMN (10). NDS appeared to be hydrophilic, acid, and heat stable, and, by filtration, exhibited an approximate molecular weight of 500 or less. Inhibitor and/or extraction studies suggested NDS activity was not attributable to “likely” PMN-derived candidate products such as those of the respiratory burst, proteases, or protease products, or arachidonic acid metabolites. Lastly, the Isc response to NDS exhibited a polarity opposite to that reported by others for potential small mediators such as adenosine (15) and monochloramine (9) which, in purified form, preferentially elicit an Isc response in T84 cells when applied basolaterally. NDS elicited a Isc response when applied apically, which would be the polarity correct for a PMN effect within a crypt abscess.

Here we report that the NDS-elicited Isc response does not coincide with a detectable rise in Ca²⁺, cAMP, or cGMP, the known major signaling pathways involved in electrogenic Cl⁻ secretion triggered by diverse agents such as cholera toxin, *Esch-*

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1. Abbreviations used in this paper: DNDS, 4, 4'-dinitrostilbene-2, 2'-disulfonic acid; IBMX, 3-isobutyl-1-methyl xanthine; Isc, short-circuit current; NDS, neutrophil-derived secretagogue; PMA, phorbol myristate acetate.

erichia coli heat stable toxin, vasoactive intestinal peptide, carbachol (14), and others including monochloramine (9). However, we show that the NDS-elicited Isc response indeed represents a Cl^- secretory response. The Cl^- secretory response elicited by NDS is cAMP-like, as shown by its effects on anion, but not Rb^+ efflux, and by its potentiating interaction with the Ca^{2+} -mediated Cl^- secretory pathway. Furthermore, we show that NDS added to guinea pig crypt epithelial cells caused volume changes prevented by a Cl^- channel blocker, consistent with NDS activity not being restricted to intestinal cell lines. Such data suggest that NDS may elicit Cl^- secretion in T84 cells either by stimulating a small undetectable and localized cAMP pool or by acting at a point in the signaling cascade distal to cAMP. Such requirements may be the basis for the unique apical polarity by which NDS exerts its Cl^- secretagogue activity.

Methods

Approximately 400 monolayers were used for these studies. Confluent monolayers of the human intestinal epithelial cell line T84 were grown on collagen-coated permeable supports and maintained until steady-state resistance was achieved, as we have previously described (11). The majority of monolayers were like those for which we have developed a microassay (16). Measurements of transepithelial resistance, voltage, and short-circuit current were as previously described (11, 16, 17), and were carried out after monolayers were washed in HBSS. HBSS was used in both reservoirs. Bidirectional flux studies were conducted in modified Ussing chambers, as previously detailed (11, 18). ^{125}I and ^{86}Rb efflux studies were performed, and rate constants for efflux obtained exactly as described by Venglarik et al., using T84 cells grown on 35-mm plastic petri dishes (19). These investigators have previously shown that ^{125}I and ^{86}Rb can reliably replace ^{36}Cl and ^{42}K for measurement of efflux through Cl^- or K^+ channels in T84 cells.

PMN collected by venipuncture from normal adult human volunteers were isolated (Brigham and Women's Hospital Human Subjects Committee protocol 89-1465) as previously described (20). NDS-conditioned HBSS was obtained by stimulating PMN at a density of 10^7 PMN/ml, in one of two ways: (a) with $0.1 \mu\text{g/ml}$ phorbol myristate acetate (PMA) for 20–40 min at 37°C ; or with (b) PMN coexposed to endotoxin (LPS, $0.1 \mu\text{g/ml}$) and *N*-FMLP, 10^{-6} M for 20–40 min at 37°C . NDS-conditioned HBSS was passed through a 1,000 nominal molecular weight Amicon filter, by pressure under N_2 , to partially purify NDS activity (10).

Measurements of cAMP and cGMP were performed on ethanol extracts of cells obtained from monolayers grown on permeable supports, using radioimmunoassay kits as directed by the supplier (New England Nuclear, Boston, MA). Briefly, cell-associated [cAMP] and [cGMP] were determined on ethanol extracts of monolayers incubated at 37°C with NDS-conditioned HBSS for 10 min ($n = 8$), 119 nM cholera toxin for 75 min ($n = 6$), 200 nM *E. coli* stable toxin for 10 min ($n = 8$), or HBSS alone for 10 or 75 min ($n = 8$). All extractions were carried out at 4°C and timed to coincide with peak Isc activities, as determined by electrical assays. The time course of cholera toxin-induced [cAMP] closely correlated with the time course of cholera toxin-induced Isc. In subsets of experiments, the phosphodiesterase inhibitor 3-isobutyl-1-methyl xanthine (IBMX, 1 mM) was included in a preexperimental 15-min wash (37°C), and also added with the cAMP agonist forskolin (10^{-6} M) or NDS. After 8 min, ethanol extracts were obtained as above for cAMP. Cytosolic-free calcium was measured by a Fura-2 fluorescence assay of cells on permeable supports, as detailed by Wasserman et al. (21).

Crypt epithelial cells from the jejunum of adult (200–300 g) male guinea pigs were isolated by sequential collection of a hyperosmolar, Ca^{2+} -free perfusate (22). These cells were enriched $12\times$ ($P < 0.001$) in

thymidine kinase, and had diminished alkaline phosphatase activity ($P < 0.001$) and homogenous but reduced cell size (328 ± 15 fl, $P < 0.001$), in comparison with comparably isolated villus cells (23). In HCO_3^- -buffered RPMI 1640 medium at a density of $1-2 \times 10^6$ cells/ml, $99.1 \pm 0.1\%$ ($n = 9$) of the cells excluded trypan blue 5 h after suspension. Cell volume was measured using a counter and a channelyzer (model Zm and model C-256, respectively; both are from Coulter Electronics, Hialeah, FL), as previously described (24). NDS was prepared as described (10), and added in 10^{-1} dilution to crypt cells suspended in Na^+ -medium containing (mM) 140 NaCl, 3 KCl, 1 MgCl_2 , 10 D-glucose, and 10 Hepes (pH 7.3).

All tissue culture supplies were obtained from Gibco Laboratories (St. Lawrence, MA) and isotopes and radioimmunoassay kits from New England Nuclear. Fura 2/AM was obtained from Molecular Probes, Inc. (Eugene, OR). 4,4'-dinitrostilbene-2,2-disulfonic acid was obtained from Pfaltz and Bauer, Inc. (Waterbury, CT). Other reagents were obtained from Sigma Chemical Co. (St. Louis, MO) and Calbiochem Corp. (San Diego, CA).

Results

NDS-induced Isc. As shown in Fig. 1, apical exposure of monolayers to NDS-conditioned HBSS elicited a Isc response with a relatively rapid on-rate (half maximal response ~ 70 s in the absence of stirring). As previously shown (10), PMA, or LPS-FMLP as used to stimulate PMN, had no independent effect on Isc. Peak Isc ranged from 8 to $32 \mu\text{A} \cdot \text{cm}^{-2}$ in the preparations used for these experiments with a mode of $15 \mu\text{A} \cdot \text{cm}^{-2}$ ($n \sim 300$).

NDS-elicited Isc represents Cl^- secretion. Although Isc responses from T84 cells are now commonly construed as indicative of Cl^- secretion (13), given the lack of detectable intracellular signals typically associated with Cl^- secretion (see below) and the apical responsiveness of the NDS effect, it was necessary to define the basis of the Isc response. As shown in Fig. 2, NDS elicited ^{125}I efflux from T84 monolayers, indicating opening of an anion conductive pathway ($P < 0.02$ for period 3 vs period 5, $n = 7$). The gradual decrease in the rate constant of ^{125}I efflux before addition of the agonist has been shown to be due to depletion of a small amount of label trapped in the paracellular space (16). The onset of ^{125}I efflux corresponds to the on-rate of the NDS response shown in Fig. 1.

As shown in Fig. 3, measurement of unidirectional fluxes of Na^+ and Cl^- show that NDS elicits a net secretion of Cl^-

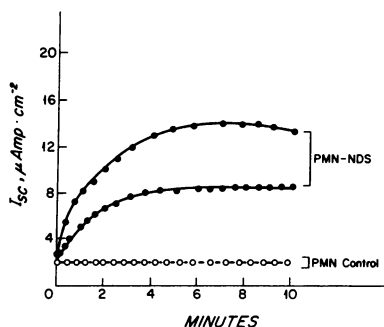


Figure 1. Time course illustrating on-rate of T84 Isc response to NDS-conditioned HBSS. The upper two curves represent responses to NDS derived from PMN stimulated with agonists and obtained from two separate donors. Each point represents the average of triplicate values. The half-maximal response

is seen at 70 s. The lower curve demonstrates the lack of an Isc response in HBSS conditioned by unstimulated PMN. HBSS with agonist alone also failed to elicit an Isc response over baseline value (not shown).

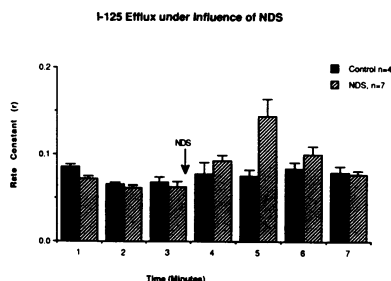


Figure 1. Sequential 1-min rate constants of ^{125}I efflux from T84 cells, as calculated by Vlengarik et al. (19). After three 1-min baseline periods, NDS was added for the following 1-min period. In response to this transient exposure, NDS-treated monolayers respond with ^{125}I release indicative of opening of an anion conductive pathway ($P < 0.02$; comparison between 3 and 5 min for NDS) ($n = 7$).

($0.30 \pm 0.04 \mu\text{eq} \cdot \text{h}^{-1} \cdot \text{cm}^{-2}$ for the period immediately after NDS addition, $P < 0.05$ compared with the period preceding NDS, $n = 8$) while not affecting net Na^+ absorption for the periods before and after NDS addition. Furthermore, as shown in the inset to Fig. 3, the observed I_{sc} elicited by NDS is largely accounted for by this measured Cl^- secretory response (0.30 ± 0.04 vs $0.36 \pm 0.06 \mu\text{eq} \cdot \text{h}^{-1} \cdot \text{cm}^{-2}$ for measured Cl^- secretion vs I_{sc} , respectively, NS).

Effects of 8-phenyltheophylline on NDS response. 8-phenyltheophylline, which can inhibit purinergic receptors, was added at 10^{-5} M (15) and 10^{-6} M with NDS to subsets of experiments. While the NDS preparation used for these experiments elicited $18 \pm 4 \mu\text{A} \cdot \text{h}^{-1} \cdot \text{cm}^{-2}$ activity, those exposed to 8-phenyltheophylline had no response ($< 2 \mu\text{A} \cdot \text{h}^{-1} \cdot \text{cm}^{-2}$ at both doses, n for each group = 6, $P < 0.05$). Preliminary characterization (data not shown) has ruled out adenosine and ATP as possible sources for NDS activity.

NDS effects on second messengers. As shown in Fig. 4, NDS did not elicit a detectable rise in $[\text{Ca}^{2+}]_i$. 10^{-4} M carbachol, used as a positive control, elicited a substantial (220–300%) rise in $[\text{Ca}^{2+}]_i$ in the same monolayers. The three NDS preparations used to establish the data shown in Fig. 4 elicited I_{sc} responses of 21 ± 5 , 16 ± 6 , and $8 \pm 3 \mu\text{A} \cdot \text{cm}^{-2}$, respectively. As shown in Fig. 5 A, NDS also did not elicit detectable elevation of intracellular cAMP or cGMP. In these experiments, positive controls for cyclic nucleotide responses in T84 monolayers were *E. coli* heat stable toxin, $0.2 \mu\text{M}$ (25), and cholera toxin $119 \mu\text{M}$ (25a).

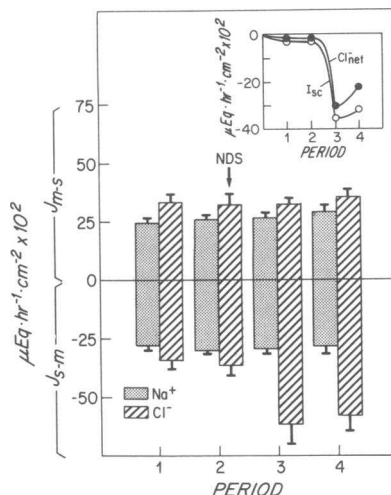


Figure 3. Bidirectional fluxes of $^{22}\text{Na}^+$ and $^{36}\text{Cl}^-$ across stimulated T84 monolayers. Absorptive flux is above and secretory flux below the origin. Four consecutive 10-min periods were assayed. Addition of NDS elicits net Cl^- secretion ($P < .05$). As shown in the inset, the measured net Cl^- secretion accounts for the observed I_{sc} generated across monolayers by NDS (all $n = 7-9$).

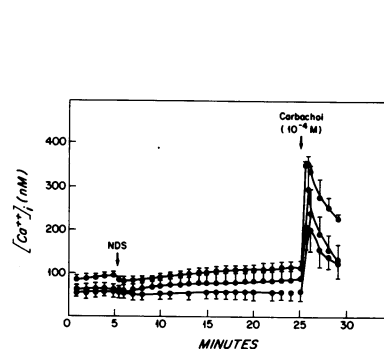


Figure 4. Fura-2 measurements of intracellular Ca^{2+} in response to NDS or, as a positive control, carbachol. Conditions of loading were identical to those described by Dharmasathaphorn and Pandol (21). In the three NDS preparations examined, NDS failed to elicit a Ca^{2+} signal (all $n = 4$).

Substantial elevations of cAMP are also readily detected at the time in which cholera toxin-elicited I_{sc} are equal in magnitude to NDS-elicited I_{sc} . For example, at a time when cholera toxin-induced current was only $3-5 \mu\text{A} \cdot \text{cm}^{-2}$ above baseline, cAMP concentration was $11.9 \text{ pmol/monolayer}$, a value readily distinguished from control ($2 \pm 1 \text{ pmol/monolayer}$). Lastly, as shown in Fig. 5 B, inclusion of IBMX did not unmask a cAMP response in NDS-exposed monolayers.

NDS elicits a Cl^- secretory pattern similar to that of cAMP agonists. Since cyclic nucleotide and Ca^{2+} -mediated signals may elicit Cl^- secretion by affecting different distal events (26), Cl^- secretion can be stimulated through one pathway even if the other is saturated (27). Indeed, stimulation through one pathway while the other is saturated has potentiating rather than simply additive effects on Cl^- secretion (27). Fig. 6 shows

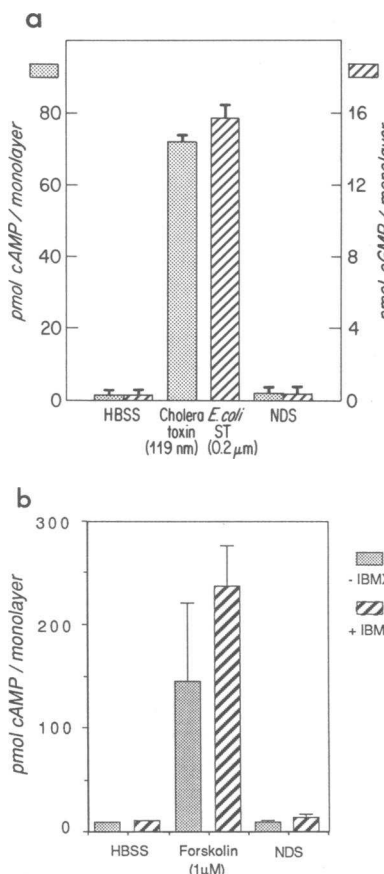


Figure 5. Measurements of cyclic nucleotides in T84 monolayers exposed to NDS or the positive controls cholera toxin and *E. coli* heat stable toxin. Whereas cyclic nucleotide responses to positive controls are easily demonstrated, responses to NDS are no different than the negative control. (a) HBSS served as the negative control (all $n = 6-8$). (b) Subset of experiments done in the presence of the phosphodiesterase inhibitor IBMX (1 mM). The use of IBMX did not uncover a cAMP response in NDS-exposed monolayers ($n = 3$; one of two experiments). Forskolin (10^{-6} M) served as the positive control (hatched bars = with IBMX).

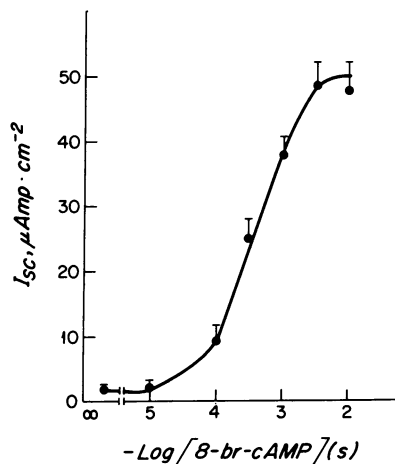


Figure 6. Isc response of T84 monolayers to graded doses of 8-Br-cAMP applied to the serosal(s) bath. Maximal responses are seen at 3 mM, a dose which was subsequently used to examine potentiation with Ca^{2+} agonists and NDS (all $n = 4$).

that Cl^- secretion elicited by basolateral 8' bromo cAMP is saturated at 3 mM. As shown in Fig. 7, NDS has no further effect on the Cl^- secretory response under conditions in which cAMP-elicited Cl^- secretion is saturated (52 ± 3 vs $51 \pm 2 \mu\text{A} \cdot \text{cm}^{-2}$ for 3 mM cAMP vs 3 mM cAMP + NDS). In these experiments NDS alone elicited a $19 \pm 3 \mu\text{A} \cdot \text{cm}^{-2}$ Isc.

In contrast to the findings above, carbachol, which stimulates Cl^- secretion via a Ca^{2+} -mediated pathway (21), elicits an additional Cl^- secretory response (27) even when the cAMP response is saturated (Fig. 8). Similarly, monolayers exposed to NDS and subsequently to carbachol responded with a substantial increase in Isc (Fig. 8). Moreover, NDS, like cAMP, potentiated the carbachol-elicited Isc ($19 \pm 6 \mu\text{A} \cdot \text{cm}^{-2}$ greater response of NDS + carbachol stimulation than predicted by a simple additive response). Fig. 9 shows that the addition of NDS and 3 mM cAMP had no effect on the subsequent response to carbachol, as compared with monolayers preexposed to cAMP alone. Lastly (Fig. 10), the PMN agonists (PMA or FMLP-LPS) used to generate NDS were irrelevant to the potentiating effect of carbachol on the NDS response, and these agonists alone had no effect on the cAMP- or carbachol-elicited Cl^- secretion (data not shown). These data indicate that, with respect to the interplay with Ca^{2+} -mediated Cl^- secretion, NDS again shares similarities to the cAMP-mediated secretory pathway.

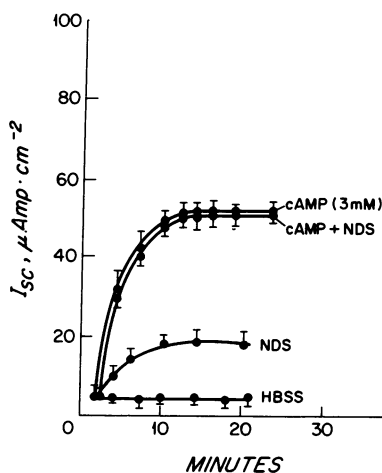


Figure 7. Isc responses elicited by maximally effective concentrations (3 mM) of 8-Br-cAMP, NDS, or NDS + 8-Br-cAMP. When the cAMP response is maximal, NDS does not further increase the Isc. Such data suggest that NDS and cAMP stimulate the same Cl^- secretory process (all $n = 8-12$).

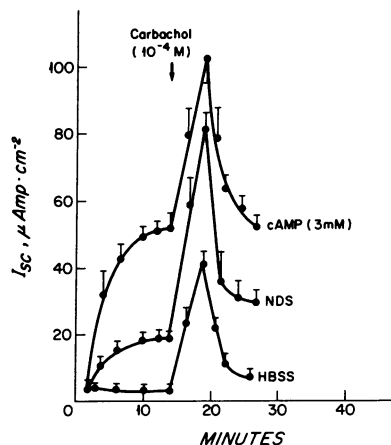


Figure 8. Isc response of T84 monolayers to the Ca^{2+} agonist carbachol after incubation with HBSS or with HBSS containing either NDS activity or 3 mM 8-Br-cAMP. Both cAMP- and NDS-induced Cl^- secretion show a potentiated response to carbachol (responses are significantly greater than expected additive responses). Such data indicate that NDS is cAMP-like in this potentiating interaction with Ca^{2+} agonists (all $n = 7-10$).

Experiments in which either PMA- or FMLP/LPS-exposed monolayers were subsequently exposed to either cAMP or carbachol showed "synergistic" responses with either agent, which were not due to these PMN agonists alone (data not shown).

To further compare the pattern of secretory activation elicited by NDS with that elicited by Ca^{2+} and cAMP agonists, ^{125}I and ^{86}Rb efflux studies were performed. As shown in Table I, NDS predominantly elicits ^{125}I , as does the cAMP agonist forskolin. In contrast, the Ca^{2+} agonist carbachol predominately effects ^{86}Rb efflux with relatively small increments in ^{125}I efflux. The positive control data (forskolin and carbachol) are consistent with those reported by Frizzell and colleagues (19). As shown in Table I, the two patterns of activation (predominantly ^{125}I efflux for cAMP and NDS; predominately ^{86}Rb efflux for Ca^{2+}) can conveniently be conveyed as a ratio relating agonist-induced ^{125}I to ^{86}Rb efflux. Thus, the pattern of NDS on ^{125}I and ^{86}Rb effluxes again mimics that of cAMP activation of Cl^- secretion.

NDS directly elicits Cl^- secretion from freshly isolated mammalian crypt cells. We next sought to examine whether NDS elicited Cl^- secretion by direct means from nontransformed mammalian crypt cells. The effect of NDS on isotonic jejunal crypt cell volume is illustrated in Fig. 11. Within 5 min

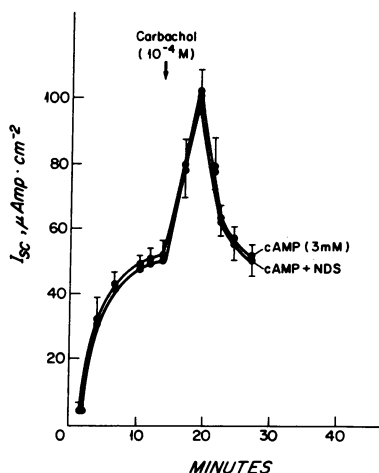


Figure 9. Isc responses of T84 monolayers to the Ca^{2+} agonist carbachol in the presence of 3 mM 8-Br-cAMP alone or in combination with NDS. NDS does not influence the cAMP/ Ca^{2+} potentiation (all $n = 7-8$).

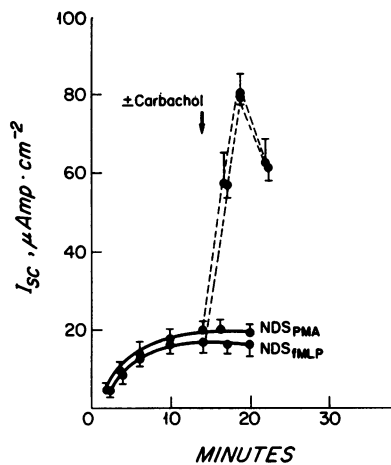


Figure 10. Isc response to the Ca^{2+} agonist carbachol after exposure to NDS activity that had been elicited from PMN with either PMA or FMLP/LPS. The dashed lines show the potentiated response to carbachol. The agent used to elicit NDS activity from PMN does not influence the observed NDS potentiation of the Ca^{2+} -elicited Isc response (all $n = 8$).

of the addition of NDS, the relative volume of the crypt cells was less, compared with controls (0.90 ± 0.01 vs 0.94 ± 0.01 , $P < 0.05$). This volume reduction continued, and at the conclusion of the experiment the relative volume of the crypt cells was less, compared with untreated controls (0.84 ± 0.01 vs 0.92 ± 0.02 , $P < 0.001$). 4,4'-Dinitrostilbene-2,2'-disulfonic acid (DNDS), an established Cl^- channel blocker in epithelia, prevented the NDS-stimulated volume reduction. The final relative volume of cells in DNDS ($250 \mu\text{M}$) and NDS was greater, compared with cells that had received NDS (0.9 ± 0.01 vs 0.84 ± 0.01 , $P < 0.001$).

Discussion

When activated by conditions similar to those normally present in the colonic lumen, PMN secrete a small hydrophilic product, trivially denoted NDS, which elicits a Isc response when applied to T84 cells (10). We, therefore, speculate that NDS activity is likely to be present in the so-called crypt abscesses that characterize many active inflammatory intestinal diseases. In the current study, we explored aspects of the intracellular signaling related to this NDS-elicited Isc response, and because our findings were unexpected, it was necessary that we clearly identify the transport event underlying the NDS-elicited Isc. ^{125}I efflux, a macroscopic assay of Cl^- conductive pathway (19) revealed that NDS stimulated anion efflux from T84 cells. Bidirectional $^{22}\text{Na} + ^{36}\text{Cl}^-$ flux studies performed under short-

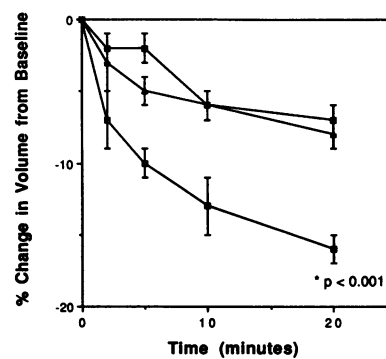


Figure 11. NDS directly affects guinea pig crypt epithelial cells. For these experiments, a supernatant from human granulocytic cells, into which NDS activity had been spontaneously released, was used (absence of any agonist). When applied to T84 cells this preparation elicited a secretory response of 17 ± 3

$\mu\text{A} \cdot \text{cm}^{-2}$. In contrast to buffer alone (triangles), the NDS preparation (open squares) elicited a significant ($P < 0.001$) volume decrease in guinea pig crypt cells. Such volume decrease represent stimulated salt loss with osmotically obligate water loss. The Cl^- channel blocker DNDS ($250 \mu\text{M}$) prevented the volume loss (closed squares), indicating the volume reduction coincided with Cl^- loss through Cl^- channels. (All $n = 4-5$.)

circuit conditions demonstrated that NDS did elicit electrogenic Cl^- secretion, and that this secretory process fully accounted for the observed Isc response elicited by NDS. T84 cells are recognized as a model system in which electrogenic Cl^- secretion occurs (12, 13). With an exception to be noted below, the plethora of secretory agonists studied in this model system elicit Cl^- secretion by acting through intracellular signaling cascades that either elevate intracellular Ca^{2+} or the cyclic nucleotides cAMP or cGMP (25-27). It is now clear that these two basic signaling pathways eventuate in Cl^- secretion through two distinctive Cl^- conductive pathways on the apical membrane (26). One, activated by cAMP, just recently has been recognized to be characterized by a linear current voltage relationship (26) comparable with that recently reported for cGMP (28). This channel is thought to either be regulated by, or directly represent, the cystic fibrosis gene product, CFTR (29). The second, activated by increases in intracellular Ca^{2+} , has now been shown by Cliff and Frizzell to be outwardly rectified and spontaneously activated by depolarization (26). We find that NDS-elicited Isc is not accompanied by detectable increases in intracellular cGMP, cAMP, or Ca^{2+} . From our sequential stimulation and efflux studies, we find that NDS behaves as though the cyclic nucleotide-activated Cl^- secretory pathway has been stimulated even though no such signal is detected (but such signals are easily detected in positive controls eliciting comparable currents to NDS). It has been shown

Table I. Rate Constants of ^{86}Rb and ^{125}I Efflux

Agonist	$r, ^{86}\text{Rb}$ efflux			$r, ^{125}\text{I}$ efflux			%ΔI/%ΔRb
	Preagonist	Postagonist	%Δ	Preagonist	Postagonist	%Δ	
NDS	0.016 ± 0.002	0.021 ± 0.002	+31	0.063 ± 0.007	0.144 ± 0.021	+129	4.16
Forskolin, $1 \mu\text{M}$	0.011 ± 0.001	0.016 ± 0.001	+45	0.062 ± 0.004	0.259 ± 0.017	+317	7.04
Carbachol, $100 \mu\text{M}$	0.027 ± 0.006	0.091 ± 0.017	+237	0.069 ± 0.004	0.097 ± 0.012	+41	0.17

Mean \pm SE; all $n = 6-15$; all $P < 0.02$ except carbachol $r, ^{125}\text{I}$, where $P = 0.05$; P values compare 1-min period preagonist with the 1-min period 60-120 s after agonist addition; %Δ, mean percent change from preagonist rate constant value; %ΔI/%ΔRb, ratio of mean percent change for ^{125}I and ^{86}Rb effluxes, a value which readily conveys the pattern of activation (i.e., predominantly ^{125}I efflux = values > 1 ; predominantly ^{86}Rb efflux = values < 1).

by others that when cAMP-elicited Cl^- secretion is maximal, a subsequent Ca^{2+} -mediated signal results in a secretory response that is greater than that predicted by the sum of the individual responses; that is, sequential stimulation results in a potentiated response (27). We find that NDS does not further increase a maximal cAMP-elicited response. In contrast, sequential NDS/ Ca^{2+} agonist exposures show a potentiated response, again suggesting NDS acts in a cAMP-like fashion. Efflux studies further substantiated this cAMP-mimetic effect of NDS. ^{86}Rb efflux studies that mark K^+ channel activity (19) reveal that Ca^{2+} agonists elicit a large K^+ efflux, while K^+ efflux elicited by cAMP agonists are not as substantial (19). In contrast, cAMP elicits large $^{36}\text{Cl}^-$ or ^{125}I efflux relative to Ca^{2+} signals (19). We show NDS to predominantly elicit a ^{125}I efflux with minimal ^{86}Rb efflux, a pattern similar to that elicited by cAMP agonists.

Suspensions of highly purified crypt jejunal epithelial cells lost volume when treated with NDS. Such volume loss is attributable to salt efflux from isolated cells, together with osmotically obligated water (33). This isotonic volume reduction was prevented by an established Cl^- channel blocker, dinitrostilbene disulfonic acid (DNDS) (30), suggesting NDS also activated a Cl^- conductance in natural crypt cells. Moreover, we selected the approach of examining Cl^- secretion in isolated crypt cells, since it allows one to conclude that the NDS-elicited response in natural crypt epithelial cells is due to a direct effect, rather than an indirect one acting through subepithelial components. We conclude the secretagogue activity of NDS is not restricted to intestinal cell lines such as T84.

The ability of NDS to elicit substantial cAMP-like currents without detectable increments in cAMP is reminiscent of adenosine-induced Cl^- secretion (32). The data previously reported by us compared with that reported by Barrett et al. (15) would suggest that NDS is distinct from adenosine, since the reported sidedness of the responses would appear to differ. More importantly, however, preliminary characterization has ruled out free adenosine or ATP as possible identities for NDS activity in the isolated PMN supernatants. Further studies on the molecular isolation and characterization of NDS are underway. However, since 8-phenyltheophylline inhibits the NDS affect, it is possible NDS, or a metabolic product of NDS generated at the cell surface, affects purinergic receptors, as does adenosine. It is also clear currently that NDS is small (< 500 nominal mol wt), heat and acid stable (unlike ATP), hydrophilic, and not representative of PMN-derived oxidants or arachidonic acid metabolites (10). The above findings are compatible with distinctive hypotheses to be tested in the future: (a) NDS elicits a unique Cl^- current distinct from cAMP, but with many cAMP-like characteristics; (b) NDS elicits cAMP-mediated Cl^- secretion by affecting a restricted cAMP pool that cannot be detected with the assay used; (c) NDS stimulates the cAMP-regulated Cl^- channel, but acts in the signaling cascade at a site distal to cAMP, perhaps by directly affecting this channel or a closely associated regulatory element. Given our previous findings of preferential apical effects of NDS, this last possibility is intriguing. Whichever hypothesis proves correct, these data indicate that NDS elicits Cl^- secretion in a potentially novel fashion, an observation which may in itself provide insights into regulation of Cl^- secretion. We speculate that this PMN-derived bioactivity may contribute to the secretory diarrhea observed in patients with active intestinal inflammation.

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