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Effect of Staphylococcal Enterotoxins A, B, and C, on Ion Transport and Permeability Across the Flounder Intestine[1] (38320)

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Staphylococcal enterotoxins, produced by certain strains of *Staphylococcus aureus*, are the common cause of food poisoning. They can produce cholera-like symptoms, including vomiting, nausea and diarrhea (6). Studies on the action of cholera enterotoxin on intestinal fluid and electrolyte transport have been well documented (1, 7). However, little information is available on the mechanism of action of those staphylococcal enterotoxins. It has been shown that staphylococcal enterotoxin B stimulates chloride transport in rat intestine by nonelectrogenic mechanism (16).

Using the Ussing chamber technique, we have shown previously that a negative potential difference (PD) exists across the winter flounder intestine and a net chloride absorption plays a significant role in these unique electrical properties (9). The present investigation was conducted using the same technique to study the effect of three staphylococcal enterotoxins on electrical properties and ion transport across the winter flounder intestine, also to elucidate the toxin effect on membrane permeability to small solute.

Method and Procedure. Winter flounder, *Pseudopleuronectes americanus*, were caught in the Gulf of Maine during the period of July and August. They were kept alive in a sea water tank for a week before used in experiments. Fish were sacrificed by decapitation; a piece of intestine, 3 cm below the stomach and 4 cm above the cloaca, was removed and divided into 4 sections. Each was washed with teleost Ringer solution and mounted in an Ussing chamber. The details of the method and chamber design have been described previously by us (5, 9).

Forster's teleost Ringer solution (9) containing 5.5 m*M* dextrose was freshly prepared, gassed with 95% O_2–5% CO_2 and used as the bathing solution for both sides of the mounted intestine. During the experiment, the bathing fluid was bubbled continuously with the same gas. All experiments were carried out at room temperature. At steady state, the PD across the control intestinal membrane ranged from 2 to 7 mV, serosa being negative with respect to the mucosa and the short circuit current (I_{sc}) ranged from 30 to 80 μA. These control values remained constant over a 2.5 hr period.

Steady state unidirectional fluxes of Na and Cl were measured using ^{22}Na and ^{36}Cl as described previously (5, 9). Transmural solute flow was measured by using ^{14}C-urea and ^{14}C-thiourea and the membrane permeability to these solutes are expressed as K_{trans}, calculated according to the Fick equation (4).

Purified staphylococcal enterotoxins A, B, and C (SEA, SEB, SEC) in powder form were obtained from the U. S. Army Institute of Infectious Disease. They were kept at −70° and diluted daily to the appropriate concentration with 0.01 *M* PO_4 buffer (pH 7.4). The enterotoxins were added to both the serosal and mucosal bathing solutions. The amount used ranged from

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1 to 20 μg/ml.

Results. The effects of various concentrations of staphylococcal enterotoxins A, B, and C on the change of I_{sc} (ΔI_{sc}) across the flounder intestinal membrane are shown in Fig. 1. One hour after the addition of enterotoxins to both sides, the calculated percentage increases of PD for SEA, SEB and SEC at the concentrations of 2.5 μg/ml are 45%, 54% and 47% respectively, while the increments in I_{sc} after addition of SEA, SEB and SEC are 38%, 47% and 38% respectively. In the dose-response study, the concentration of the toxins used here vary from 2.5 to 20 μg/ml and the response was expressed as percent change of I_{sc}. As shown here, judging from the average of 3 experiments, only SEC showed a dose-response relationship where the peak of increase was at the concentration of 10 μg/ml. However, SEA and SEB did not further enhance the short circuit current of PD at a higher concentration level. In all experiments with either enterotoxin, the resistance did not vary significantly throughout the studies.

Table I summarizes the staphylococcal enterotoxin effect on sodium and chloride ion transport. A net absorption of Na and Cl ions was observed in control studies with the J^{Cl}_{net} exceeding the J^{Na}_{net}, thus contributing the negative PD across the intestinal membrane. This confirms our previous observations in both flounder and catfish (4, 9). After the addition of enterotoxin A, B, or C, a significant increase in the Na flux, both J^{Na}_{ms} and J^{Na}_{sm}, was observed. In contrast, the unidirectional Cl ion flux from mucosal to serosal side did not vary significantly after the addition of the enterotoxins; however, these toxins did enhance the serosal-to-mucosal chloride flux significantly, *i.e.*, stimulate the

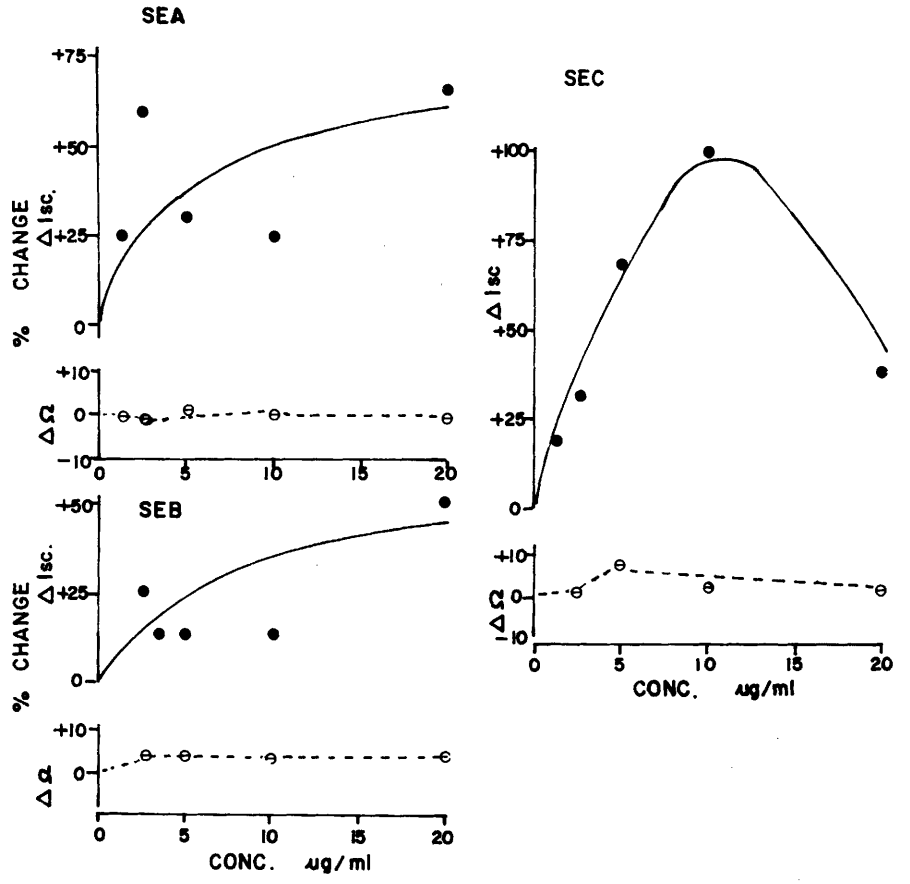

FIG. 1. Dose response curve of staphylococcal enterotoxins A, B, and C on electrical measurement across the flounder intestine. The ordinate is percentage change of short circuit current (I_{sc}) and resistance at one hr after the toxin was added. The abscissa is the final concentration. Each point was the average of three experiments and the lines were drawn arbitrarily.

TABLE I. Effect of Staphylococcal Enterotoxins A, B, and C on Ion Transport Across the Flounder Intestine.

	Conc.	No. of fish	^{22}Na flux			^{36}Cl flux					
			J^{Na}_{ms}	J^{Na}_{sm}	J^{Na}_{net}	J^{Cl}_{ms}	J^{Cl}_{sm}	J^{Cl}_{net}	J_{net}	I_{sc}	J^{R}_{net}*
			(μEq. cm^{-2} hr^{-1})			(μEq/cm^{-2} hr^{-1})			(μEq. cm^{-2} hr^{-1})		
Control		32	6.78 ± 0.64**	4.48 ± 0.38	2.30	5.88 ± 0.60	2.92 ± 0.30	2.96	−0.66	−1.24	−0.58
SEA	1 μg/ml	10	7.36 ± 0.39 (P = 0.5)***	6.22 ± 0.38 (P < 0.01)	1.14	5.29 ± 0.53 (P = 0.5)	3.87 ± 0.39 ($P \leq 0.05$)	1.42	−0.28	−0.86	−0.58
SEB	1 μg/ml	10	9.71 ± 0.60 (P < 0.01)	7.52 ± 0.58 (P < 0.01)	2.19	7.27 ± 0.66 (P = 0.2)	4.92 ± 0.67 (P < 0.01)	2.35	−0.16	−0.73	−0.57
SEC	1 μg/ml	10	8.54 ± 0.46 (P = 0.05)	6.77 ± 0.54 (P < 0.01)	1.77	6.21 ± 0.57 (P = 0.7)	4.06 ± 0.48 (P < 0.05)	2.15	−0.38	−0.78	−0.40

* $J^{R}_{net} = I_{sc} - J_{net}$.

** Mean ± SE.

*** P value is the t-test between the control and enterotoxin-treated data.

chloride secretion, resulting in an upward movement of both PD and I_{sc}. Since the control value of both PD and I_{sc} was electronegative to the serosal side, a stimulation of chloride secretion would move the electrical measurement from negative toward positive, therefore, the magnitude of measured I_{sc} became smaller as shown in Table I after the addition of the enterotoxins. Although SEA, SEB and SEC all affect the Na and Cl ion flux, but the calculated residual flux ($J^{R}_{net} = I_{sc} - J^{Na}_{net} + J^{Cl}_{net}$) did not change significantly.

The permeability characteristics of the control fish intestine and those treated with enterotoxins are shown in Table II. At enterotoxin concentration of 2.5 μg/ml, the calculated permeability coefficients (K_{trans}) for urea and thiourea were not significantly higher than that of the controls. This finding is similar to that observed in canine loop after challenge with cholera toxin (14).

Discussion. The results of present studies demonstrate that staphylococcal enterotoxins A, B, and C increase the fluxes of Na and Cl ions across fish intestine, with the greater increase being in the serosal-to-mucosal flux for each ion and resulting in an observed upward movement of both PD and I_{sc}. A similar effect was demonstrated by Field *et al.*, in studying the cholera enterotoxin effect on isolated rabbit ileal mucosa (7). They showed a net secretory flux of Cl ion and a progressive increase in transmural electrical PD and I_{sc}. This was confirmed by Curran *et al.*, recently (13). The data in the present study also demonstrated that the residual flux (J^{R}_{net}) of the staphylococcal enterotoxin treated intestine was not significantly different from that of the control and the resistance did not vary in control studies as well as in enterotoxin studies. This suggests that the enterotoxins increase the PD and I_{sc} by stimulating Na and Cl active secretion without effect on bicarbonate or other anion flux (12) and the secretory mechanism seems to be electrogenic. Sullivan *et al.*, demonstrated that staphylococcal enterotoxin B induced a net secretion of Na, K, Cl and water in rat small intestine without any observable changes in PD and I_{sc} (16). This suggests that staphylococcal enterotoxins may have a different effect on electrical properties of the intestinal membrane in mammals and fish. In general, the three staphylococcal enterotoxins exert qualitatively similar effects on electrical properties and ion transport across the fish intestine; they only vary quantitatively.

Despite other controversial results of cholera toxin on the mammalian intestinal membrane permeability (11, 14), our data on fish membrane permeability as shown in Table II indicate that there is no significant increase in permeability coefficients to small solutes such as urea and thiourea after the addition of staphylococcal enterotoxins. This is in agreement with the findings of Rohde and Chen (14). They reported there is no change in membrane permeability for urea and arabinose in canine loops after challenge with cholera toxin and no significant change in equivalent pore size was observed.

The molecular mechanisms by which staphylococcal enterotoxins produce the diarrhea and electrolyte changes is unknown. Recent evidence suggest that the effect of cholera toxins on net secretory ion and water flux

TABLE II. Effect of Staphylococcal Enterotoxins A, B and C on Flounder Intestinal Permeability.

	No. of fish	K_{trans} cm $sec^{-1} \times 10^7$			
		M→S	% of control	S→M	% of control
^{14}C-Urea					
Control	26	75 ± 7*		60 ± 6	
SEA 2.5 μg/ml	6	87 ± 7	116	75 ± 5	125
SEB 2.5 μg/ml	6	89 ± 9	119	67 ± 5	112
SEC 2.5 μg/ml	6	91 ± 6	121	74 ± 6	123
^{14}C-Thiourea					
Control	16	87 ± 9		78 ± 8	
SEA 2.5 μg/ml	6	112 ± 10	129	106 ± 8	135
SEB 2.5 μg/ml	6	106 ± 8	122	100 ± 9	128
SEC 2.5 μg/ml	4	114 ± 13	131	92 ± 17	118

* Mean ± SE.

across the guinea pig and rabbit ileum (10, 15), canine ileum (8) and human ileum (3) is mediated by enhanced activity of the adenyl–cyclase/cyclic AMP system. Based on the similar symptomatic manifestations produced by cholera and staphylococcal enterotoxins and their effects on ion transport and electrical properties of the intestinal membrane, one may propose the similar mode of action for both toxins.

We would like to point out here that under short circuited condition the ion transport properties across the fish intestine was different from that found in mammalian intestine, especially concerning chloride ion flux (2, 12). For example, the $J^{Cl}_{M\to S}$ in fish intestine is greater than the $J^{Cl}_{S\to M}$, resulting in a net Cl ion absorption and a negative PD. Both cholera and staphylococcal enterotoxins cause a stimulation of Cl secretion without affecting the $J^{Cl}_{M\to S}$ and J^{R}_{net}; this indicates that the fish intestine moves the chloride ion bidirectionally by two separate systems: a dominant mucosal-to-serosal flux which is probably carried by Cl-anion exchange system and is not influenced by the enterotoxins, and a chloride ion secretory process which is regulated by a cyclic AMP system and stimulated by bacterial enterotoxins.

Summary. Purified staphylococcal enterotoxins A, B, and C added to the mucosal and serosal bathing solution of winter flounder intestine *in vitro* caused changes in ion fluxes and electrical properties. An increase in Na and Cl fluxes was observed after the addition of these enterotoxins, with the greatest increase being in the serosal to mucosal flux for each ion resulting in an upward movement of both PD and I_{sc}. In the staphylococcal enterotoxin treated intestine, the membrane permeability to small solute such as urea and thiourea did not increase significantly. The tissue resistance and the residual flux (J^{R}_{net}) did not change significantly after adding the enterotoxins, suggesting that staphylococcal enterotoxins increase PD and I_{sc} by stimulating active Na and Cl secretion via an electrogenic mechanism.

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