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INHIBITION OF EPITHELIAL CHLORIDE CHANNELS BY A SEMI-PURIFIED FRACTION OF CROTALUS ATROX VENOM

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Abstract: Rattlesnake venom has a strong effect on the ionic permeability of plasma membranes. Rattlesnake venom and one of its semipurified fractions (CAV-C2) has been implicated in affecting both sodium and chloride transfer across epithelia. The mechanism of CAV-C2 action on epithelia, however, is still open to question. Aplysia californica intestine has been shown to contain both chloride conductive channels and sodium dependent chloride uptake mechanisms in the mucosal membrane of its enterocytes which makes it an ideal model to differentiate the CAV-C2 action on either of these membrane transport processes. In the absence of sodium in the bathing medium, chloride is the only ion actively transported. CAV-C2 and piretanide have similar types of inhibitory effects on net chloride transport while furosemide had no effect on chloride movement across the intestine. It was concluded that CAV-C2 action is on chloride conductive channels located in the mucosal membrane of the enterocytes.

Introduction: Rattlesnake venom has a strong effect on the ionic permeability of the nerve (1) and muscle (2) plasma membrane. More recently, it has been demonstrated that Crotalus atrox venom (CAV) affected both Na^+ and Cl^- transport across epithelial membranes (3). Furthermore CAV-C2, a semipurified hemolytic toxin from CAV (4), was shown to directly affect Cl^- transport across frog skin (5). Clearly, however, the effect of CAV-C2 on either Cl^- conductive pathways or Na^+-dependent Cl^- transport mechanisms could not be rigorously defined because all active transcellular Cl- movement across the frog skin is ultimately linked to the movement of Na^+ (6). Since the Aplysia californica (sea hare) intestine can actively absorb Cl^-, in the presence or absence of Na^+ (7), it can serve as a unique model to discern the mechanism by which CAV-C2 affects Cl^- transport across plamsa membranes.

Materials and Methods: The adult Aplysia californica (a seaborne mollusc) was sacrificed, its intestine removed and mounted as a flat sheet between the mirror-image compartments of a conventional lucite Ussing chamber. Transmural potential difference (PD) and short-circuit current (SCC) were measured by methods previously described (3, 6). The intestine was oxygenated and mounted between identical Na^+ free, seawater Ringer that had the following constituents in mM: Tris Cl, 462; $MgSO_4.7H_2O$, 2.4; KCl, 9.7; $KHCO_3$, 2.4; $MgCl_2.6H_2O$, 9.8; $CaCl_2$, 1.5. The medium osmolality was 1000 mosm/l and it had a pH of 7.8 at 25°C. When both PD and SCC were stabilized, CAV-C2 was added, unless otherwise stated to the mucosal bathing medium. CAV-C2 was obtained as a gift from Dr. Anthony T. Tu, Department of Biochemistry, Colorado State University, Fort Collins, CO (4). The application of radiotracer technique for the unidirectional mucosal to serosal (J_{ms}) flux of $^{36}Cl^-$ was as described by Quay and Armstrong (8).

Results: The first series of experiments was designed to determine the site of CAV-C2 action. Typically, when CAV-C2 was added to the mucosal

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bathing solution at a concentration of 40 μg/ml, both PD and SCC were inhibited. These electrical characteristics were partially restored upon rinsing and replacing the mucosal solution with fresh, oxygenated toxin-free medium (N = 5). The addition of 40 μg/ml CAV-C2 to the serosal solution had virtually no effect on PD or SCC (N = 3).

In order to discern the CAV-C2 effect on Cl^- transport, determination of the unidirectional J_{ms} $^{36}Cl^-$ flux, in both the absence and presence of CAV-C2, was carried out under short-circuited conditions. As shown in Table 1, there was an equivalent Cl^- inhibition of both mean J_{ms} of Cl^- and the average SCC after CAV-C2 (100 μg/ml) addition to the mucosal medium.

The addition of 0.1 mM piretanide to the mucosal bathing solution inhibited both PD and SCC. Rinsing and replacing the mucosal medium with piretanide-free medium restored both PD and SCC (N = 4). However, there was no effect when 0.1 mM piretanide was added to the mucosal medium after 40 μg/ml CAV-C2 had inhibited SCC (Fig. 1).

Mucosal or serosal furosemide (0.1 mM) had no effect on PD or SCC.

The next series of experiments was designed to test the effects of Ca^{2+} on CAV-C2 inhibited Cl^- transport. Increasing mucosal and serosal Ca^{2+} concentrations to 11.2 mM abolished any CAV-C2 induced effects on PD and SCC. However, adding 1 mM ethylene diamine tetraacetic acid (EDTA) to the mucosal bathing solution partially restored the inhibition of PD and SCC due to CAV-C2 (N = 5).

Discussion: In a Na^+-containing bathing solution, the SCC across *Aplysia californica* intestine is accounted for by active absorptive fluxes for both Na^+ and Cl^- (9). The secretory fluxes of these ions are predominantly or wholly paracellular and therefore passive (10). In the absence of Na^+ in the bathing medium, the SCC is a pure Cl^- absorptive current as has been demonstrated in Table 1 where CAV-C2 abolished the SCC which was identical to a component of the J_{ms} Cl^- flux.

The very fast response time of the electrical effects evoked by mucosal CAV-C2, and the partial inhibition of these effects by Ca^{2+} strongly suggest interaction of these agents with specific components of the mucosal membrane as has been suggested previously (5) rather than an intracellular site of action.

In the presence of Na^+, furosemide inhibits both PD and SCC in the *Aplysia* intestine (12) suggesting the existence of a Na^+-Cl^- symport process in this preparation. In contrast, the finding that mucosal furosemide had no effect on PD and SCC in the absence of Na^+ strongly suggests that Cl^- transit through the *Aplysia* enterocyte is not mediated by a Na^+-coupled transport process for furosemide is a known Na^+-Cl^- symport inhibitor (11). The observation that mucosal piretanide had similar inhibitory effects on PD and SCC as CAV-C2 coupled with the observation that piretanide had no

TABLE 1

UNIDIRECTIONAL MUCOSAL TO SEROSAL CHLORIDE FLUX AND SHORT CIRCUIT CURRENT IN TRIS Cl SEAWATER MEDIUM

	J_{ms}	SCC
Before CAV-C2 addition	220.3 ± 11.6 (5)	42.6 ± 6.9 (5)
After CAV-C2 addition	173.1 ± 17.2 (5)	0.8 ± 0.4 (5)

Values are means ± SEM in neq cm^{-2}-min^{-1}. Number of experiments shown in parentheses.

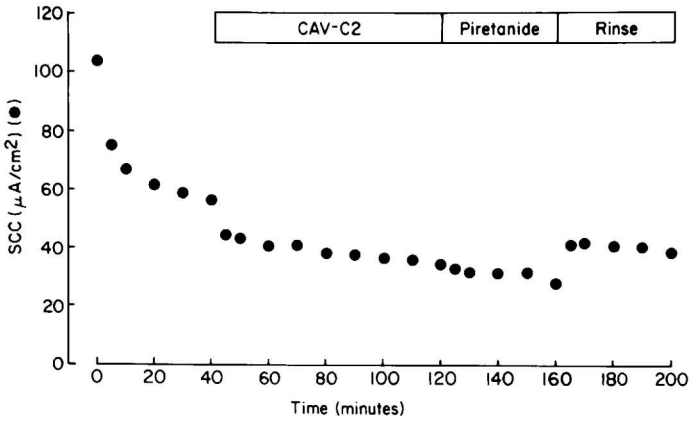

Figure 1: Comparison of the effects of piretanide and CAV-C2 on SCC in Tris Cl seawater medium.

effect on PD and SCC in the presence of CAV-C2 (Fig. 1) suggests that both agents are acting on the same site: the Cl^- conductive channels located in the mucosal membrane (7, 12). This observation compares favorably with that found in gallbladder (13) where it was demonstrated that piretanide blocked Cl^- conductive pathways.

The novel utility of the Aplysia intestine will be considered briefly. It is extraordinary that this experimental system contains several types of Cl^- transport processes which can be differentially studied by artificially manipulating the experimental environment. This experimental model provides an internal control to the study of Cl^- transport mechanisms rather than an external interspecie comparison of these different events.

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