Failure of Cholera Enterotoxin to Alter Cyclic 3',5'-Adenosine Monophosphate-Mediated Responses in Toad Urinary Bladder

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Cholera enterotoxin, which stimulates mammalian adenyl cyclase, failed to affect cyclic 3,5'-adenosine monophosphate-mediated functions in toad bladder. In addition, cyclase-mediated responsiveness to vasopressin was maintained.

Cholera enterotoxin exerts effects in intestinal and other tissues by causing raised intracellular levels of cyclic 3',5'-adenosine monophosphate (c-AMP) (8). Unless chromatographed on highly polar columns (5), the toxin also contains a potent vascular permeability factor (2) and may alter intestinal transmucosal permeability (6, 7).

To investigate possible effects of cholera toxin on epithelial permeability, independent of vascular factors, we decided to do in vitro studies on a well characterized epithelial membrane, the toad bladder. This tissue demonstrates marked enhancement of both active sodium transport and osmotic water flow (J_w) when exposed to vasopressin (4) resulting from activation of adenyl cyclase by the hormone and increased intracellular levels of c-AMP (1).

Female *Bufo marinus* hemibladders (National Reagents, Bridgeport, Conn.) were mounted in a Lucite chamber between two layers of taut nylon mesh to prevent billowing or movement of the membrane. One side of the chamber was sealed except for a calibrated horizontal pipette for measurement of volume changes every 15 min (J_w). Ringers solution (225 milliosmols/kg) bathed the serosal surface while hypotonic (75 milliosmols/kg) otherwise similarly composed solution was on the mucosal side. When identical solutions bathed each surface of the bladder of the toad, the "short-circuit" current (I_{sc}) required to reduce the spontaneous transmembrane potential to zero was electrically equivalent to the rate of net Na^+ transport. Because the solutions were not identical in the present experiments, I_{sc} determined by the method of Ussing and Zerahn (9) gave only an estimate of net Na^+ transport. After base line observations, 100 to 500 μ g of cholera

enterotoxin (3), 25 μ g of which uniformly produced fatal experimental cholera in infant rabbits, was added to the mucosal or serosal solutions (six experiments each). Later (60-180 min), vasopressin was added to the serosal bath (133 milliunits/ml) and I_{sc} and J_w were measured for an additional 15 to 30 min to assess responsiveness of the preparation. Experiments were divided between late summer and early winter to exclude seasonal influences on the bladders.

Data are shown in Fig. 1. Addition of toxin at time zero did not alter I_{sc} or J_w . Later introduction of vasopressin produced a prompt and characteristic increase in both parameters. In four additional experiments, toxin added to the mucosal or serosal bath had no effect on the spontaneous (open-circuit) transmembrane potential ($E_{T=0}$) either before or after vasopressin.

Unless equal and opposite effects on the active and passive currents had exactly balanced one another, these experiments indicate that toxin probably did not influence active Na^+ transport or passive permeability to Na^+ and Cl^- in the leak pathway of this membrane. Furthermore, no alteration of permeability (J_w) was noted, although the tissue remained responsive to subsequent challenge with vasopressin. This suggests either that cholera toxin does not affect toad bladder cellular c-AMP levels or that the membrane receptor site is specific for vasopressin (1). Further study of toad bladder and comparison with toxin responsive tissues (8) may help define the nature of the membrane-toxin interaction.

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FIG. 1. Effect of cholera enterotoxin and vasopressin on short-circuit current (I_{sc}) and osmotic water flow (J_w) in toad bladder. Toxin was added after an initial equilibration period (0 time) and vasopressin 60 to 180 min later (dotted line).

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