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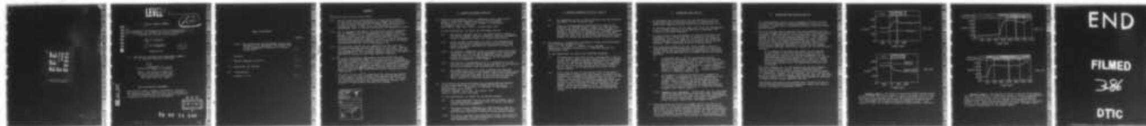

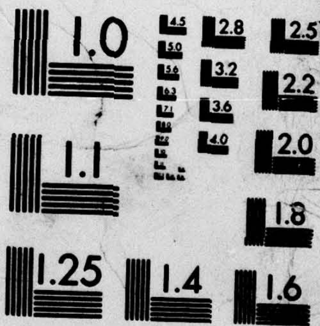
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ANNUAL SUMMARY REPORT

The Interaction of Pseudomonas Toxins with Epithelial Cell Membranes; The Primary Step in the Pathogenetic Sequence of Cellular Intoxication

W.A. Brodsky

28 August 1979

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ABSTRACT

During the past year, we have found:

(i) how the electrical characteristics of the apical membrane of the turtle bladder epithelium are changed after exposure to each of three extracellularly-added, ADPR transferase containing bacterial toxins (e.g., the proenzymatic form of Pseudomonas toxin A, Cholera enterotoxin and Diphtheria toxin);

(ii) how to segregate the apical from the basal-lateral membranes, how to identify each kind of membrane by uniquely-located enzymatic markers (e.g., ouabain-sensitive ATPase and a DIDS-binding protein mark the basal-lateral membrane; while a catecholamine-sensitive adenylate cyclase, a cyclic nucleotide-sensitive phosphoprotein kinase, and a concanavalin A-binding glycoprotein mark the apical membrane);

(iii) This membrane separation and identification has provided a necessary technique for the next phase of this work - that of testing for the effects of these toxins on the electrical and flux parameters of the isolated membranes (which are obtained in the form of inside-out and right side-out vesicles.

(iv) In recent preliminary experiments, we have found that the transport-stimulating effect of cholera toxin is eliminated when this transport has been pre-stimulated with norepinephrine; and conversely, the stimulatory effect of norepinephrine cannot be evoked when the transport parameter has been pre-stimulated with cholera toxin. Both agents (cholera toxin and norepinephrine) stimulate HCO_3 reabsorption without changing Na or Cl reabsorption.

This part of the work is preparatory for testing the effects of both agents on the transport and enzymatic parameters of isolated apical membrane vesicles, which in turn, is crucial for characterizing the toxin induced membrane changes independent of toxin-induced intracellular changes.

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I. RESULTS OBTAINED (1978-79)

(a) Effects of pseudomonas toxin A, diphtheria toxin, and cholera toxin on electrical characteristics of turtle bladder.
Brodsky, W.A., J.C. Sadoff, J.H. Durham, G. Ehrenspeck, M. Schachner and B.H. Iglewski
Proc. Nat'l. Acad. Sci. U.S.A., 76:3562-3566, 1979

In this publication, we report the following results:

(i) the apical (mucosal) but not the basal-lateral surface of the turtle bladder contains receptors sites for Pseudomonas toxin A, diphtheria toxin, and cholera enterotoxin

(ii) toxin A binds irreversibly to sites in the neighborhood of the Na-selective conductance path and the anion pump path in the apical membrane with resultant decreases in these parameters.

(iii) in contrast, diphtheria toxin or cholera enterotoxin binds reversibly to sites in the neighborhood of the anion pump-containing path of the apical membrane without changing either the electrical conductance or the Na selective transport parameters of the bladder.

(iv) the effect of Pseudomonas toxin A is blocked by its pre-mixture with specific rabbit serum antitoxin, by pre-heating or by pre-treatment with dithiothreitol and urea.

(v) the proenzymatic form of toxin A induces the observed electrical responses of the bladder, while the enzymatically-activated form (either made active with the DTT-urea treatment or originally obtained in the enzymatically-active form) induces no electrical response in the bladder.

(b) Localization and characterization of transport-related elements in the plasma membrane of turtle bladder epithelial cells.
Brodsky, W.A., Z.I. Cabantchik, N. Davidson, G. Ehrenspeck, E. Kinne-Saffran and R. Kinne.
Biochim. Biophys. Acta, 556: (In Press), 1979
(Xerox of galley proof enclosed)

In this publication, we report the following results:

(i) The mixed membrane fraction of the turtle bladder can be electrophoretically separated into its apical and basal-lateral membrane components.

(ii) The basal-lateral membranes are identified by their enrichment in ouabain-inhibitable (Na + K)·ATPase and in a high affinity disulfonic stilbene binding protein (DIDS).

(iii) The apical membranes are identified by their enrichment in a catecholamine-stimulable adenylate cyclase and a cyclic AMP-stimulatable protein kinase.

(iv) The membranes in all of these fractions and sub-fractions are mainly in the vesicular form.

(v) There is a physiological counterpart for each of the enzymatic or binding markers: ouabain or DIDS acts only on the basal-lateral surface of the intact bladder to produce a decrease in Na transport or a decrease in anion transport respectively; norepinephrine or cyclic AMP derivatives acts only on the apical surface of the intact bladder to produce an increase in anion transport alone.

(c) The pattern of concanavalin a binding to intact and dissociated turtle bladder epithelial cells.
Cohen, B., J.H. Durham, J. Gennaro & W.A. Brodsky
(Manuscript enclosed; submitted to J. Membrane Biology for publication, 1979)

In this manuscript, we report the following findings:

(i) The addition of the plant lectin, concanavalin A (Con A) to the muuosal and/or serosal fluid of the intact bladder results in a methyl glucoside-inhibitable Con A binding to sites on the apical membrane surface of the granular cells, but not to sites on the basal-lateral surface, nor to sites on the mitochondrial-rich cells of the intact system.

(ii) Con A binds to part of the surface (35%) of isolated suspensions of dissociated (but intact) epithelial cells (obtained by incubation of epithelium in Ca-free, Mg-free media). Part of this distribution (20%) can be attributed to stability of the apical membrane after separation of cells from interstitial fluid while the remainder (15%) is ascribed to lateral diffusion of components of the apical membrane (e.g., lectin-binding sites) within the confines of the plasma membrane envelope.

II. OBJECTIVES FOR 1979-80

1. To determine the reversibility (or irreversibility) of the rapidly-developing transient increases in sodium-selective conductance (G_{Na}) and related short-circuiting current (Isc).

2. To evaluate the nature of the long term (12-24 hr) Pseudomonas toxin A-induced decreases in Na-selective conductance, Na transport, and anion transport.

3. To characterize long-term effects of cholera enterotoxin and diphtheria toxin, along with and in comparison to those of Pseudomonas toxin A, in order to elicit those electrophysiological characteristics which are or might be due to the NAD-dependent, ADPR transferase activity of these toxins.

4. Since the apical membrane of the turtle bladder contains a catecholamine-sensitive adenylate cyclase activity and since certain electrical properties of this membrane are reversibly changed by the mucosal addition of cholera toxin, the related aims are:

   (i) to determine how or if the electrophysiological response of the bladder to cholera toxin is modified by the prior mucosal addition of other effectors of the membrane-bound cyclase-kinase system -- e.g., adrenergic agonists and antagonists, phosphodiesterase blockers (the methylxanthines), and nicotinic acid or niacinamide (which is supposed to inhibit the adenylate cyclase activity in skeletal muscle); and

   (ii) to determine if and how the electrical response of the bladder to Pseudomonas toxin A or diphtheria toxin is modified by effectors of the adenylate cyclase-protein kinase system in the apical membrane of the bladder. Direct determinations of these enzymatic activities will be performed on the membrane fraction or fractions isolated from toxin A-treated and control bladders (see item 6, below).

   (iii) Figures 1 to 4 inclusive. Recent work (not yet published) indicates that cholera toxin increases HCO_3 reabsorption almost exclusively; and that this toxin and norepinephrine can compete with each other in evoking a stimulatory effect on HCO_3 reabsorption (see pages 6 and 7).

5. Since the observed electrical changes can be ascribed to the interactions of each toxin with amino groups, acyl groups or mannosides on the apical membrane surface, to determine whether any of the toxin-induced responses can be modified or eliminated after pre-exposure of the mucosal surface to: (i) SITS or DIDS (4,4'-isothiocyano-substituted disulfonic stilbenes which react covalently with accessible amino groups to form thiocarbamates);

(ii) hydroxylamine (which reacts covalently with acyl groups on amino acid residues to form hydroxammates); and (iii) concanavalin A (which binds to mannoside groups of membrane-bound glycoproteins).

6. Since we now know how to separate the mixed membrane fraction of turtle bladder epithelial cells into apical and basal-lateral components, the next step is to determine the toxin-induced changes in the isolated membrane preparations. This requires the use of lectin affinity chromotography or other techniques (Steck and Kant) for producing inside-out and right side-out vesicles of isolated apical membrane fragments (and/or of basal-lateral membrane fragments). Restricting our comments to the apical membrane (the initial target site of the bacterial toxins),

   it is planned to determine the effect of the addition of each toxin to the fluid bathing inside-out and/or right side-out vesicles of the apical membrane on the transmembrane electrical potential difference ($\Delta\Psi$) and on the ion concentration gradients (e.g., ΔpH, ΔCl, ΔHCO_3) which are achieved after energizing the proton or bicarbonate pump elements with the appropriate substrate and co-factors.

   This aim depends on: (i) the survival of ion pump elements during the procedures used to isolate membrane vesicles; (ii) the choice of the proper substrate for initiating proton or bicarbonate transport in the vesicles, and (iii) the ability to elicit toxin-induced changes superimposed upon the substrate-energized production of transmembrane electrochemical gradients in right side-out apical membrane vesicles.

   Preliminary experiments have already been started with one of the electrical potential-detecting probes, namely the organic cation, tetraphenyl phosphonium (TPP^+).

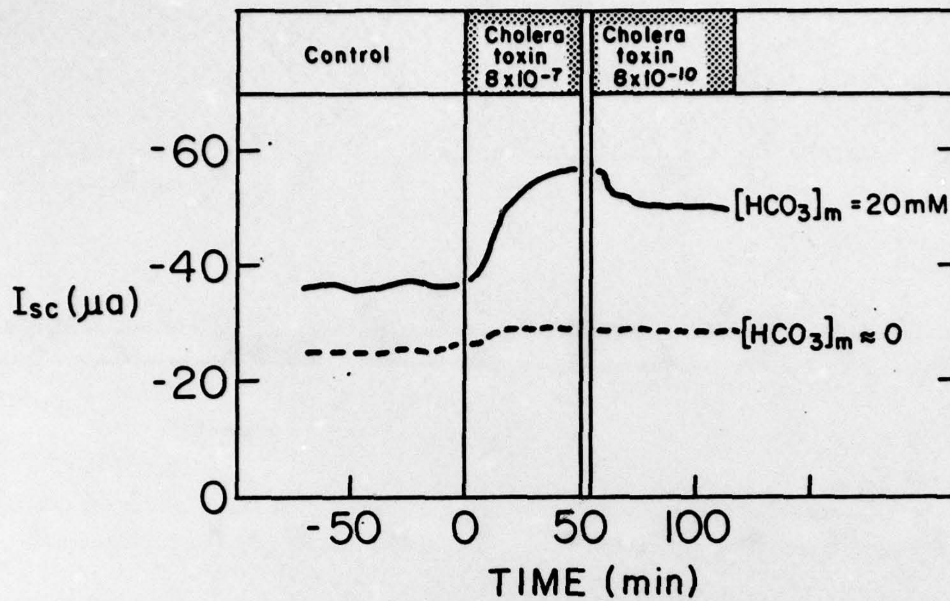

FIG. (1)

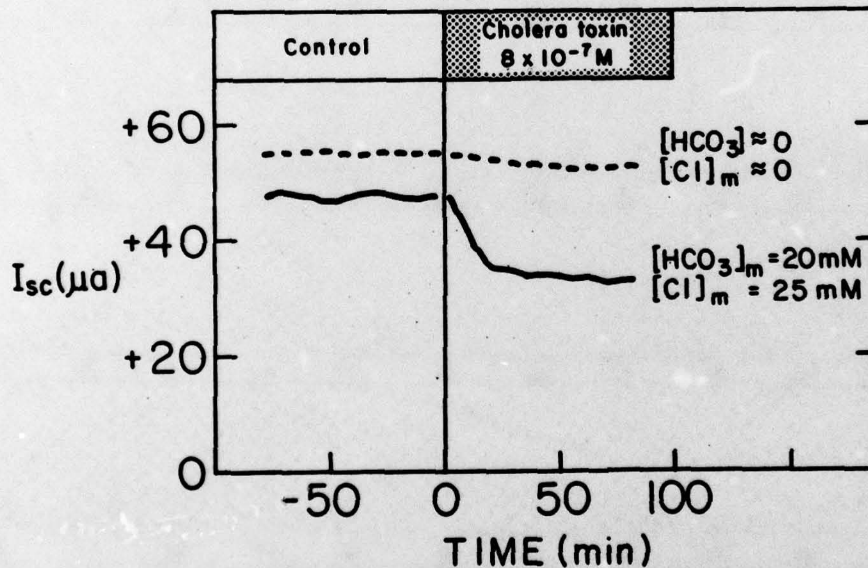

FIG. (2)

Figures 1 and 2. The effect of mucosally-added cholera toxins is to increase the flow of negative short-circuiting current across bladders in Na-free media (Fig. 1) and Na-rich media (Fig. 2); and that under both bathing conditions, the presence of mucosal HCO_3 is a requirement for evoking a cholera-induced increase - presumably in the rate of HCO_3 reabsorption (and/or proton secretion).

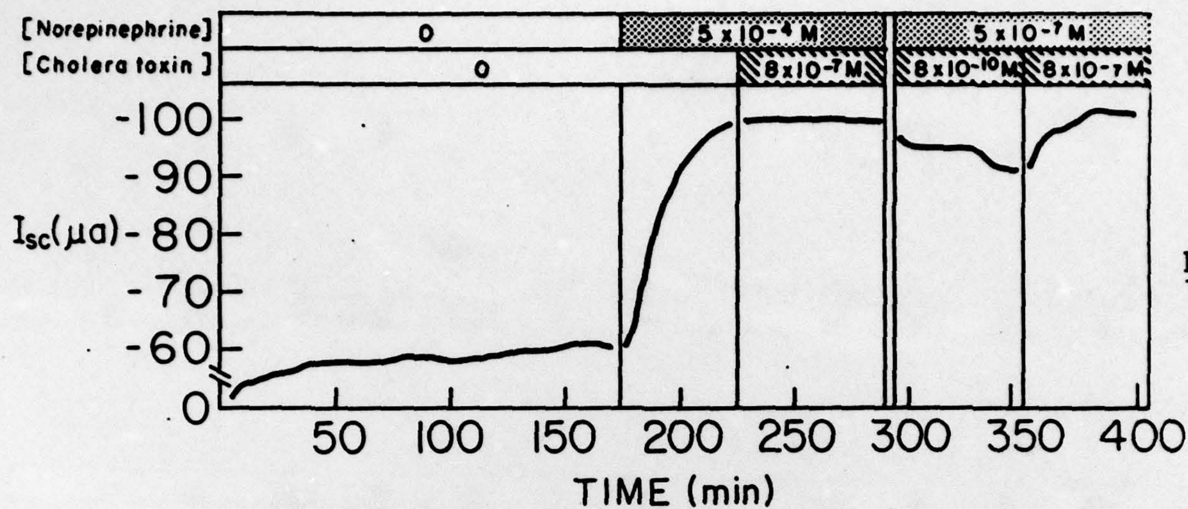

FIG. (3)

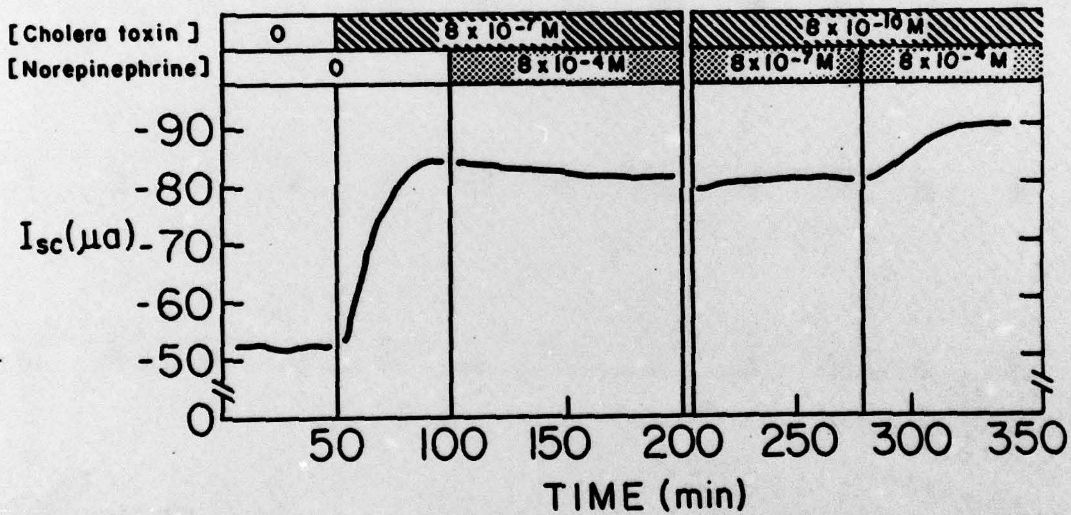

FIG. (4)

Figures 3 and 4. The failure to elicit a cholera toxin-induced increase in the Isc of a bladder pre-exposed to mucosal norepinephrine (Fig. 3); and conversely, the failure to elicit a norepinephrine-induced increased in the Isc of a bladder pre-exposed to mucosal cholera toxin. Both interfering effects are only partially reversible after a 1:1000 dilution of the interfering agent. Bladders bathed in ouabain-supplemented Na-rich Ringer media.

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