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STIMULATION OF Na^+ TRANSPORT ACROSS THE TOAD URINARY BLADDER BY *p*-CHLOROMERCURIBENZENE SULFONATE

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Summary

The sulfhydryl reagent *p*-chloromercuribenzene sulfonate increased the I_{sc} across substrate-replete toad urinary bladder when applied to the mucosal (apical) surface. This increase was accounted for by an increased mucosal to serosal net flux of Na^+. In the absence of substrate, the rise in I_{sc} was accompanied by an irreversible increase in tissue conductance which was not apparent in the replete preparation. These findings suggest that *p*-chloromercuribenzene sulfonate may be useful in marking mucosal functions associated with the Na^+ transport apparatus.

In earlier studies, certain "mild" sulfhydryl reagents, notably PCMBS and NTCB, stimulated the I_{sc} across toad urinary bladder when applied to the mucosal, but not the serosal, epithelial surface [1]. Frenkel et al. [1] also observed that at concentrations that produce a 25—50% increase in I_{sc}, neither of these reagents apparently penetrates the apical plasma membrane, as judged by the titration of total trichloroacetic acid-soluble intracellular SH content. The increase in I_{sc} usually terminated spontaneously within 1 h after application. Both reagents significantly modified the subsequent response of the bladder to either vasopressin or the combination of cyclic adenosine 3′:5′-monophosphate plus theophylline. These findings suggested that the effects of the SH reagents on I_{sc} might be attributable to specific modifications of apical SH functions as-

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Abbreviations: I_{sc}, short-circuit current; PD, potential difference; J^{Na^+}, Na^+ flux; PCMBS, *p*-chloromercuribenzene sulfonate; MalNEt, *N*-ethylmaleimide; NTCB, 2-nitro-5-thiocyanatobenzoic acid; Nbs_2, 5,5′-dithiobis-2-nitrobenzoic acid.

TABLE I

EQUIVALENCE OF PCMBS-STIMULATED I_{sc} AND THE FLUX OF Na^+

Na^+ fluxes and I_{sc} are expressed as μequiv./h/2.5 cm^2 (means ± S.E., n = 8 pairs). Quarterbladders were incubated throughout in 10 mM glucose Ringers. Following preincubation, fluxes and I_{sc} were measured for a 1 h control period (Period I). PCMBS ($3.75 \cdot 10^{-4}$ M) was then added to mucosal solutions of group A and diluent alone to the mucosal solutions of group B. Measurements were continued for 1 h (Period II).

	A. PCMBS	B. Control	$\bar{x}\Delta$ (A − B)
Period I			
$J^{Na^+}_{m\to s}$	6.53 ± 0.71	6.30 ± 0.82	0.22 ± 0.47
$J^{Na^+}_{s\to m}$	0.45 ± 0.07	0.66 ± 0.19	−0.21 ± 0.19
$J^{Na^+}_{net}$	6.07 ± 0.75	5.64 ± 0.85	0.43 ± 0.43
I_{sc}	5.64 ± 0.67	5.36 ± 0.59	0.29 ± 0.28
Period II			
$J^{Na^+}_{m\to s}$	7.10 ± 0.65	5.50 ± 0.55	1.60 ± 0.46*
$J^{Na^+}_{s\to m}$	0.51 ± 0.07	0.69 ± 0.16	−0.18 ± 0.15
$J^{Na^+}_{net}$	6.59 ± 0.68	4.82 ± 0.55	1.77 ± 0.39*
I_{sc}	6.40 ± 0.74	5.10 ± 0.57	1.30 ± 0.31*

*$P < 0.02$ for paired Student "t" comparison between groups A and B. The overall difference between I_{sc} and $J^{Na^+}_{net}$ (0.16 ± 0.17, n = 32) suggest their equivalence during both periods.

sociated with the active transport of Na^+. In these earlier experiments [1] however, no information was provided on whether changes in net Na^+ transport account for the entire effect on I_{sc}. The present studies were undertaken to determine if the response to PCMBS is specifically related to effects on the transport of Na^+ as opposed to other ions, and to evaluate the substrate and conductance relationships of the response.

Pairs of hemibladders, obtained from *Bufo marinus* of Colombian origin (Tarpon Zoo, Tarpon Springs, Fla.) were mounted in split chambers and the I_{sc}, PD and transepithelial resistance (PD/I_{sc}) were determined as described previously [2]. The bathing media contained: 90 mM NaCl, 25 mM $NaHCO_3$, 3 mM KCl, 1 mM $CaCl_2$, 0.5 mM KH_2PO_4, and 0.5 mM $MgSO_4$ supplemented with 800 units/ml penicillin, 0.5 mg/ml streptomycin and 10 μg/ml chloramphenicol [3]. The hemibladders were continuously gassed with 97% O_2 + 3% CO_2 and the temperature (23—25°C), pH (7.5—7.8), and osmolarity (220—230 mosM) were maintained within the indicated ranges. All bathing solutions were routinely replaced with 10 ml of fresh media 1—2 h before introducing any of the reagents.

Bidirectional Na^+ flux measurements were performed with ^{22}Na and ^{24}Na (New England Nuclear). ^{24}Na (5—10 μCi/ml, ≈ 4 Ci/g) was added to each mucosal compartment and a similar addition of ^{22}Na (1—2 μCi/ml, carrier free) was made to each serosol compartment. 45 min was then allowed for equilibration. Thereafter, 0.5 ml samples were withdrawn from each compartment every 20 min for analysis in a Nuclear Chicago 1185 gamma system. ^{24}Na was counted in a 3.0—4.0 Mev window and, after 25—30 ^{24}Na half-lives, ^{22}Na was counted in a 0.78—1.78 Mev window. Appropriate corrections were made for

radioactive decay of ^{24}Na and for changes in specific activities in the media resulting from samplings and additions. PCMBS was purchased from Sigma. The conventional reagents, obtained from Baker and Adams were all analytical grade or spectroquality.

The relationship between the effect of PCMBS on I_{sc} and Na^+ transport was explored by simultaneously comparing bidirectional isotopic Na^+ fluxes with the integrated I_{sc} across continuously short-circuited hemibladders. The results in Table I show that during an initial 1 h control period, net Na^+ flux and I_{sc} were equivalent and of equal magnitude in both hemibladders. After addition of a maximally effective concentration of PCMBS to one hemibladder, significant differences were recorded in $J^{Na^+}_{m\rightarrow s}$, $J^{Na^+}_{net}$, and I_{sc}. The absolute increases in $J^{Na^+}_{net}$ and $J^{Na^+}_{m\rightarrow s}$ agreed reasonably well, but appear somewhat higher than the increase in I_{sc}. This difference, however, was not significant. This result suggests that the effect of PCMBS on I_{sc} is primarily a consequence of increased mucosal to serosal active transport of Na^+, although small effects on the transport of other ions cannot be excluded.

To evaluate the possibility that PCMBS might also impair intracellular processes required for metabolic support of active Na^+ transport, we examined the I_{sc} response to exogenous substrate after pre-incubation for 18 h in substrate-free media. In these experiments, quarterbladders were either pretreated with PCMBS or the diluent before challenge with glucose. Addition of substrate (e.g., glucose or pyruvate) to substrate-deprived bladders of Colombian toads evoked a rapid and sustained increase in I_{sc} [4].

The results in Fig. 1 indicate that glucose and PCMBS gave prompt and

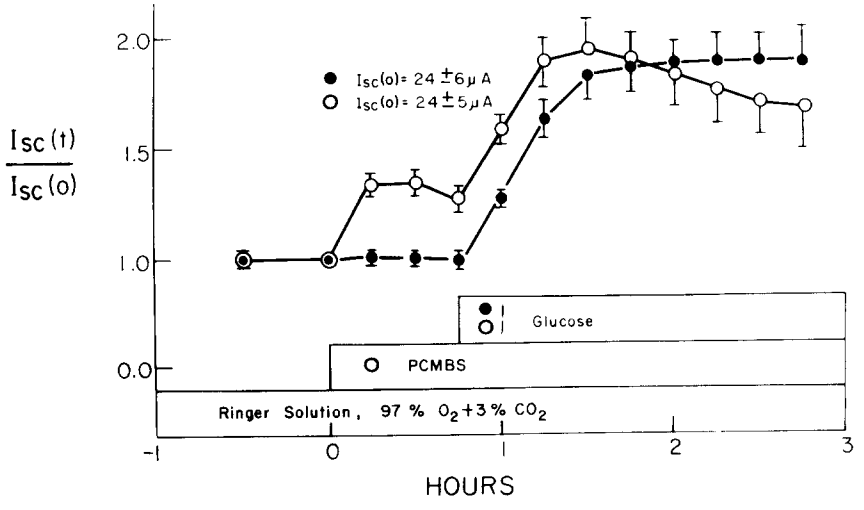

Fig. 1. Effect of PCMBS on the I_{sc} response to glucose in substrate-depleted bladders. Quarterbladders (8 pairs) were preincubated 18 h without substrate. PCMBS (final concentration = $3.75 \cdot 10^{-4}$ M) was added to the mucosal solutions of test quarterbladders at time, t = 0. Controls received only diluent. An isosmotic glucose solution (final concentration = 10^{-2} M) was added to both serosal and mucosal solutions of all groups at t = 45 min. Ordinate ratio expresses the I_{sc} at any time to that at t = 0. I_{sc_0} denotes the absolute I_{sc} recorded at time zero for the test (○——○) and the control (●——●) groups. (Mean ± S.E.). The verticals represent ± 1 S.E.

additive rises in I_{sc}, suggesting that little impairment in the Na^+ transport system had occurred, at least during the early phase of the response. In contrast to earlier experiments using bladders supplied with ample exogenous substrate (cf. Table I), however, we noted that the I_{sc} response to PCMBS obtained as shown in Fig. 1 was accompanied by a progressive decline in total electrical resistance of the bladder.

Accordingly, a direct comparison was made of the effect of PCMBS on I_{sc} and transepithelial electrical resistance in both substrate-depleted and substrate-repleted bladders. The results in Table II confirm that the rise in I_{sc} was accompanied by a significant decline in resistance only in the substrate-deprived hemibladders. It is possible, therefore, that groups required for the functional integrity of the epithelium are exposed in the substrate-depleted state and the possibility is raised of multiple effects in the depleted preparation. A similar elevation in I_{sc} was seen both in the presence and absence of detectable changes in total resistance, suggesting that the primary effect of PCMBS on Na^+ transport involves a pathway that constitutes only a fraction of total tissue conductance.

TABLE II

SUBSTRATE DEPENDENCE OF THE PCMBS EFFECT ON TRANSEPITHELIAL RESISTANCE

I_{sc} and resistance (R) values are given as μA and ohms · 2.5 cm (means ± S.E., n = 8 pairs). Time points are denoted in subscripts (min). Ratios denote values at numerator-subscripted times compared with those at time zero. Quarterbladders were incubated for 18 h either in the presence (A) or absence (B) of 10 mM glucose. PCMBS ($3.75 \cdot 10^{-4}$ M) was added to the mucosal solution of both groups at time zero. Controls received only diluent.

	I_{sc_0}	R_0	$I_{sc_{30}}/I_{sc_0}$	R_{30}/R_0	$I_{sc_{60}}/I_{sc_0}$	R_{60}/R_0
A. Glucose present						
Control	64 ± 12	685 ± 58	0.99 ± 0.01	0.98 ± 0.02	0.97 ± 0.02	0.98 ± 0.02
PCMBS	69 ± 13	665 ± 52	1.26 ± 0.04*	0.94 ± 0.03	0.92 ± 0.05	0.94 ± 0.03
B. Glucose absent						
Control	12 ± 2	772 ± 55	0.99 ± 0.03	1.01 ± 0.01	0.97 ± 0.04	1.01 ± 0.01
PCMBS	15 ± 4	747 ± 51	1.36 ± 0.03*	0.86 ± 0.03*	1.10 ± 0.06	0.79 ± 0.04*

*$P < 0.01$ for paired Student "t" comparison between PCMBS and control groups.

Thus, the effect on mucosal to serosal Na^+ transport probably involves augmentation of conductance properties of SH elements in the mucosal surface, including those involved in Na^+ uptake at this barrier. Alternatively, processes coupling the uptake of Na^+ across the apical boundary with active extrusion at the basal-lateral surfaces may be modulated.

Earlier studies have shown that PCMBS and similar reagents alter both cation and substrate transport at the level of the cell plasma membrane in a variety of cell types [5, 6]. In toad bladder, studies by Frenkel et al. [1] defined the relative actions of several SH reagents on basal I_{sc} and the subsequent response to vasopressin. Thus, titration with PCMBS or NTCB elevated basal I_{sc}, whereas Nbs_2 had no effect and MalNEt depressed basal transport levels. Although these studies do not delineate the mechanism whereby PCMBS alters the I_{sc}, our present findings indicate that the response is the result of alterations in active Na^+ transport. Thus, the selective application of PCMBS and other SH reagents now provide a useful method for dif-

ferentially labeling and isolating SH functions associated with the transit of Na^+ across the apical boundary.

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