BRIEF COMMUNICATION

p-CHLOROMERCURIBENZENE SULFONATE BLOCKS AND REVERSES THE EFFECT OF AMILORIDE ON SODIUM TRANSPORT ACROSS RABBIT COLON IN VITRO

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ABSTRACT The addition of 10^{-3} M *p*-chloromercuribenzene sulfonate (PCMBS) to the solution bathing the mucosal surface of rabbit colon has no effect on the rate of active Na transport but blocks or reverses the inhibitory action of amiloride. The tissue must be exposed to PCMBS for 20–30 min for a complete blocking effect, and removal of PCMBS from the mucosal solution after this period of exposure does not restore the sensitivity of the tissue to amiloride. The slow time-courses of the blocking and reversal effects suggest that PCMBS does not irreversibly interact with groups directly involved in the binding of amiloride.

The processes involved in active transepithelial absorption of Na by descending rabbit colon, in vitro, resemble those responsible for Na transport by isolated frog skin and toad urinary bladder inasmuch as (*a*) the transepithelial electrical potential difference and short-circuit current are entirely attributable to active Na transport; (*b*) Na transport is stimulated by aldosterone after a characteristic 60-min lag period; (*c*) Na entry into the transporting cells across the mucosal membrane is completely blocked by the potent pyrazine diuretic, amiloride; and (*d*) Na transport is abolished by ouabain in the serosal solution and stimulated by amphotericin B in the mucosal solution (Frizzell et al., 1976; Schultz et al., 1977; Frizzell and Schultz, 1977; Frizzell and Turnheim, manuscript submitted for publication). Recently it has been reported that *p*-chloromercuribenzoic acid (PCMB) or its sulfonated derivative (PCMBS) rapidly stimulate Na transport by frog skin (Dick and Lindemann, 1975; Lindemann and Voute, 1976)

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and toad urinary bladder (Frenkel et al., 1975; Spooner and Edelman, 1976). The present studies were initiated to determine whether these sulfhydryl-reactive agents similarly affect Na transport by rabbit colon.

Segments of descending colon removed from white rabbits were stripped of underlying musculature and mounted as flat sheets in a short-circuit apparatus as described previously (Frizzell et al., 1976). Both surfaces of the tissue were bathed with identical solutions whose millimolar concentrations were: Na, 140; Cl, 124; HCO_3, 21; K, 5.4; HPO_4, 2.4; H_2PO_4, 0.6; Mg, 1.2; Ca, 1.2; and glucose, 10. The solutions were maintained at 37°C, and were aerated with a mixture of 95% O_2–5% CO_2; the pH was 7.4. All experiments were carried out under short-circuit conditions, interrupted briefly at 10-min intervals for measurement of the transepithelial electrical potential difference (ψ_{ms}).

Unlike the results reported for frog skin and toad urinary bladder, the addition of 10^{-3} M PCMBS (Sigma Chemical Co., St. Louis, Mo.) to the mucosal solution did not significantly affect the ψ_{ms} or the rate of active Na transport as measured by the short-circuit current, I_{sc}, (Frizzell et al., 1976) during the ensuing 60–90 min. Addition of PCMBS to the serosal solution brought about a marked transient increase in I_{sc} which then declined to zero within 10 min. However, the most surprising finding was that after exposure of the tissue to 10^{-3} M PCMBS for 20–30 min, active transepithelial Na transport was no longer inhibitable by amiloride even at concentrations as high as 10^{-4} M. The absence of a significant effect of PCMBS on the I_{sc} and the ability of this agent to block the effect of amiloride are documented in Fig. 1. When the concentra-

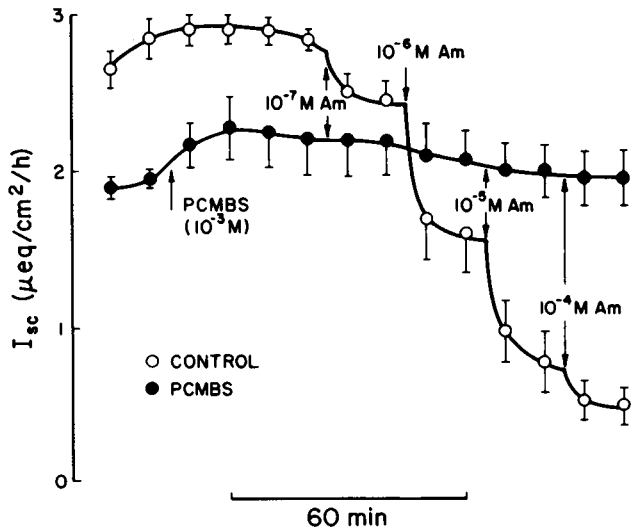

FIGURE 1 The effect of graded doses of amiloride (Am) on the I_{sc} across rabbit colon in the absence (○) and presence (●) of 10^{-3} M PCMBS. Data represent means and SEM of four experiments in the absence of PCMBS and five experiments in the presence of PCMBS. Note that a half-maximal inhibition of the I_{sc} in control tissues is elicited by approximately 10^{-6} M amiloride.

tion of PCMBS in the mucosal solution was less than 10^{-3} M and/or when the tissue was exposed to this agent for less than 15 min, the inhibitory effect of amiloride on the I_{sc} was partially but not completely blocked.

The average results of four experiments in which PCMBS (10^{-3} M) was added to the mucosal solution after the I_{sc} was almost entirely abolished by amiloride (10^{-4} M) are illustrated in Fig. 2 (open circles). Clearly, PCMBS reverses the inhibitory effect of amiloride; the onset of this reversal is rapid but the restoration of the I_{sc} to the pre-amiloride level is slow (45–60 min). In two experiments, bidirectional and net fluxes of Na were determined as described previously (Frizzell et al., 1976) to ensure that the "restored I_{sc}" in fact represents the restoration of active Na transport in the presence of amiloride; the net flux of Na measured using ^{22}Na (2.9 ± 0.4 μeq/cm^2 per h) was in excellent agreement with the "restored I_{sc}" (2.8 ± 0.7 μeq/cm^2 per h).

To exclude the possibility that the effect of PCMBS could be due to an interaction with amiloride, which directly inactivates this diuretic agent, PCMBS and amiloride were mixed in the same stock solution for 30 min and were then added simultaneously to the mucosal solution (final concentrations: 10^{-3} M PCMBS; 10^{-4} M amiloride). As shown in Fig. 2 (closed circles), the I_{sc} rapidly fell to near zero but subsequently increased, and control values were restored within 30–45 min.

Finally, to determine whether the effect of PCMBS is readily reversible, two experiments were performed in which PCMBS (10^{-3} M) was added to the mucosal solution and, 30 min later, the mucosal solution was replaced with the standard (PCMBS-free) electrolyte solution. The addition of amiloride to the mucosal solution of these "pre-treated" tissues had no effect on the I_{sc}.

Amiloride very rapidly inhibits the I_{sc} across rabbit colon but this effect is just as rapidly reversed by removal of the diuretic from the mucosal solution (Schultz et al., 1977; Turnheim et al., 1978). Thus, this agent seems to form loose bonds with readily accessible sites on the mucosal membrane. Although it is known that the im-

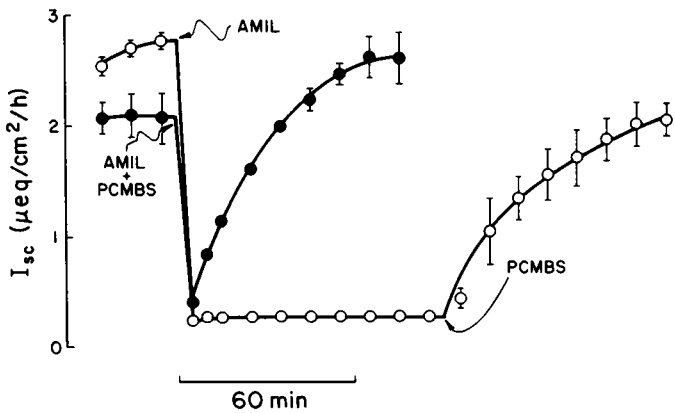

FIGURE 2 The ability of PCMBS to reverse the effect of amiloride when added after (○) or simultaneously with (●) the diuretic. Open circles represent the means of four experiments and closed circles are the means of two experiments.

portant structural groups of this diuretic include the charged amidino group, an amino group at position 5 and Cl at position 6 (Benos et al., 1976; Cragoe et al., 1967), the nature of its interaction with the mucosal membrane of Na-transporting epithelia is unknown. In marked contrast, rabbit colon must be exposed to PCMBS for 20–30 min to completely block the effect of amiloride, and the blocking-action is not reversed by removing PCMBS from the mucosal solution. Also, the reversal of the amiloride effect by PCMBS is a relatively slow process with a half time of 10–15 min. Thus, the action of PCMBS must be the result of the formation of relatively tight bonds with groups that are not readily accessible.[1] It follows that the effect of PCMBS cannot be the result of direct interactions with groups involved in amiloride binding and, by inference, it seems likely that the binding of amiloride does not necessarily involve superficial or readily accessible sulfhydryl groups. However, at the same time, it is unclear whether PCMBS prevents the binding of amiloride or whether it blocks and reverses the action of *bound* amiloride. The resolution of this question could provide important insight into the mechanism of action of amiloride.

We cannot, at this time, reconcile our findings with those reported for frog skin (Dick and Lindemann, 1975; Lindemann and Voute, 1976) and toad urinary bladder (Frenkel et al., 1975; Spooner and Edelman, 1976) where (*a*) PCMBS stimulates Na transport and (*b*) in frog skin, Na transport in the presence of PCMB is inhibited by amiloride[2] (the papers by Frenkel et al. [1975] and Spooner and Edelman [1976] do not indicate whether amiloride inhibits the increased I_{sc} across toad urinary bladder in the presence of PCMBS). However, it is of interest that Li and de Sousa (1977) found that the addition of Ag (10^{-4} M) to the solution bathing the outer surface of frog skin blocks or reverses the inhibitory effect of amiloride (2×10^{-4} M) on the I_{sc}; their figures indicate that the action of amiloride is rapid and that the reversal by Ag is considerably slower. Thus, the effects of Ag on frog skin resemble the effects of PCMBS on rabbit colon. The apparent inability of PCMB to block the action of amiloride in frog skin may be due to the fact that it does not readily gain access to the membrane groups responsible for this effect.

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[1]As discussed by Rothstein (1970), PCMBS is a highly specific sulfhydryl-reactive agent. Nonetheless, one cannot a priori exclude the possibility that its effect in rabbit colon is due to interactions with other membrane constituents, particularly carboxylate or phosphate groups for which this agent has an appreciable affinity. An analogous situation has been reported by Sutherland et al. (1967) for erythrocytes where the effect of PCMBS on cation permeability develops slowly. Studies of the uptake and desorption of PCMBS by erythrocytes supported the notion that this agent does not interact with readily accessible membrane sites (Rothstein, 1970).

[2]The reports by Dick and Lindemann (1975) and Lindemann and Voute (1976) do not indicate the duration of exposure of the tissue to PCMB. Thus, it is possible this agent failed to block the action of amiloride because the exposure was too brief.

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