A ROLE FOR PROTEIN KINASE C IN THE CONTROL OF K SECRETION AND Na ABSORPTION BY THE URINARY BLADDER OF THE WINTER FLOUNDER, PSEUDOPLEURONECTES AMERICANUS

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The urinary bladder of the winter flounder absorbs NaCl and secretes K^+. The absorptive process is electrically silent presumably due to the presence in the apical membrane of a thiazide sensitive NaCl cotransporter (Stokes, J. Clin. Inv. 74:7-16, 1984). In contrast, K^+ secretion is associated with a serosa to mucosa current which is a measure of the rate of conductive K^+exit across the apical membrane via barium-sensitive K^+channels (Dawson & Frizzell, Pflügers Arch 414:393-400, 1989). Although the apical transport steps for the absorptive and secretory processes have been characterized, there are, as yet, no known hormonal regulators of salt transport in this tissue, and nothing is known about intracellular events which might regulate ion flows at the apical or basolateral membrane. The purpose of these experiments was to investigate the possibility that the ubiquitous enzyme, protein kinase C, might have some role in the regulation of salt transport by the bladder. The results provide evidence for inhibitory control of both NaCl absorption and K^+secretion.

Urinary bladder from winter flounder was mounted as a flat sheet as previously described in either Ussing chambers (0.287 cm^2) for $^{22}Na^+$ flux studies or in perfusion chambers for electrophysiological studies in the absence of radioisotope (Wilkinson & Dawson, Bull. M.D.I.B.L. 29:108-109, 1990). All fluxes were measured under short circuit conditions (serosal bath as reference) using the sample and replace paradigm of Dawson (J. Membr. Biol. 37:213-233, 1977), modified as previously described (Post & Dawson, Bull. M.D.I.B.L., this issue). Solutions for flux measurements consisted of (in mM): 140 Na^+, 147.5 Cl^-, 2.5 K^+, 1.5 Ca^{2+}, 1.0 Mg^{2+}, 15 HEPES, and 10 glucose, at a pH of 7.5. 100 µM verapamil was present in the serosal bath to minimize smooth muscle activity. Solutions for perfusion experiments were similar except that, where indicated, 10 mM K Gluconate was added to the mucosal perfusate to effect changes in mucosal K^+concentration.

Figure 1 presents evidence that phorbol esters inhibit both K^+ secretion and Na^+ absorption. I_{SC}, a direct measure of K^+secretion (Dawson & Frizzell, Pflügers Arch 414:393-400, 1989), and the mucosal to serosal $^{22}Na^+$ rate coefficient ($\lambda^{\bullet}_{MS}$), a measure of Na^+ absorption (Stokes, J. Clin. Inv. 74:7-16, 1984), are plotted versus time for a bladder secreting K^+. The polarity of the current was consistent with positive charge movement from the serosal (S) to the mucosal (M) bath. The rate coefficient for $^{22}Na^+$ flow was indicative of a transmural pathway for M to S Na^+ movement. Addition of 10 nM 4-β-phorbol 12,13 myristate acetate (PMA) to the mucosal bath decreased both I_{SC} and $\lambda^{\bullet}_{MS}$. The relatively minor effect of the subsequent mucosal addition of 100 µM hydrochlorothiazide (HCT), a known inhibitor of

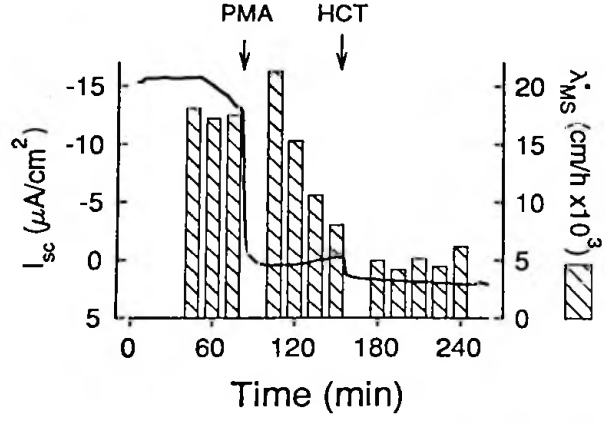

Figure 1 10 nM 4-β-phorbol 12,13 myristate acetate (PMA) inhibited most of the hydrochlorothiazide (HCT) sensitive Na^+ absorption and K^+ secretion in flounder urinary bladder.

the NaCl cotransporter (Stokes, J. Clin. Inv. 74:7-16, 1984), is consistent with the notion that the phorbol ester eliminated most of the NaCl cotransport activity as well as K^+ secretion.

Inhibition of electroneutral NaCl cotransport by HCT or Hg^{2+} inhibits K^+ secretion , presumably by decreasing the driving force for K^+ exit across the apical membrane (Wilkinson & Dawson, Bull. M.D.I.B.L. 29:108-109, 1990). Thus PMA could inhibit K+ secretion either indirectly by inhibiting the NaCl cotransporter or by a direct effect on the apical K^+ conductance (or both). Figure 2 shows I_{sc} for a urinary bladder mounted in a perfusion chamber. Apical K^+ conductance was assessed by determining the effect of a 10mM step increase of the mucosal K^+ concentration (indicated in figure 2 as a solid bar along the time axis). As shown in the figure, 0.5 µM Hg^{2+} reduced I_{sc} but, as indicated by the response to the step increase in mucosal K^+ concentration, the apical K^+ conductance was, if anything, increased as has been previously reported. In contrast, after inhibition of I_{sc} by 10 nM PMA apical K^+ conductance was reduced or absent. Exposure to up to 1 µM 4-α-phorbol 12,13 didecanoate, an inactive phorbol ester, was without effect on either I_{sc} or G_T (not shown). These results support the notion that PMA decreased K^+ secretion via inhibition of the apical K^+ conductance.

Figure 3 shows that 100 µM 1,2 dioctanoyl-sn-glycerol (C:8) (DAG), a diacylglycerol analogue, also inhibited both Na^+ transport and K^+ secretion. The subsequent addition of 100 µM HCT to the mucosal bath revealed that the DAG exerted a more striking effect on K^+ secretion than on Na^+ absorption.

The profound inhibitory action of 10 nM PMA and the weaker effects of 100 µM DAG contrasts sharply with the ineffectiveness of 1 µM 4-α-phorbol 12,13 didecanoate at inhibiting transmural ion movement. This pharmacological profile is consistent with a mechanism of action involving protein kinase C (Evans et al., Bioch. Soc. Trans. 19:397-402, 1991). The data presented in this report are consistent with the notion that both apical K^+ channels and thiazide sensitive NaCl cotransporters are under the inhibitory control of protein kinase C.

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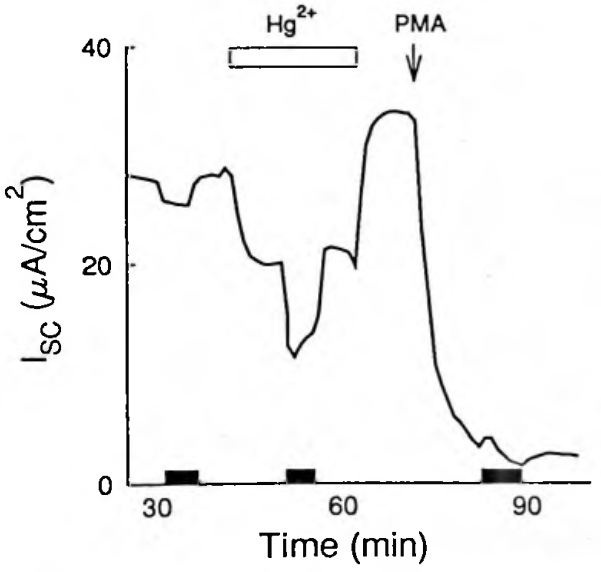

Figure 2 Phorbol esters (10 nM) reduced the apical K^+ conductance of flounder urinary bladder.

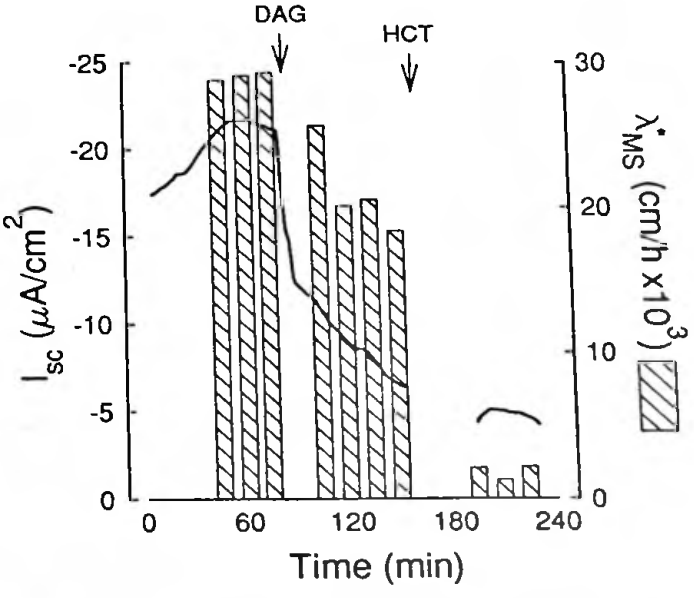

Figure 3 100 µM 1,2 dioctanoyl-sn-glycerol (C:8) inhibited both K^+ secretion and Na^+ absorption by flounder urinary bladder.