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Methylation Increases Sodium Transport into A6 Apical Membrane Vesicles: Possible Mode of Aldosterone Action

Abstract. *When isolated apical membrane vesicles prepared from cultured A6 epithelia were incubated in vitro with the methyl donor* S-*adenosylmethionine, the control rate of amiloride-inhibitable sodium transport was doubled. The methylation inhibitors 3-deazaadenosine and* S-*adenosyl homocysteine returned the* S-*adenosylmethionine–stimulated sodium transport to control levels. Neither these agents nor adenosine affected sodium transport into control vesicles. In vesicles incubated with* S-*adenosyl-[*^{3}*H-methyl]methionine, both membrane phospholipids and proteins were labeled, and this labeling was inhibited by deazaadenosine. In vesicles prepared from A6 cells treated with aldosterone, sodium transport was twice the control value and* S-*adenosylmethionine did not cause any further stimulation of transport. In those vesicles, both lipid and protein methylation were increased. These results suggest that methylation, which increases the rate of amiloride-sensitive sodium transport, is involved in the action of aldosterone at the apical membrane level in epithelia.*

Aldosterone increases sodium transport across responsive epithelia, including the epithelia formed in tissue culture by the amphibian kidney cell line A6 (*1*). Part of this action is an increase in sodium flux into the cells across their apical membranes (*2*), possibly caused by activation of previously quiescent sodium channels in those membranes (*3–5*). We found earlier that apical membrane vesicles prepared from cultured A6 epithelia contain amiloride-sensitive sodium channels (*6, 7*). Amiloride-sensitive sodium transport into these vesicles was twice as rapid when the A6 epithelia were incubated with aldosterone before vesicle preparation (*7*). This result was the starting point for the present studies. Wiesmann *et al.* (*8*) observed that aldosterone increased methylation in intact cultured TB-6c epithelia (a continuous toad bladder cell line) and that inhibition of the methylation prevented the increase in sodium transport caused by aldosterone. To test whether aldosterone acts by increasing methylation of the phospholipids surrounding sodium channels or by methylation of some proteins—perhaps the channels themselves—thereby increasing sodium flux through the channels, we methylated isolated apical membrane vesicles from A6 cells in vitro. Amiloride-sensitive sodium transport was measured and compared to the rate of transport observed in vesicles prepared from aldosterone-treated A6 cells (*9*).

Incubation of vesicles from control cells with *S*-adenosylmethionine ($10^{-4}M$) increased the amiloride-sensitive transport of sodium into the vesicles from 0.20 ± 0.02 to 0.37 ± 0.02 nmol/min per milligram of protein ($n = 9$; $P < 0.05$). In contrast, in vesicles prepared from cells incubated with aldosterone ($10^{-7}M$ for 4 hours) amiloride-sensitive sodium transport [0.42 ± 0.03 nmol/(min · mg)] was not further increased by *S*-adenosylmethionine [0.39 ± 0.02 nmol/(min · mg); $n = 5$]. In other words, the effects of aldosterone and *S*-adenosylmethionine were not additive. From the lack of effect of *S*-adenosylmethionine in vesicles made from aldosterone-treated cells, we infer that the methylating system was already fully activated in those cells and that this activation was not lost during the membrane isolation procedure.

Since methylating enzymes are associated with plasma membranes (*10*) and methyl groups can be incorporated into plasma membrane phospholipids (*11*), it seemed likely that addition of the methyl donor *S*-adenosylmethionine would increase methylation in the vesicles. As shown in Fig. 1, addition of *S*-adenosyl-[^{3}H-methyl]methionine resulted in labeling of phospholipids, and this labeling was inhibited by 3-deazaadenosine, an inhibitor of methylation. Protein carboxymethylation (*12*) was also observed (502 ± 25 dpm per milligram of protein) and was reduced (156 ± 12 dpm/mg) by the addition of $3 \times 10^{-4}M$ deazaadenosine. To test whether aldosterone indeed increased methylation at the apical membrane level, we incubated intact A6 epi-

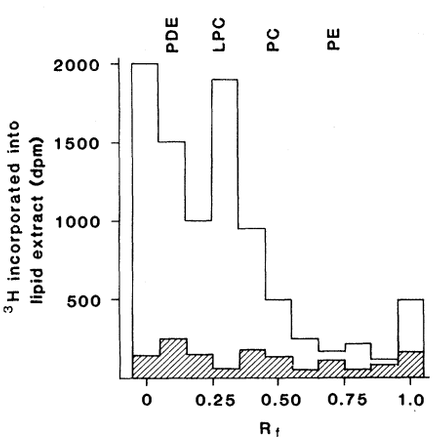

Fig. 1. Methylation by $10^{-4}M$ *S*-adenosylmethionine in vitro. Chromatographically pure *S*-adenosyl-[^{3}H-methyl]methionine (5 μCi/ml) was added to vesicles suspended in either diluent (open bars) or deazaadenosine (300 μ*M*) (hatched bars) and incubated for 35 minutes at room temperature. The suspension was then centrifuged at 17,000*g* for 10 minutes at 4°C, and the pellet was rinsed twice with ice-cold 145 m*M* NaCl. Lipids were extracted as described earlier (*17*). The lipid extract was chromatographed on silica gel plates (Baker) in a mixture of chloroform, propanol, propionic acid, and water (2:3:2:1). One-centimeter sections were scraped from the thin-layer plates directly into vials for liquid scintillation counting. Phospholipid standards run on the same plate were made visual with I_2 vapor. They had the following relative mobilities (R_f): phosphatidylcholine (PC), 0.52; phosphatidylethanolamine (PE), 0.73; lysophosphatidylcholine (LPC), 0.27; and phosphatidyldimethylethanolamine (PDE), 0.13.

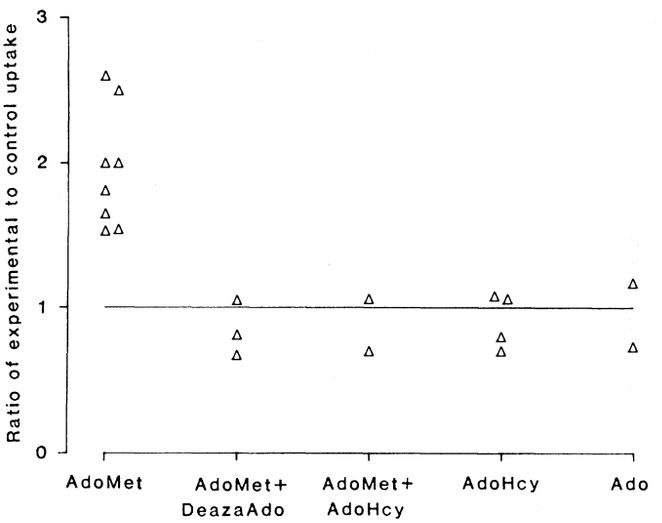

Fig. 2. Effect of *S*-adenosylmethionine, its metabolites, and inhibitors of methylation on sodium transport by A6 vesicles. The vesicles were incubated for 12 minutes at room temperature with no additions (control) or with the combinations of agents shown. Concentrations used were: *S*-adenosylmethionine (AdoMet), $10^{-4}M$; deazaadenosine (DeazaAdo), $10^{-4}M$; *S*-adenosylhomocysteine (AdoHcy) (with *S*-adenosylmethionine), $10^{-3}M$; *S*-adenosylhomocysteine (alone), $10^{-5}M$ and $10^{-4}M$; adenosine (Ado), $10^{-4}M$. Only the addition of *S*-adenosylmethionine by itself caused a significant change in sodium uptake in comparison with the control.

thelia for 5 hours with [^{3}H]methionine (5 μCi/ml) in the absence (control) or presence of aldosterone ($10^{-7}M$). Vesicles were then prepared, and the incorporation of the radioactive label into membrane lipid and protein was measured. Protein labeling in vesicles from aldosterone-treated cells (416 ± 143 dpm/mg) was significantly greater than that in control vesicles (80 ± 35 dpm/mg; $n = 3$). Label incorporation into phosphatidylcholine was 6875 ± 324 dpm/mg in vesicles from aldosterone-treated cells as compared to 4777 ± 463 dpm/mg in control vesicles ($n = 3$).

In order to test whether methylation was responsible for the increase in sodium transport after the addition of *S*-adenosylmethionine, we incubated vesicles with *S*-adenosylmethionine metabolites and inhibitors of methylation (Fig. 2). *S*-Adenosylhomocysteine and adenosine, which are nonmethylating metabolites of *S*-adenosylmethionine, did not affect the amiloride-sensitive sodium transport. Both deazaadenosine ($10^{-4}M$) and *S*-adenosylhomocysteine ($10^{-3}M$), which inhibit methylation (*13*), prevented the increase in sodium transport with *S*-adenosylmethionine. Furthermore, in vesicles prepared from aldosterone-treated A6 cells, the addition of deazaadenosine ($10^{-4}M$) as well as *S*-adenosylhomocysteine ($10^{-3}M$) caused a significant decrease in amiloride-sensitive flux from 0.48 ± 0.034 to 0.25 ± 0.023 nmol/(min · mg) ($n = 3$), a value close to that of controls.

Methylation of membrane phospholipids has been reported to modify the function of receptors and carriers in membranes (*10, 14*), and carboxymethylation of proteins has been reported to modify enzyme activity (*15*). At this point we do not know whether either of these reactions is responsible for the effects of *S*-adenosylmethionine and aldosterone.

In summary, a number of observations lead us to suggest that methylation of apical membrane phospholipids or proteins (or both) contributes to the increase in apical sodium transport that aldosterone causes in cultured amphibian urinary tract epithelia and presumably in other responsive epithelia. These observations include the following. (i) Methylation of apical membrane vesicles in vitro increases sodium transport in vesicles from A6 cells grown without aldosterone. (ii) This increase in amiloride-sensitive sodium transport matches the stimulation of sodium transport measured in vesicles prepared from cells treated with aldosterone. (iii) Methylation in vitro does not further increase the already elevated sodium transport in vesicles prepared from A6 cells incubated with aldosterone. (iv) Inhibitors of methylation abolish the stimulation of sodium transport both in vesicles treated with *S*-adenosylmethionine and in vesicles prepared from aldosterone-treated A6 cells. (v) Lipid and protein methylation is increased in vesicles prepared from aldosterone-treated A6 cells.

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