Pflügers Arch (1985) 403:236–239

Pflügers Archiv
European Journal of Physiology
© Springer-Verlag 1985

Aldosterone does not stimulate the Na:K pump in isolated turtle colon

Dan R. Halm [1] **and David C. Dawson** [2]

[1] Department of Physiology and Biophysics, University of Alabama in Birmingham, Birmingham, AL 35294, USA
[2] Department of Physiology, University of Michigan, Ann Arbor, MI 48109, USA

Abstract. The study of the mechanisms by which mineralocorticoids stimulate sodium absorption across distal epithelia has focused on three possible sites of action: apical sodium permeability, the basolateral Na:K pump, and the production of high-energy substrates. Recently we developed a method for direct measurement of the current generated by the basolateral Na:K pump of the turtle colon [15]. In the presence of mucosal amphotericin-B and serosal barium the short-circuit current across the colon can be equated with the current produced by active electrogenic exchange of sodium for potassium across the basolateral membrane. This pump current is a measure of the transport capacity of the epithelial Na:K pump that is uncomplicated by changes in apical membrane sodium permeability. Pump currents, thus defined, were compared in control tissues and tissues treated with aldosterone in vitro. After 9 h Na absorption was increased 4-fold in the aldosterone-treated tissues but the values of the pump current were identical in the two groups. This result indicates that acute stimulation of sodium absorption by aldosterone does not occur by stimulating the Na:K pump directly.

Key words: Aldosterone – Na:K pump – Colon – Amphotericin B – Barium

Introduction

Aldosterone has been shown to stimulate sodium absorption across distal epithelia such as the renal collecting tubule [9], urinary bladder [7, 16, 22] and colon [10]. The process by which aldosterone stimulates sodium transport has been studied extensively [3, 8, 9, 22], and three possible mechanisms for aldosterone stimulation of sodium absorption have been considered: 1) an increase of apical Na permeability, 2) direct stimulation of the Na:K pump, 3) an increase in the availability of high-energy substrates to the pump. Though a great deal of evidence indicates that aldosterone induces an increase in the Na conductance of the apical membrane [1, 4, 16–18, 25], the existence of a direct action of aldosterone on the Na:K pump has been more difficult to determine.

Offprint requests to: D. R. Halm at above address

The demonstration of a direct effect of aldosterone on the Na:K pump in epithelia is complicated by the series arrangement of the apical membrane Na channels and basolateral membrane pumps. In this configuration, changes in transepithelial Na absorption due to a primary alteration of Na:K pump activity could be accompanied by secondary changes in apical membrane Na conductance and basolateral membrane K conductance. Possible mechanisms for regulating these conductive elements have been discussed recently [21]. Specifically, a decrease of apical Na conductance has been observed in connection with increases in either the cellular calcium activity [2] or the cellular Na activity [18, 24]. One approach to the question of possible basolateral effects of mineralocorticoids has been to study Na:K ATPase isolated from mineralocorticoid treated animals [6, 19, 20]. A second approach has been to functionally eliminate the apical membrane as a barrier to transport by using polyene antibiotics, such as amphotericin-B or nystatin [10, 22] to increase apical cation permeability. In toad bladder and rabbit colon this artificial increase of apical membrane permeability eliminated the difference in Na absorption between control and aldosterone-treated tissues, suggesting that the primary effect of the mineralocorticoid was at the apical membrane. In these experiments, however, the polyene-induced I_{sc} was not identified with the Na:K pump. Recently we showed that in the presence of mucosal amphotericin and serosal barium, I_{sc} across the turtle colon could be equated with the turnover of the electrogenic Na:K pump [13, 15]. This procedure thus permitted us to measure directly the transport function of the basolateral Na:K pump in the presence and in the absence of exogenous aldosterone. The results presented here demonstrate clearly that aldosterone does not have a short-term effect on the transport rate of the Na:K pump in the turtle colon.

Methods

The colon of the freshwater turtle was stripped of surrounding musculature and bathed by aerated Ringer's solution in Ussing chambers with an aperture of 5.2 cm^2 [5]. The transmural P.D. was measured with calomel half-cells connected by Ringer-agar bridges to both chamber halves; current could be passed across the tissue with Ag-AgCl electrodes also connected by Ringer-agar bridges to both chamber halves. The transepithelial electrical PD was maintained at zero by automatic voltage clamp circuits, with provision for fluid resistance compensation. The Ringer solution contained (in mM): 114 Na, 3 K, 1 Ca, 114.5 Cl,

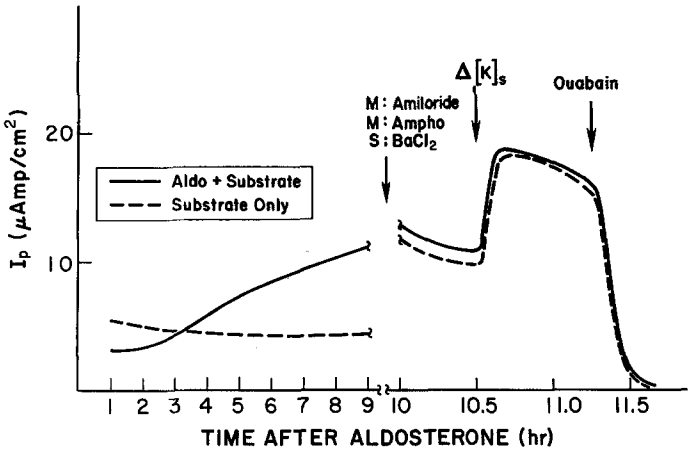

Fig. 1. Pump current: response to aldosterone. The current responses of a representative pair of tissues are shown (pair 1 from Table 1). The I_{pump} was calculated from the I_{sc} as described in the appendix: I_{pump} equals one-third the I_{sc} before amphotericin B addition, and I_{pump} equals I_{sc} after mucosal amphotericin B and serosal barium

2.5 HCO_3^-, 2 pyruvate, 5 glucose, 5 mannitol. The major anion of the bathing solutions was either chloride or benzene sulfonate. Benzene sulfonate Ringer was used during treatment with amphotericin B to aid the maintenance of stable tissue conductance, presumably by reducing osmotic lysis that results from net salt entry into epithelial cells during polyene treatment.

The response of I_{sc} to amphotericin B was maximal at a concentration of 8 μmol/l [13], so that all measurements of I_{pump} were made at this polyene concentration. Amphotericin B was added as a concentrated solution in dimethyl sulfoxide (DMSO had no effect alone). Aldosterone was obtained from Sigma, St. Louis, MO, USA, amphotericin B from Squibb, Princeton, NJ, USA, and amiloride was a gift from Merck, Sharp and Dohme, Rahway, NJ, USA.

Results

Portions of stripped turtle colon were incubated with aerated Ringer solution in Ussing chambers for 15 h without the standard energy substrates, glucose and pyruvate. This procedure was employed to deplete endogenous aldosterone and reduce Na absorption to basal levels. Replacement of the incubation solutions with Ringer solutions containing substrate produced a small rise in I_{sc} of about 2 μA/cm^2, to a value of 8 ± 2 μA/cm^2 (mean ± SEM, $n = 4$). Addition of 5 μM aldosterone to the serosal solution produced an increase in the I_{sc}, beginning after a quiescent period of 60–120 min. Nine hours after aldosterone addition, the I_{sc} of aldosterone-treated tissues ranged from 30–65 μA/cm^2 (42 ± 8 μA/cm^2), while the I_{sc} of control tissues had increased by 4 ± 3 μA/cm^2, to a mean value of 14 ± 3 μA/cm^2. Figure 1 shows a representative experiment.

The pump current (see Appendix) was measured after 9 h, when sodium absorption by aldosterone treated tissue was maximally stimulated. The bathing solutions were replaced with benzene sulfonate Ringer solutions, mucosal amiloride (10^{-4} M) was added to inhibit the native apical sodium conductance, and serosal barium (5 mM) was added to inhibit the native basolateral potassium conductance. The subsequent addition of mucosal amphotericin B (8 μM) induced identical pump currents in both control and treated tissues (Fig. 1). Serosal addition of 7 mM KCl resulted in identical increases in I_{pump} in both tissues, consistent with the dependence of the Na:K pump on serosal potassium [13, 15]. The observed I_{pump} was completely inhibited by serosal addition of ouabain (10^{-4} M), confirming that the current represented Na:K pump activity. The similarity of maximal pump rates for control and aldosterone-treated tissues indicated that the Na:K pump was not the primary site for aldosterone stimulation, but rather suggests that the low native apical Na permeability in the control tissue limited the rate of pump turnover.

Discussion

The measurement of the current produced by active, electrogenic exchange of Na for K provides a direct assay of pump activity which focuses on the transport function, rather than the ATPase function, of the epithelial Na:K pump. The pump currents measured in this study were stimulated to near maximal levels by a mucosal solution sodium concentration about 30 times higher than the apparent dissociation constant of Na (K_{Na}) for pump activation and by increasing the serosal potassium concentration to 10 mmol/l, a value three times higher than the K_K of the pump [13]. Therefore, a difference in I_{pump} would reflect a true difference in maximal pump rate, presumably related to the number of pump sites. Alterations in cellular ionic composition brought about by amphotericin-induced changes in apical cation permeability could alter the number of operating pumps. Presumably, however, both control and aldosterone-treated tissues would respond similarly to such non-specific effects. The ability of control and aldosterone treated tissues to produce identical rates of pump turnover is consistent with the notion that the initial effect of aldosterone on the turtle colon was not to stimulate the Na:K pump directly[1]. The response of I_{pump} to serosal potassium also suggests that the kinetics of potassium activation were unchanged by aldosterone. The increase in I_{sc} produced by aldosterone probably resulted from an increase in apical sodium entry as shown by other investigators [18, 22] rather than an effect on the Na:K pump.

The response of the Na:K ATPase to aldosterone has been studied in the kidney by measuring differences in enzymatic activity in isolated nephron segments of experimentally treated animals [6, 20]. A decrease of nephron Na:K ATPase activity in rabbits was observed 8–14 days after adrenalectomy [20]. Three hours after aldosterone addition to adrenalectomized rabbits, the Na:K ATPase activity in isolated cortical collecting tubules was elevated above the adrenalectomized control levels. However, if amiloride, a blocker of epithelial Na channels, was administered to a rabbit prior to aldosterone addition, then Na:K

1 It should be noted that the aldosterone concentration employed in this study was above the presumed physiological level, 10^{-9}–10^{-8} M [22]. However, a concentration in the region of 10^{-6} M has been used for *in vitro* studies with both amphibian [4] and mammalian [7, 10] tissues, to maximize the response. The use of a high concentration raises the possibility of glucocorticoid effects, but should not have precluded observation of mineralocorticoid effects

Table 1. Pump current: response to aldosterone

Tissue pair	I_{pump} (aldosterone treated tissue) / I_{pump} (control tissue)			
	A After initial incubation	B 9 h after aldosterone addition	C After amphotericin B stimulation	D After serosal addition of 7 mM KCl
1	0.55	2.57	1.11	1.05
2	0.87	3.43	1.14	0.94
3	0.89	3.30	1.05	0.99
4	0.54	3.10	1.22	1.11
Mean ± SEM	0.71 ± 0.10	3.10 ± 0.19	1.13 ± 0.04	1.02 ± 0.04

ATPase activity did not increase. This might have resulted from a direct inhibiton of the Na:K ATPase by amiloride, as reported for the proximal tubule [23]. However, the effective plasma concentration of amiloride was probably lower than that needed to cause such an inhibition of the Na:K ATPase. Amiloride inhibition of aldosterone-induced increases in Na:K ATPase is consistent with the notion that the latter effect was secondary to the increase in sodium entry.

The results presented here show that an increase of apical sodium entry caused by amphotericin-B can eliminate the difference in sodium transport observed between control and aldosterone-treated tissues. It is important to note that the conditions for the isolated kidney tubule studies were different than those for the isolated turtle colon. In the kidney Na:K ATPase experiments, the animals had been adrenalectomized for nearly 2 weeks, but the turtle colon was without mineralocorticoids and glucocorticoids for less than 24 h, so that a decline in Na:K ATPase activity may not have occurred in the turtle colon as occurred in the tubules form adrenalectomized animals. Also, it is difficult to determine if an Na:K ATPase activity assay includes latent non-basolaterally located enzyme activity which is not directly correlated with transport [19]; whereas, the present study provides an direct measure of transport. These recent findings with renal tubules and the colon support the hypothesis that the initial site of action for aldosterone is not the Na:K ATPase, but rather the apical membrane sodium conductance. However, these findings do not preclude the possibility that aldosterone may induce long-term changes in either Na:K pump activity [3, 25] or the availability of energy substrates to the pump [3, 8, 11].

Appendix

The rate of net sodium absorption across the turtle colon has been shown to equal the I_{sc} [5] and be dependent on an electrogenic basolateral membrane Na:K exchange pump [15]. The pump current [eq. (1)] equals the difference in Na and K flows through the pump.

$$I_{pump} = I^{Na}_{pump} - I^{K}_{pump} = (n^{Na}_{pump} - n^{K}_{pump})I_{pump}. \quad (1)$$

The I_{sc} is equal to the sum of the ionic currents across either cellular membrane, apical or basolateral, and the cations Na and K have been shown to carry these currents [12, 15],

$$I_{sc} = I^{Na}_{apical} - I^{K}_{apical}. \quad (2)$$

At steady-state I^{Na}_{apical} equals I^{Na}_{pump}, since the basolateral membrane sodium conductance is small, as reported previously [14]. The size of I^{K}_{apical} will depend on the relative potassium conductances of the apical and basolateral membranes,

$$I^{K}_{apical}\,[g^{K}_{a}/(g^{K}_{a} + g^{K}_{b})]\; I^{K}_{pump} = I^{K}_{pump}/(1+\alpha),\ \alpha = g^{K}_{b}/g^{K}_{a}. \quad (3)$$

Combining equations (1, 2 and 3) yield a relationship between I_{sc} and I_{pump}.

$$I_{sc} = I^{Na}_{pump} - I^{K}_{pump}/(1+\alpha)$$
$$= \{[v(1+\alpha)-1]/(1+\alpha)(v-1)\}I_{pump},\ v = n^{Na}_{pump}/n^{K}_{pump} \quad (4)$$

where v is the pump exchange stoichiometry.

The native turtle colon exhibits a g^{K}_{a} much smaller than g^{K}_{b} [12], such that α approaches infinity,

$$\lim_{\alpha\to\infty} I_{sc} = [v/(v\text{-}1)]\, I_{pump} = 3\ I_{pump},\ v = 3/2. \quad (5)$$

Therefore, the stoichiometry of the Na:K exchange pump will define the relation between I_{pump} and I_{sc}, such that the previously observed 3 Na:2 K stoichiometry [15] results in the I_{pump} equaling one-third of the measured I_{sc}. When g^{K}_{b} is inhibited with barium and amphotericin B increases g^{K}_{a} (α approaches zero), I^{K}_{apical} equals I^{K}_{pump} such that I_{pump} equals I_{sc} independent of the exchange stoichiometry,

$$\lim_{\alpha\to 0} I_{sc} = I_{pump}. \quad (6)$$

Acknowledgements. This research was supported by grants from the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases (AM29786) and the Michigan Heart Association. Dr. Dawson was the recipient of a Research Career Development Award from NIADDK (AM00994).

References

1. Civan MM, Hoffman R (1971) Effect of aldosterone on electrical resistance of toad bladder. Am J Physiol 220:324–328
2. Chase HS, Al-Awqati Q (1983) Calcium reduces the sodium permeability of luminal membrane vesicles from toad bladder: Studies using a fast-reaction apparatus. J Gen Physiol 81: 643–665
3. Cox M, Geheb M (1984) Aldosterone-induced proteins in renal epithelia. In: Wade JB, Lewis SA (eds) Current topics in membranes and transport, vol 20. Academic Press, New York, pp 271–293

4. Crabbe J (1980) Decreased sensitivity to amiloride of amphibian epithelia treated with aldosterone: Further evidence for an apical hormonal effect. Pflügers Arch 383:151–158
5. Dawson DC (1977) Sodium and chloride transport across the isolated turtle colon: Parallel pathways for transmural ion movement. J Memb Biol 37:213–233
6. Doucet A, Katz AI (1981) Short-term effect of aldosterone on Na-K-ATPase in single nephron segments. Am J Physiol 241:F273–F278
7. Eaton DC (1981) Intracellular sodium ion activity and sodium transport in rabbit urinary bladder. J Physiol 316:527–544
8. Edelman IS (1975) Mechanism of action of steroid hormones. J Steroid Biochem 6:147–159
9. Fanestil DD, Park CS (1981) Steroid hormones and the kidney. Ann Rev Physiol 43:637–649
10. Frizzell RA, Schultz SG (1978) Effect of aldosterone on ion transport by rabbit colon *in vitro*. J Memb Biol 39:1–26
11. Garty H, Edelman IS, Lindemann B (1983) Metabolic regulation of apical sodium permeability in toad urinary bladder in the presence and absence of aldosterone. J Memb Biol 74: 15–24
12. Halm DR, Dawson DC (1984) Potassium transport by turtle colon: Active secretion and active absorption. Am J Physiol 246:C315–C322
13. Halm DR, Dawson DC (1983) Cation activation of the basolateral sodium-potassium pump in turtle colon. J Gen Physiol 82:215–329
14. Kirk KL, Dawson DC (1983) Mechanism of epithelial lithium transport: Evidence for basolateral Na:Na and Na:Li exchange. J Gen Physiol 82:497–510
15. Kirk KL, Halm DR, Dawson DC (1980) Active sodium transport by turtle colon via an electrogenic Na:K exchange pump. Nature 287:237–239
16. Lewis SA, Wills NK (1983) Apical membrane permeability and kinetic properties of the sodium pump in rabbit urinary bladder. J Physiol 341:169–184
17. Nagel W, Crabbe J (1980) Mechanism of action of aldosterone on active sodium transport across toad skin. Pflügers Arch 385:181–187
18. Palmer LG, Li J, Lindemann B, Edelman IS (1982) Aldosterone control of the density of sodium channels in the toad urinary bladdder. J Memb Biol 64:91–102
19. Park CS, Edelman IS (1984) Effect of aldosterone on abundance and phosphorylation kinetics of Na-K-ATPase of toad urinary bladder. Am J Physiol 246:F509–F516
20. Petty K, Kokko J, Marver D (1981) Secondary effect of aldosterone on Na:K ATPase activity in the rabbit cortical collecting tubule. J Clin Invest 68:1514–1521
21. Schultz SG (1981) Homocellular regulatory mechanisms in sodium-transporting epithelia: Avoidance of extinction by "flush-through". Am J Physiol 241:F579–F590
22. Sharp G, Leaf A (1966) Mechanism of action of aldosterone. Physiol Rev 46:593–633
23. Soltoff SP, Mandel LS (1983) Amiloride directly inhibits the Na, K-ATPase activity of rabbit kidney proximal tubules. Science 220:957–959
24. Turnheim K, Thompson SM, Schultz SG (1983) Relation between intracellular sodium and active sodium transport in rabbit colon: Current-voltage relations of the apical sodium entry mechanism in the presence of varying luminal sodium concentrations. J Memb Biol 76:299–309
25. Will P, Lebowitz J, Hopfer U (1980) Induction of amiloride-sensitive sodium transport in the rat colon by mineralocorticoids. Am J Physiol 238:F261–F268

Received July 24/Accepted December 3, 1984