0031-3998/86/2003-0242$02.00/0
PEDIATRIC RESEARCH

Vol. 20, No. 3, 1986
Printed in U.S.A.

Role of Aldosterone for Control of Colonic NaKATPase Activity in Weanling Rats

YIGAEL FINKEL AND ANITA APERIA

Department of Developmental Physiology, St. Göran's Children's Hospital, Stockholm, Sweden

ABSTRACT. Experiments were performed to examine how aldosterone modulates colonic NaKATPase activity during the weaning period. NaKATPase activity was determined in proximal and distal colon in control rats aged 16, 20, and 40 days, in rats aged 20 and 40 days fed low sodium diet for 4 days and in 20-day-old rats adrenalectomized on day 16. In some protocols net sodium and water transport was determined with *in vivo* perfusion technique. In control rats colonic NaKATPase activity increased significantly between day 16 and 20. This increase was abolished by adrenalectomy but restored by aldosterone substitution, 5 μg/100 g body weight/12 h. No significant increase in NaKATPase activity occurred between day 20 and 40. Serum levels of both aldosterone and of corticosterone were low until day 14 and increased to peak level at day 18–20. In 20- and 40-day-old rats fed a low sodium diet, NaKATPase activity increased significantly in proximal and distal colon in both age groups but the increases were significantly greater in the 20- than the 40-day-old animals. A low sodium diet increased serum aldosterone, but not serum corticosterone levels in both age groups: also the low sodium diet significantly increased net sodium and water transport in 20- but not in 40-day-old rats. Aldosterone is of physiological importance for the regulation of NaKATPase activity in the colon at the time of weaning. The immature colon may have an enhanced sensitivity to aldosterone. (*Pediatr Res* 20: 242–245, 1986)

Abbreviation

Pi, inorganic phosphorous

At the time of weaning there is an accelerated maturation of gastrointestinal enzymes and this maturation is attenuated by adrenalectomy (1). It is generally assumed that it is the glucocorticoid hormone which triggers the maturation. The serum levels of glucocorticoid hormones increase rapidly at the time of weaning (2) and treatment with large doses of glucocorticoid in suckling rats results in precocious development of enzymes in the gastrointestinal tract (3–6).

There are few developmental studies of the large intestine. The adult large intestine has a high concentration of mineralocorticoid receptors (7, 8), and the activity of NaKATPase enzyme in the adult colon can be modulated by mineralocorticoid (9). The present study was designed to explore the influence of mineralocorticoid hormone on colonic NaKATPase activity at the time of weaning.

Received March 26, 1985; accepted October 31, 1985.
Correspondence Dr. Yigael Finkel, Department of Developmental Physiology, St. Göran's Children's Hospital, Box 12500, S-112 81 Stockholm, Sweden.
Supported by funds from the Swedish Medical Research Council, B84-19X-03644-13A and the Swedish Infant Food Industry.

MATERIALS AND METHODS

Animals. Sprague-Dawley rats from Anticimex, Sollentuna, Sweden were used. The animal quarters were air-conditioned (21 ± 1° C) with a 12-h light cycle (0700–1900 h) and a 12-h dark cycle. The litter sizes were maintained at four to six animals.

Experimental Groups. Intact rats. The animals were kept with lactating dams. They had free access to ordinary rat food (Ewos R3) containing 0.13 mmol Na/g and tap water *ad libitum* until 20 days. Thereafter they were kept in litters without dams. Their body weight increased from a mean of 21.5 g at 10 days to a mean of 170 g at 40 days.

Sodium depletion. These animals were fed a low sodium diet (Ewos) for 4 days. The diet was similar to the ordinary rat food but contained 0.009 mmol Na/g. Animals aged 16 days were separated from the dams and fed a low salt diet and tap water *ad libitum* until the day of study. The weight gain of these animals was the same as in intact rats of corresponding ages.

Adrenalectomy. Sixteen-day-old rats were subjected to bilateral adrenalectomy under light ether anesthesia. Following a midline incision, the adrenals were gently dissected free from the perirenal fat and the vascular pedicle was clamped. After adrenalectomy the rats were kept with lactating dams. They had free access to ordinary rat food and tap water *ad libitum.* In addition they were given 0.5 ml 0.9% NaCl intraperitoneally daily. Their weight gain was lower than the weight gain of intact rats of corresponding age. One group of adrenalectomized rats received aldosterone (Aldocorten, CIBA-GEIGY, Basel, Switzerland) 5 μg/100 g body weight/12 h intramuscularly. The weight gain of the latter animals was slightly lower than for intact animals of corresponding age.

Experimental Protocols. To obtain blood samples for the determination of serum levels of corticosterone and aldosterone, cardiac puncture was performed on nonanesthetized animals. The blood samples were obtained between 0900–1000 h, and great precaution was taken to minimize stress to the animals.

To obtain samples for NaKATPase determinations the animals were anesthetized with Inactin 80 mg/kg body weight. The abdomen was opened and the entire colon was gently removed. Small pieces of tissue, including mucosa and muscular wall, were taken from the proximal and distal colon. There are distinct anatomical differences between the proximal and distal colon (10), which are easy to detect in both 20- and 40-day-old rats. The pieces were weighed and kept frozen at −70° C until the day of analysis.

An in vivo perfusion technique was used to determine the net colonic fluid and sodium transport. The rats were anesthetized with Inactin 80 mg/kg body weight and a tracheostomy was performed. One of the carotid arteries was cannulated for blood sampling and a jugular vein was cannuated for fluid replacement with Ringer's solution, 1% of body weight/h by continuous infusion. The abdomen was opened and the colon was cannulated at the cecalcolonic junction. The colon was rinsed and flushed with 10–20 ml of warm air. Thereafter a rectal catheter was inserted with the tip just above the anal sphincter and the colon perfused with a warm solution containing 147 mM Na, 4

mM K, 2.3 mM Ca, and 155 mM Cl. ^{14}C PEG (5 g/liter) was used as a nonabsorbable marker. Recovery of ^{14}C-PEG was 100% or more in all perfusates. There was no ^{14}C-PEG in the blood samples taken after *in vivo* perfusion. The perfusion rate was 15 ml/h. After 30 min of equilibration, effluents from three periods of 20 min each were collected. The technique of *in vivo* perfusion has been described in detail previously (11).

Analysis. Serum corticosterone concentrations were determined by radioimunoassay as described by Nilsen *et al.* (12). Serum aldosterone concentrations were determined using a radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA).

For the determination of colonic NaKATPase activity the tissue was homogenized in a solution (w/v = 1:10) containing 250 mM sucrose, 30 mM histidine, 3 mM EDTA. Sodium deoxycholate 1.2 mmol was added to this solution and the pH was adjusted to 6.8. After homogenization the samples were centrifuged for 2 min. The supernatant was used for analysis.

The supernatant was diluted in a solution containing 120 mM NaCl, 30 mM KCl, 5 mM Mg-Cl_2, 30 mM histidine, 0.2% SBA; the pH was adjusted to 7.4. For determination of ATPase, 3 mM ATP was added to the solution after 5 min preincubation at 37° C. The incubation at 37° C was continued for 15 min, then stopped by cooling and the addition of 50 μl of ice-cold 50% trichloroacetic acid. For the determination of Mg ATPase, parallel incubations were made in the presence of 1 mM ouabain. The samples were centrifuged at 3,000 rpm for 10 min and 250 μl of the supernatants were taken from each sample and diluted in distilled water for the determination of Pi using the method described by Baginsky *et al.* (13); total protein content was measured using Lowry's method (14). NaKATPase was calculated as the difference between total ATPase and Mg ATPase activity and expressed as μmol Pi released/h/mg protein.

Net Fluid and Sodium Transport. The effluents from the perfusion were analyzed for electrolyte and ^{14}C-PEG concentrations. The radioactivity of the effluent perfusate was determined in a Packard scintillation counter. Na was determined by net flamephotometry.

Net water transport was determined from the concentration of ^{14}C-PEG, using the formula: Net water transport = PR(1-PEG_{in}/PEG_{eff}), where PR = perfusion rate in ml/min, PEG_{in} = ^{14}C-PEG concentration in the infusion solution, and PEG_{eff} = ^{14}C-PEG in the effluent collection.

Net sodium transport was determined from the formula: Net sodium transport = PR[I_{in}-I_{eff} (PEG_{in}/PEG_{eff})] I_{in} and I_{eff} were the sodium concentrations in microeq in the infusion solution and the effluent perfusate, respectively.

Calculations. The Student's *t* test and one-way analysis of variance were used in the statistical analysis. Values are given as the mean ± SEM. *p* values less than 0.05 are considered significant. The data were calculated by the computer program STATPAC (15).

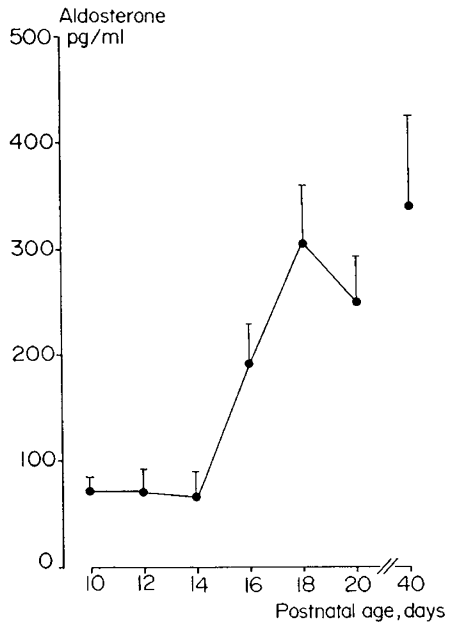

Fig. 1. Serum aldosterone levels in rats during postnatal development. Each value represents the mean of six to eight rats. The *bars* represent SEM.

RESULTS

Hormone levels. Age-related changes in serum aldosterone levels are shown in Figure 1. Serum levels of aldosterone were low in rats aged 10–14 days. They thereafter increased sharply to reach adult levels at 18 days (304 ± 59 pg/ml). Serum levels of aldosterone were not significantly different in 18- to 20- and 20- to 40-day-old rats.

Serum levels of corticosterone (data not shown) were low in 10- to 14-day-old rats, increased at 18 days of age and were higher in 40 day than 20-day-old rats. In both 20- and 40-day-old rats 4 days of a low salt diet significantly increased serum aldosterone levels but had no effect on the serum corticosterone levels (Fig. 2). In both age groups the serum aldosterone levels increased above the maximal value which could be determined by the assay used (1200 pg/ml). SD could therefore not be calculated.

In adrenalectomized 20-day-old rats, the serum concentrations of aldosterone and corticosterone were not detectable with the assays used.

Enzymes. The developmental changes in NaKATPase activity

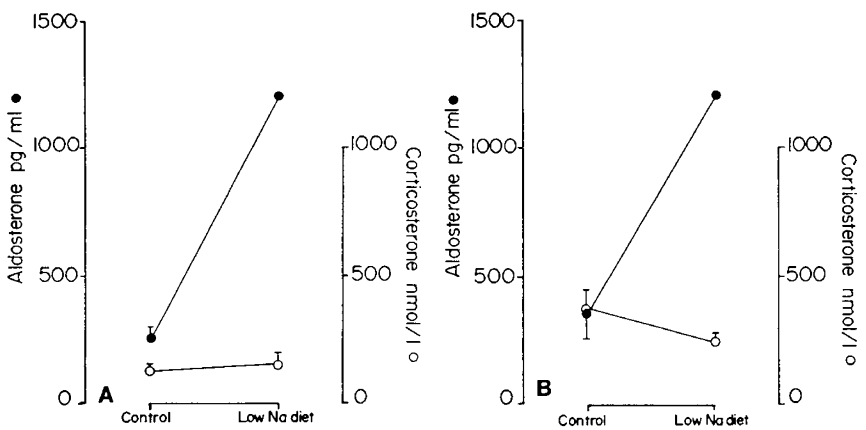

Fig. 2. Serum aldosterone and corticosterone levels in 20-day-old rats (*A*) and 40-day-old rats (*B*) rats fed normal and low salt diet. The *bars* represent SEM.

from 16 to 20 days of age are summarized in Table 1. In intact animals the NaKATPase activity increased significantly in both the proximal and the distal colon from 16 to 20 days of age.

Adrenalectomy at the age of 16 days abolished the development of both proximal and distal colonic NaKATPase. However, if the rats adrenalectomized at 16 days received aldosterone supplementation until the age of 20 days, the NaKATPase activity was the same in both the proximal and the distal colon as in control 20-day-old rats.

The effect of low sodium diet on colonic NaKATPase activity are shown in Figure 3. In 20-day-old rats fed a low sodium diet for 4 days NaKATPase activity (μmol Pi/mg protein/h) increased significantly in both the proximal and distal colon. In the proximal colon NaKATPase increased from 6.3 ± 0.5 μmol Pi/mg protein/h in normal diet rats to 13.7 ± 1.4 in low sodium diet rats. In the distal colon of 20-day-old rats NaKATPase increased from 8.6 ± 0.8 in normal diet rats to 14.0 ± 1.3 in low sodium diet rats. In 40-day-old rats which were fed a low sodium diet for 4 days NaKATPase in proximal colon increased from 10.6 ± 0.8 in normal diet rats to 13.7 ± 2.1 in low sodium diet rats. In the distal colon NaKATPase increased from 7.9 ± 0.7 in normal diet rats to 11.0 ± 0.7 in low sodium diet rats. The increases in the enzyme activity were significantly greater in 20-day than in 40-day-old animals.

Table 1. *Development of colonic NaKATPase activity in 16- to-20-day-old rats under control conditions and following adrenalectomy with and without aldosterone supplementation, 10 μg/100 g/day (mean ± SEM; n = 6–8 in each group)*

	NaKATPase (μmol Pi/mg protein/h)	
	Proximal colon	Distal colon
16 day intact	2.18 ± 0.36	2.11 ± 0.46
20 day intact	6.33 ± 0.54*	8.57 ± 0.77*
20 day adx	3.54 ± 0.85†	4.02 ± 0.62*,†
20 day adx + aldo	8.43 ± 0.57*	7.17 ± 0.63*

* Significantly different from 16-day-old rats.
† Significantly different from 20-day-old intact rats.

Perfusion studies. Perfusion studies were carried out in 20- and 40-day-old rats which had received a normal or a low sodium diet. The results are shown in Table 2.

Net sodium absorption was significantly higher in 20-day-old rats fed a low sodium diet than in 20-day-old rats fed a normal diet. Similarly, net water absorption was significantly higher in 20-day-old low sodium rats than in 20-day-old control rats.

In 40-day-old rats net sodium absorption was the same as in control rats and net water absorption was actually somewhat lower than in control rats.

In accordance with previous studies we found significantly higher net sodium absorption in 20- than in 40-days old rats (16). We have previously attributed this finding to a higher paracellular transport in young than in adult rats.

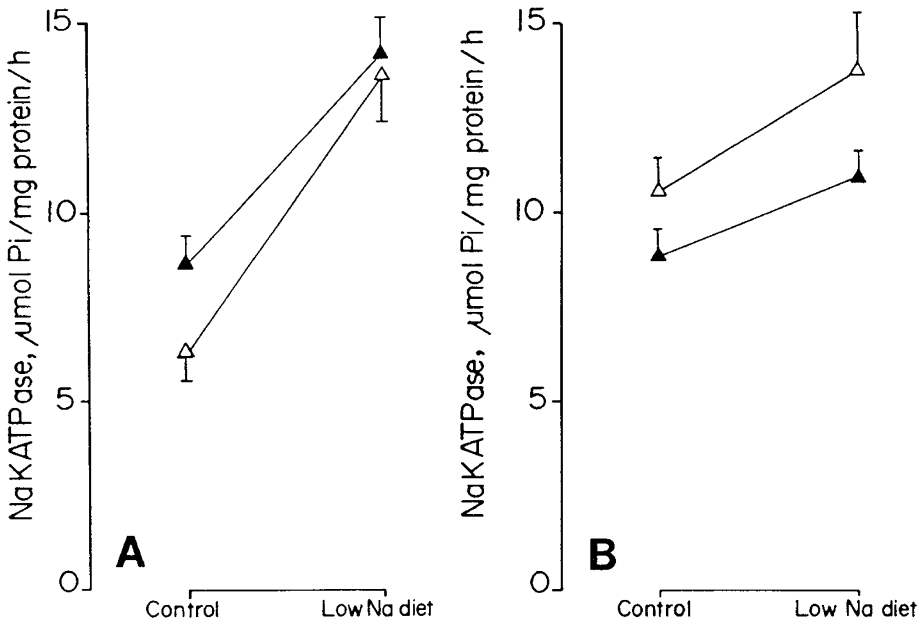

Fig. 3. NaKATPase in 20-day-old rats (*A*) and 40-day-old rats (*B*) fed normal and low sodium diet. △, proximal colon; ▲, distal colon. Each value represents the mean of six to eight rats. The *bars* represent SEM.

Table 2. *Effect of 4 days low sodium diet on colonic Na and water transport in 20-day and 40-day-old rat (mean ± SEM; n = 6–8 in each group)*

	20 days		40 days	
	Low sodium diet	Control	Low sodium diet	Control
Net water absorption (ml/min/g dry tissue)	2.64 ± 0.75*	1.02 ± 0.67	0.47 ± 0.05†	0.81 ± 0.02
Net sodium absorption (μmol/min/g dry tissue)	425 ± 99*	191 ± 91	79 ± 13	116 ± 7

* Significantly higher than 20-day-old control rats.
† Significantly lower than 40-day-old control rats.

DISCUSSION

The period of weaning is critical for the development of the gastrointestinal tract (1). The postnatal changes in serum corticosterone occurring at the time of weaning have attracted much interest, since there is convincing evidence that the glucocorticoid hormone plays a key role in the enzymatic changes in the gastrointestinal tract at that time (17). In the present study we found that at the time of weaning in rats serum levels of aldosterone followed the same developmental pattern that has previously been described for corticosterone (2, 18). This raised the question whether aldosterone might be of physiological importance for the development of the colon at the time of weaning, since the colon is rich in aldosterone receptors (7, 8).

Several findings in the present study suggest that aldosterone is of physiological importance for the regulation of NaKATPase activity in the developing colon. NaKATPase activity in 16-day-old rats was significantly lower than in 20-day-old rats. If the rats were adrenalectomized at the age of 16 days the developmental increase in NaKATPase activity was abolished; it was restored if the adrenalectomized rats were given supplementary low doses of aldosterone.

To examine the effect of endogenous fluctuations of aldosterone levels on colonic NaKATPase activity in young and adult rats we stimulated endogenous aldosterone production by giving a low sodium diet. We found a significant increase in proximal and distal colonic NaKATPase activity in both young and adult rats fed a low sodium diet. Since the low sodium diet did not change the serum corticosterone levels, we feel justified to attribute the increase in NaKATPase activity to the secondary hyperaldosteronism.

Aldosterone receptors are present both in the proximal and the distal colon. Since it has been reported that the mode of action of aldosterone might be different in proximal and distal colonic tissue (19), we studied those segments separately. However, we did not detect any differences between the two segments with regard to enzyme response to aldosterone.

The increases in enzyme activities induced by low salt diet were significantly higher in the young than in the adult rats. This observation may indicate that immature colonic tissue has an enhanced sensitivity to the inductive effect of aldosterone. Enhanced sensitivity to the inductive effect of glucocorticoid hormones has previously been observed in the immature small intestine (20) as well as in the immature renal proximal tubule (21). In the renal proximal tubule, the enhanced sensitivity to glucocorticoid hormones could be attributed to increased concentration of available hormone receptors (22).

We found that in the young rat secondary hyperaldosteronism was accompanied both by an increase in NaKATPase activity and in net Na and water absorption. It is well documented from studies in adult animals that aldosterone can modulate both sodium permeability and the sodium pump. Adrenalectomy reduces both NaKATPase activity and transcellular sodium transport. In adrenalectomized animals replacement with aldosterone but not synthetic glucocorticoid has been shown to restore both the enzyme activity and the sodium transport capacity to normal in tissues rich in mineralcorticoid receptors (23). The mechanisms by which aldosterone influences transcellular sodium transport and NaKATPase activity are not fully understood. There is evidence that aldosterone increases the sodium permeability of the apical cell membrane (21) as well as NaKATPase activity in the basolateral cell membrane.

The increase in the sodium permeability of the luminal cell membrane will cause an increase in intracellular sodium content. It has been suggested that this increase in intracellular sodium stimulates the recruitment of NaKATPase to the basolateral cell membrane. However, there are other studies suggesting that NaKATPase synthesis is also directly stimulated by aldosterone (25). The increase in net colonic sodium and water absorption that we observed in the young rats with secondary hyperaldosteronism might be due to an increase in apical Na permeability as well as to an increase in NaKATPase activity and our results are compatible with the hypothesis that aldosterone may induce NaKATPase activity by increasing intracellular sodium availability.

REFERENCES

1. Henning S 1981 Postnatal development: coordination of feeding, digestions and metabolism. Am J Physiol 241:G199–G214
2. Henning SJ 1973 Plasma concentrations of total and free corticosterone during development of the rat. Am J Physiol 235:E451–E456
3. Doell RG, Kretchmer N 1964 Intestinal invertase: precocious development of activity after injection of hydrocortisone. Science 143:42–44
4. Galand G, Forstner GG 1974 Isolation of microvillus plasma membranes from suckling rat intestine. The influence of premature induction of digestive enzymes by injection of cortisol acetate. Biochem J 144:293–302
5. Raul F, Simon PM, Kedinger M, Grenier JF, Haffen DK 1978 Separation and characterization of intestinal brush border enzymes in adult rats and in suckling rats under normal conditions and after hydrocortisone injections. Enzyme 23:89–97
6. Sasaki R, Mura M, Takeuchi T, Furihata C, Matsushima T, Sugimura T 1976 Premature induction of amylase in pancreas and parotid gland of growing rats by dexamethasone. Biochem Biophys Acta 428:619–626
7. Marver D 1984 Assessment of mineralcorticoid activity in the rabbit colon. Am J Physiol 246:F437–F446
8. Marusic ET, Hayslett JP, Binder HJ 1981 Corticosteroid-binding studies in the cytosol of colonic mucosa of the rat. Am J Physiol 240:G417–G423
9. Will PC, DeLisle RC, Cortright RN, Hopfer U 1981 Induction of amiloride-sensitive transport in the intestines by adrenal steroids. Ann NY Acad Sci 372:64–78
10. Lindström CG, Rosengren, J-E, Fork F-T 1979 Colon of the rat, an anatomic, histologic and radiographic investigation. Acta Radiol [Diagn] (Stockh) 20:523–535
11. Finkel Y, Aperia A, Eklöf A-Ch 1985 Development of colonic fluid and electrolyte transport, influence of weaning pattern. J Pediatr Gastroenter Nutr 4:457–462
12. Nilsen OG, Toftgård R, Eneroth P 1980 Effects of acrylonitrile on rat liver cytochrome P-450 benzo(a)pyrene metabolism and serum hormone levels. Toxicol Lett 6:399–404
13. Baginski ES, Foa PP, Zak B 1967 Determination of phosphate; study of labile organic phosphate interference. Clin Chem Acta 15:155–158
14. Lowry OH, Kroserough NJ, Farr AL, Randall RJ 1951 Protein measurement with the Folin reagent. J Biol Chem 193:265–275
15. STATPAC, Manual 1983 Department of Medicine Information Processing (IMI), Stockholm
16. Marin L, Aperia A 1984 Colonic water and electrolyte transport in young and adult rats. J Pediatr Gastroenterol Nutr 3:471–475
17. Henning S, Sims JM 1979 Delineation of the glucocorticoid sensitive period of intestinal development in the rat. Endocrinology 104:1158–1163
18. Koldovsky O, Jirsova V, Heringova A 1965 Effect of aldosterone and corticosterone on beta-galactosidase and invertase activity in the small intestine of rats. Nature (Lond) 206:300–301
19. Hirsch D, Pace P, Binder HJ, Hayslett JP 1985 Evidence that aldosterone influences transport in target tissue by dissimilar mechanisms. Am J Physiol 248:F507–F512
20. Buts JP, DeMeyer R, Kolanowski J 1983 Ontogeny of cell proliferation and DNA synthesis in rat colon; role of glucocorticoids. Am J Physiol 244:G469–G474
21. Aperia A, Larsson L, Zetterström R 1981 Hormonal induction of NaKATPase in developing proximal tubular cells. Am J Physiol 241:F356–F360
22. Aperia A, Haldosén L-A, Gustafsson J-Å 1985 Ontogeny of triamcinolone-acetonide binding sites in outer cortical tissue from rat kidney. Am J Physiol 249:F891
23. Horster M, Lückhoff A 1983 Aldosterone on sodium transport of rat distal colon in long-term adrenalectomy during acute and chronic substitution. J Physiol 340:503–511
24. Garg LC, Knepper MA, Burg MB 1981 Mineralcorticoid effects on NaKATPase in rabbit nephron segments. Ann NY Acad Sci 372:30–38
25. Lewis SA 1983 Control of Na^+ and water absorption across vertebrate "tight" epithelia by ADH and aldosterone. J Exp Biol 106:9–24