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Sodium-Chloride Transport in the Medullary Thick Ascending Limb of Henle's Loop: Evidence for a Sodium-Chloride Cotransport System in Plasma Membrane Vesicles

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Summary. Sodium transport mechanisms were investigated in plasma membrane vesicles prepared from the medullary thick ascending limb of Henle's loop (TALH) of rabbit kidney. The uptake of ^{22}Na into the plasma membrane vesicles was investigated by a rapid filtration technique. Sodium uptake was greatest in the presence of chloride; it was reduced when chloride was replaced by nitrate, gluconate or sulfate. The stimulation of sodium uptake by chloride was seen in the presence of a chloride gradient directed into the vesicle and when the vesicles were equilibrated with NaCl, KCl plus valinomycin so that no chemical or electrical gradients existed across the vesicle (tracer exchange experiments). Furosemide decreased sodium uptake into the vesicles in a dose-dependent manner only in the presence of chloride, with a K_i of around 5×10^{-6} M. Amiloride, at 2 mM, had no effect on the chloride-dependent sodium uptake. Similarly, potassium removal had no effect on the chloride-dependent sodium uptake and furosemide was an effective inhibitor of sodium uptake in a potassium-free medium. The results show the presence of a furosemide-sensitive sodium-chloride cotransport system in the plasma membranes of the medullary TALH. There is no evidence for a Na^+/H^+ exchange mechanism or a $Na^+-K^+-Cl^-$ cotransport system. The sodium-chloride cotransport system would effect the uphill transport of chloride against its electrochemical potential gradient at the luminal membrane of the cell.

Key Words renal medulla · thick ascending limb of Henle's loop · chloride transport · sodium transport · sodium-chloride cotransport · furosemide

Introduction

The mechanism for 'active' chloride transport in the medullary thick ascending limb of Henle's loop (TALH) appears to be a coupling of sodium and chloride fluxes via a sodium-chloride cotransport system located in the luminal membranes of the cell (Eveloff, Bayerdörffer, Silva & Kinne, 1981; Hebert, Culpepper & Andreoli, 1981). Coupled transport of sodium and chloride fluxes has been proposed since 1962 in the gallbladder and has subsequently been proposed for sodium chloride transport in many mammalian epithelia. Using *in vitro* and *in vivo* electrical and flux measurements, epithelia such as the trachea, small intestine, gallbladder and colon are thought to possess an electroneutral sodium-chloride cotransport system which is energized by the electrochemical potential gradient for sodium across the cell membrane (Diamond, 1962; Nellans, Frizzell & Schultz, 1973; Frizzell, Koch & Schultz, 1976; Welsh & Widdicombe, 1980). However, recently, epithelia such as the small intestine, gallbladder, and *Necturus* proximal tubule, which have been postulated to possess tightly coupled sodium-chloride cotransport systems, are now thought to be 'loosely' coupled via two exchange systems acting in concert: Na^+/H^+ and Cl^-/OH^- (HCO_3^-) exchangers (Seifter, Kinsella & Aronson, 1980; Liedtke & Hopfer, 1982*a b*; Weinman, Hamm & Reuss, 1981) which would effect an electroneutral transport of sodium and chloride across the cell membrane without a net bicarbonate or proton movement. And in the cortical TALH, during 1981, sodium chloride movement has been described as a coupled sodium-chloride cotransport, both electroneutral (Greger, 1981) and electrogenic (Greger & Frömter, 1980), a $Na^+-K^+-2Cl^-$ cotransport system (Greger & Schlatter, 1981) and a combination of a coupled NaCl cotransport system with the Na^+/H^+ and Cl^-/OH^- exchangers (Andreoli, Hebert, Culpepper & Friedman, 1980; Friedman & Andreoli, 1981).

The purpose of this study was to elucidate the mechanisms of sodium and chloride transport in the medullary TALH. Thus, we have prepared plasma membrane vesicles from isolated cells of the medullary TALH and investigated the properties of sodium transport at the membrane level. This allowed us to manipulate the ionic composition on both sides of the membrane and control

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the effects of membrane potential on sodium movement which circumvents these unrecognizable variables present in more intact preparations. The results provide direct evidence for a furosemide-sensitive sodium-chloride cotransport system in the plasma membranes of the medullary TALH. No evidence was found for a Na^+/H^+ exchange mechanism or a $Na^+-K^+-Cl^-$ cotransport system. This sodium-chloride cotransport system would facilitate the 'uphill' movement of chloride across the luminal membrane of the TALH cell.

Materials and Methods

Initial Preparation of Medullary Thick Ascending Limb of Henle's Cells

Cells from the medullary thick ascending limb of Henle's loop (TALH) were isolated from rabbit kidney according to the method of Eveloff, Haase and Kinne (1980). In short, rabbits were killed by a blow to the head and exsanguination, the kidneys removed and placed in ice-cold Joklik's buffer, a commercial cell culture medium (Grand Island Biological Co.). It was used unmodified except for the addition of 10% fetal or newborn calf serum. The kidneys were perfused through the renal artery to remove as many red blood cells as possible and the cortex was removed. Tubules were released from the medullary tissue by incubation with 0.2% collagenase-0.25% hyaluronidase (wt/vol) in Joklik's buffer for 1 h at 37 °C, gassed with 95% O_2-5% CO_2. Single cells were released from the tubules by eight treatments with 0.25% trypsin (wt/vol) for 20 min at room temperature with oxygenation. The cells were separated on a continuous Ficoll gradient (2.7–30.1% wt/wt) at $1400 \times g_{max}$ for 45 min. The TALH cells were located in the densest portion of the gradient and identified by their calcitonin-sensitive adenylate cyclase activity, Na, K-ATPase activity, morphology and furosemide-sensitive respiration (Eveloff et al., 1980, 1981).

Preparation of the Plasma Membrane Fraction

Plasma membrane vesicles were prepared from the TALH cells derived from the kidneys of 8 rabbits. The cells were frozen at −80 °C in ST buffer, in mM: 250 sucrose, 10 triethanolamine, pH 7.6 with HNO_3, which served to initially break up the cell membrane. Upon thawing the cells were homogenized 40 times in a glass/Teflon homogenizer.

The plasma membrane vesicles were isolated as a classical light microsomal membrane preparation (Evans, 1978), that is the membranes which sedimented after 1 h of centrifugation at $150{,}000 \times g$ and thus, consisted of both luminal and contraluminal plasma membranes.

The plasma membranes were assayed by previously described methods for the marker enzymes Na,K-ATPase, a basal-lateral membrane marker (Schoner, von Ilberg, Kramer & Seubert, 1967), alkaline phosphatase, a proximal tubule luminal membrane enzyme (Bessey, Lowry & Brock, 1947) and perhaps also found in the luminal membranes of the TALH (Bonting, Pollack, Muehrcke & Kark, 1958), filipin-sensitive Mg^{++}-ATPase (Kinne-Saffran & Kinne, 1979), a potential luminal enzyme marker for the TALH, NADH dehydrogenase (Wallach & Kamat, 1966), an endoplasmic reticulum enzyme and succinic dehydrogenase (Gibbs & Reimer, 1965), a mitochondrial enzyme marker. Protein was measured by the method of Lowry, Rosebrough, Farr and Randall (1951) after precipitation by 10% trichloracetic acid (wt/vol).

Transport Studies

The uptake of ^{22}Na was followed into the plasma membrane vesicles by a rapid filtration technique as described previously (Eveloff et al., 1978).

The plasma membrane vesicles were suspended in a vesicle buffer containing, in mM: 100 sucrose, 1 Mg $(NO_3)_2$, 20 triethanolamine (pH 7.4 with HNO_3). The incubation medium contained generally, in mM: 100 sucrose, 20 triethanolamine (pH 7.4 with HNO_3), 1 Mg $(NO_3)_2$, 0.5 Na salt and 49.5 K salt. The exact experimental manipulations are indicated in the Figure legends. The uptake of sodium was investigated at 25 °C and initiated by adding 20 µl of membranes to 150 µl of incubation medium containing 12 µCi of ^{22}Na and 40 µCi of ^3H-mannitol. The mannitol served as a control to monitor changes in vesicular size and general membrane permeability. The reaction was terminated at timed intervals by the removal of 20 µl of the reaction mixture and rapid dilution into 1 ml of cold stop solution consisting of, in mM: 100 sucrose, 1 $Mg(NO_3)_2$, 20 triethanolamine, pH 7.4, 50 K gluconate, 1 furosemide. The diluted sample was immediately filtered onto a 0.45 µm cellulose nitrate filter and the collected membranes were washed rapidly with 3 ml of stop solution. The filters were placed in scintillation fluid and counted by standard liquid scintillation techniques.

Materials

$^{22}NaCl$ (carrier free) and ^3H-mannitol were obtained from New England Nuclear. The Joklik's buffer (Minimum Essential Medium), fetal and newborn calf serum and trypsin (0.25% lyophilized) were purchased from Grand Island Biological Company, Grand Island, New York. Collagenase (grade II) was purchased from Worthington Biochemical Corp., Freehold, N.J.; Ficoll-400 from Pharmacia, Inc., Piscataway, N.J.; hyaluronidase from Boehringer-Mannheim, Indianapolis, Indiana. The furosemide was a gift from Hoechst Pharmaceuticals, Frankfurt, West Germany. Amiloride was a gift from Prof. Dr. E. Frömter, Frankfurt, West Germany.

Results

General Characteristics of the TALH Plasma Membrane Vesicles

The plasma membrane vesicles were prepared as a light microsomal preparation and consisted of both basal-lateral and luminal membranes. The enzymatic activities of the vesicles compared to the TALH cells and the total medullary cell population are shown in Table 1. The Na,K-ATPase activity in the plasma membrane vesicles was enriched 4.4 and 17.6 times the activities found in the TALH cells and medullary cell population. The alkaline phosphatase activity in the vesicles was enriched 1.5 and 0.2 times the activity of the TALH cells and the medullary cells, respectively. Alkaline phosphatase has been histochemically identified in the TALH (Bonting et al., 1958) but is probably

Table 1. Enzymatic characteristics of plasma membrane vesicles prepared from cells of the medullary thick ascending limb of Henle's loop

	Na,K-ATPase	Alkaline phosphatase	Filipin-sensitive Mg^{++}-ATPase	Succinic dehydrogenase	NADH dehydro-genase
Medullary cells	0.13±0.04	3.15±0.83	n.d.	0.0043 ±0.0015	1.39±0.43
TALH cells	0.54±0.12	0.35±0.22	1.28±0.34	0.0051 ±0.0022	1.03±0.21
Plasma membrane vesicles	2.29±0.56	0.54±0.16	2.05±0.21	0.00036±0.00018	0.95±0.12

The enzyme activities are expressed as μmol/mg protein·min.
The values are the means±SEM The number of experiments was four.
n.d. = not determined.

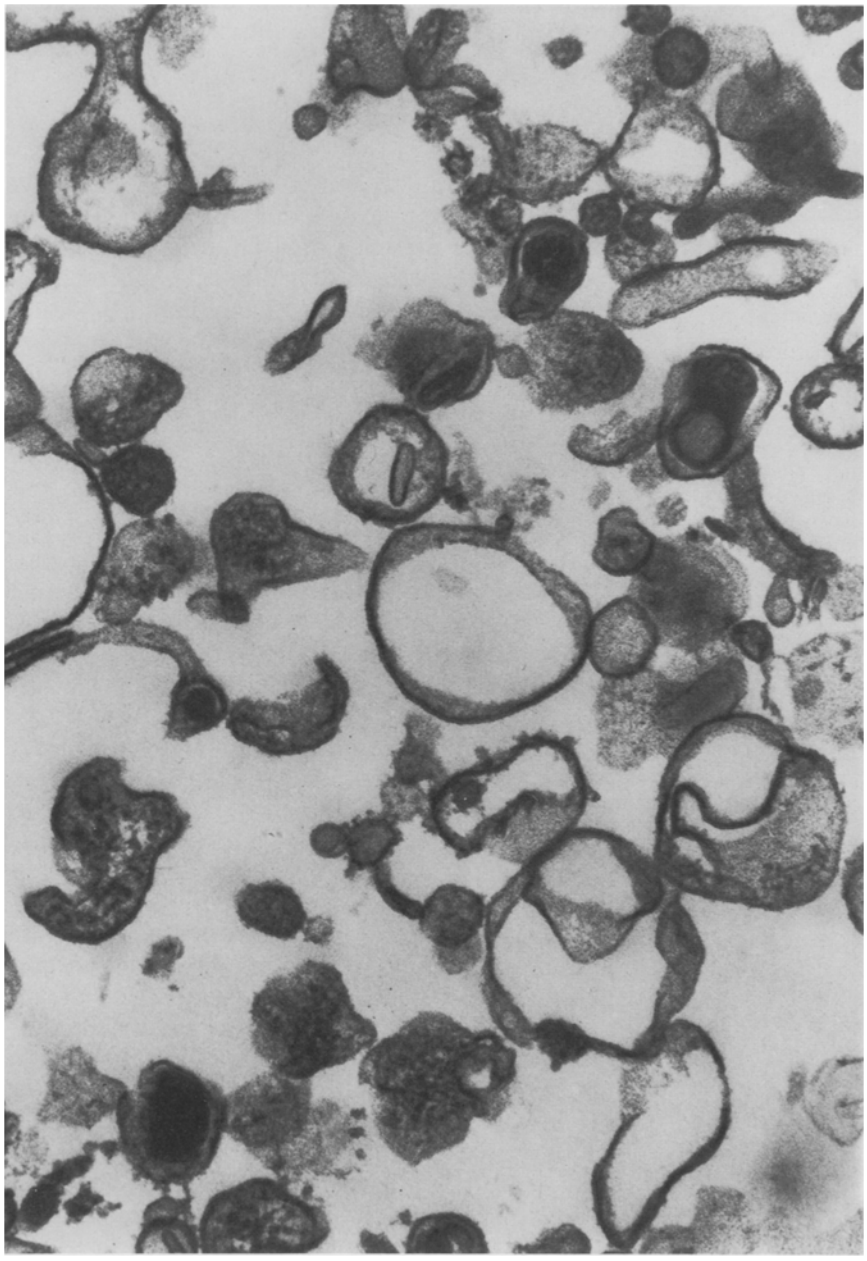

Fig. 1. Electron micrograph of the plasma membrane vesicles prepared from isolated cells of the rabbit renal medullary thick ascending limb of Henle's loop. 72,600×

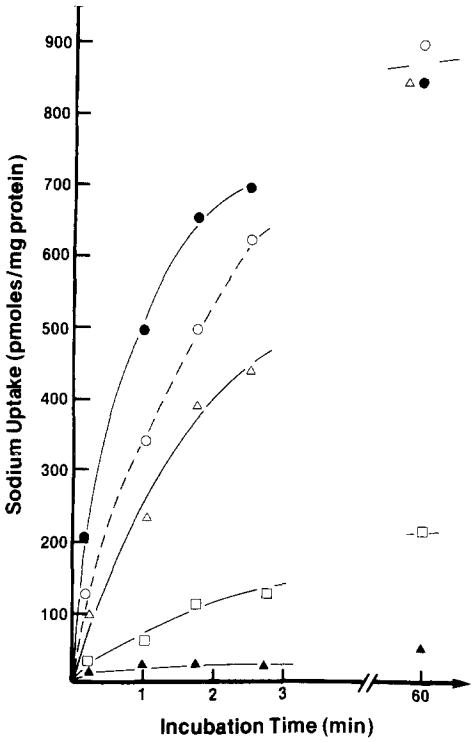

Fig. 2. Effect of anions on sodium uptake into plasma membrane vesicles from the medullary thick ascending limb of Henle's loop. The vesicles were prepared in 100 mM sucrose, 20 mM triethanolamine, pH 7.4, 1 mM $Mg(NO_3)_2$, and the incubation medium contained in addition, 0.5 mM ^{22}Na salt, 49.5 mM K salt: NaCl–KCl (●), $NaNO_3$–KNO_3 (○), Na gluconate-K gluconate (△), Na_2SO_4–K_2SO_4 (□). To assess the amount of ^{22}Na binding relative to the total sodium uptake values the plasma membrane vesicles were boiled for 1 min and then sodium uptake was followed in a NaCl–KCl incubation medium (▲). The values are the means of six experiments

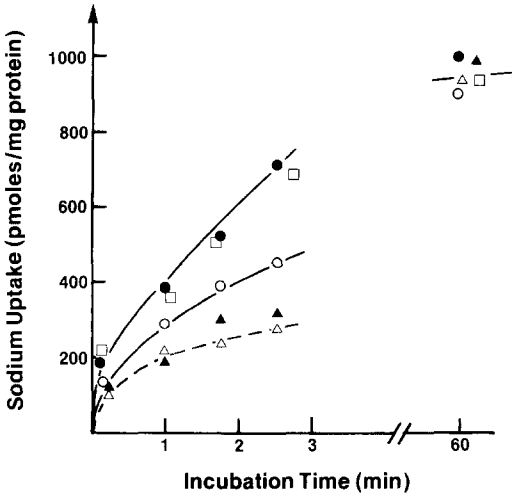

Fig. 3. Effect of anions on sodium uptake into medullary TALH plasma membrane vesicles in the absence of anion gradients. The vesicles contained, in mM: 100 sucrose, 20 triethanolamine, pH 7.4, 1 $Mg(NO_3)_2$, 50 K salt and the incubation medium contained in addition: 0.5 mM ^{22}Na salt and 1 mg/ml valinomycin. The salts used were: NaCl–KCl (●) NaBr–KBr (□), NaI–KI (▲), $NaNO_3$–KNO_3 (○) and Na gluconate-K gluconate (△). The values are the means of 4 experiments

mainly due to medullary *pars recta* contamination of the TALH cell preparation. The activity of the filipin-sensitive Mg^{++}-ATPase in the vesicles was 1.6 that of the TALH cells. Succinic dehydrogenase activity in the vesicles was 0.07 and 0.08 times and NADH dehydrogenase activity was 0.9 and 0.7 times the activity in the TALH cells and medullary cells, respectively, indicating that mitochondria and endoplasmic reticulum were not major contaminants in the plasma membrane vesicle preparation.

Electron micrographs of the plasma membrane vesicles (Fig. 1) show a preparation rich in smooth vesicles which are uniform in size and show little contamination by mitochondria or other cellular organelles, thus confirming the enzymatic analysis.

Anion Dependence of Sodium Uptake

The effect of anions on sodium uptake is shown in Fig. 2. The uptake of sodium (0.5 mM) was stimulated in the presence of a chloride gradient directed into the vesicle. When nitrate, gluconate or sulfate were the accompanying anions, sodium uptake was reduced. Mannitol uptake was followed as a control and was unchanged by anion substitution (*data not shown*) indicating that chloride did not stimulate sodium uptake through an indirect effect on membrane permeability or vesicular size.

The contribution of the binding of ^{22}Na to the total measured sodium uptake values was estimated by boiling the membranes prior to transport (Fig. 2) or by placing the vesicles in a distilled H_2O stop solution for 1 min after the transport reaction was complete. Both maneuvers indicated that approximately 2 to 5% of the sodium was bound to the membranes in the normal incubation media. No corrections were made to the uptake values for the binding component.

Since the anions may have different membrane permeabilities and thus may elicit diffusion potentials which would affect sodium uptake, the membrane vesicles were pre-equilibrated with potassium salts plus valinomycin. This allows charge compensating movements of potassium to occur and thus, effectively short-circuits the membrane. Figure 3 shows that under equilibrated conditions, the uptake of sodium was still greatest in the presence of chloride. The halide bromide could also fully support sodium uptake. Sodium uptake was reduced in the presence of the anions gluconate, nitrate or iodide. Thus, the data suggest a direct coupling of sodium and chloride fluxes across the plasma membrane of the medullary TALH.

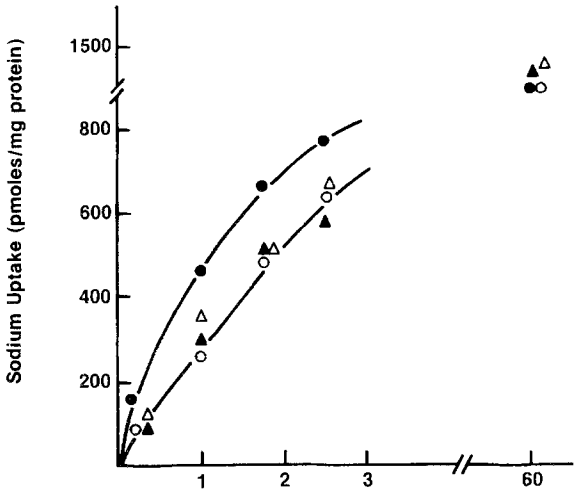

Fig. 4. Tracer exchange experiments in plasma membrane vesicles prepared from medullary TALH cells. The plasma membranes and the incubation medium contained, in mM: 100 sucrose, 20 triethanolamine, pH 7.4, 1 $Mg(NO_3)_2$, 50 K salt and 0.5 Na salt plus valinomycin (1 mg/ml). The uptake of ^{22}Na (12 μCi/150 μl) was followed in the presence of the salts NaCl–KCL (●), $NaNO_3-KNO_3$ (▲), NaCl–KCl plus 10^{-4} M furosemide (○) or, $NaNO_3-KNO_3$ plus 10^{-4} M furosemide (△). The values are the means of 3 experiments

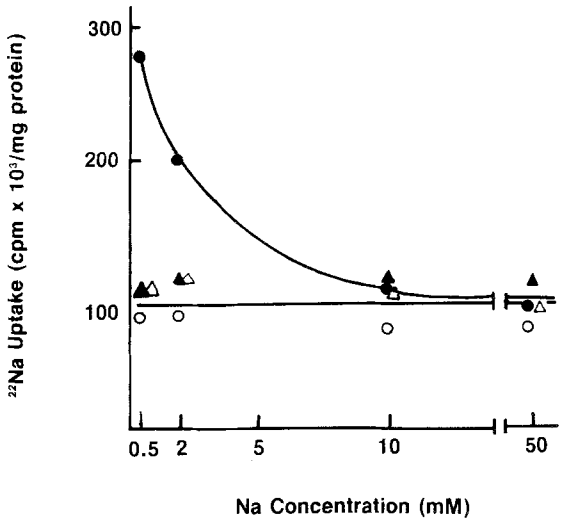

Fig. 5. Tracer replacement of ^{22}Na uptake into medullary TALH plasma membrane vesicles. The membranes were prepared in, in mM: 100 sucrose, 20 triethanolamine, pH 7.4, 1 $Mg(NO_3)_2$, 50 KCl or KNO_3. The incubation medium contained, in mM: 100 sucrose, 20 triethanolamine, pH 7.4, 1 $Mg(NO_3)_2$, and increasing concentration of NaCl (●) or $NaNO_3$ (▲) from 0.5 to 10 mM (total salt concentration was 50 mM with KCl or KNO_3) plus valinomycin (1 mg/ml) and ^{22}Na (12 μCi/150 μCi). The open symbols are sodium uptake in the presence of 10^{-4} M furosemide. The reaction was terminated at 10 s. The results are the mean of 4 experiments

Further Proof for the Coupling of Sodium and Chloride Fluxes via a Cotransport System

Additional evidence for direct coupling of sodium and chloride fluxes are tracer exchange experiments, where all solutes are equilibrated across the membrane so that no chemical or electrical gradients exist. In Fig. 4, it is seen that ^{22}Na uptake is again greater when Cl is the anion than compared to nitrate, and that further, furosemide inhibited sodium uptake only in the presence of sodium chloride. Rate constants can be calculated from the curves by plotting the $\ln(C_T - C_\infty)$ versus time, where C_T and C_∞ are the concentrations of sodium taken up into the vesicles at time t and at equilibrium (Hopfer, 1977). The rate constant for sodium in the presence of chloride is 0.25 min^{-1} and in the presence of nitrate is 0.16 min^{-1}. The rate of sodium uptake is 36% slower in the absence of chloride.

In tracer replacement or saturation experiments (Fig. 5), increasing unlabeled concentrations of NaCl, from 0.5 to 50 mM decreased tracer uptake of ^{22}Na into the plasma membrane vesicles. This phenomenon was not observable with increasing concentrations of $NaNO_3$, thus suggesting that there are a limited number of transport sites for sodium which require chloride for their interaction with sodium.

Table 2. Effect of furosemide on sodium uptake into medullary TALH plasma membrane vesicles

Furosemide conc. (M)	Chloride-dependent sodium uptake[a] (pmol/mg protein)	% Inhibition
0	93±6[b]	0
10^{-6}	68±2	27
10^{-5}	22±3	76
10^{-4}	17±6	82
10^{-3}	0±2	100

[a] The chloride-dependent sodium uptake is the sodium uptake in a chloride medium minus that in a gluconate medium. The plasma membrane vesicles were prepared as in Fig. 3 and the incubation medium contained in addition 0.5 mM $^{22}NaCl$, valinomycin (1 mg/ml) and the diuretic ranging in concentration from 10^{-6} to 10^{-3} M. The uptake time was 10 s.

[b] Values are the mean ± SEM of 6 experiments

Effect of Diuretics on Sodium Uptake

Furosemide is a potent inhibitor of sodium chloride transport in the loop of Henle (Burg, Stoner, Cardinal & Green, 1973). And in concordance, furosemide inhibited the chloride-dependent sodium uptake. Furosemide, at 10^{-3} and 10^{-4} M, inhibited sodium uptake in the presence of chloride in a dose-dependent manner under gradient and equilibrated conditions (Table 2). Furosemide also in-

Table 3. Effect of amiloride on sodium uptake in plasma membrane vesicles from medullary TALH cells[a]

	Sodium uptake	
	10 s	1 min
Control	203.83 ± 22.22	496.93 ± 66.03
0.5 mM amiloride	230.33 ± 17.68	443.88 ± 72.21
2 mM amiloride	217.53 ± 30.82	484.39 ± 52.67

[a] Values are the means ± SEM of 3 experiments. The plasma membrane vesicles were prepared in mM: 100 sucrose, 20 triethanolamine, pH 7.4, 1 $Mg(NO_3)_2$, 50 KCl, and the incubation medium contained in additon 0.5 mM $^{22}NaCl$, 1 mg/ml valinomycin plus 0.5 or 2 mM amiloride.

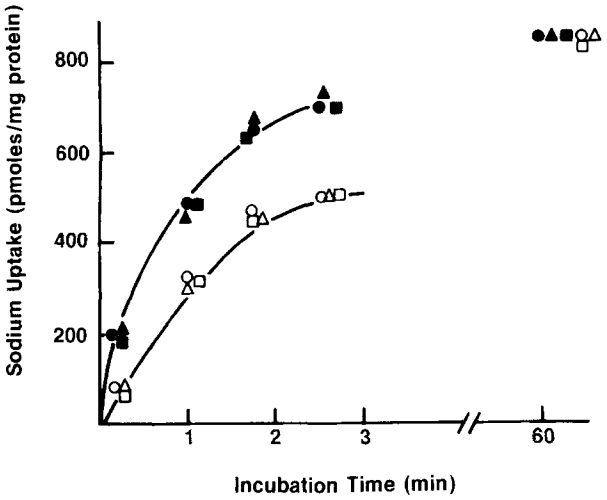

Fig. 6. The effect of potassium on sodium uptake into plasma membrane vesicles prepared from medullary TALH cells. Plasma membrane vesicles were prepared as in Fig. 1 and the incubation medium contained, in mM: 100 sucrose, 20 triethanolamine, pH 7.4, 1 $Mg(NO_3)_2$, 0.5 $^{22}NaCl$ and the following Cl salts (50 mM): KCl (●), choline Cl (▲), LiCl (■), minus or plus 10^{-4} M furosemide (open symbols). The values are the mean of 3 experiments

hibited sodium uptake in the presence of bromide as an anion (*data not shown*). However, furosemide had no inhibitory effect when nitrate was substituted for chloride (Figs. 4 and 5).

Possibility of the Existence of Na^+/H^+ Exchange or $Na^+-K^+-Cl^-$ Cotransport System

Sodium chloride transport in epithelial cells has also been reported to be effected by the concerted movements of a Na^+/H^+ plus a Cl^-/OH^- exchange mechanism or a $Na^+-K^+-Cl^-$ cotransport mechanism located in the plasma membrane (Greger & Schlatter, 1981; Hebert et al., 1981; Liedtke & Hopfer, 1982*a, b*). In order to investigate whether these mechanisms were present, in addition to the observed Na-Cl cotransport system, the effects of amiloride, an inhibitor of Na^+/H^+ exchange (Kinsella & Aronson, 1981) and potassium on sodium transport were observed. Amiloride in doses of 0.5 to 2 mM had no effect on 0.5 mM NaCl uptake into the plasma membrane vesicles, suggesting that Na^+/H^+ exchange is not a major component of Na movement across the TALH cell (Table 3). Secondly, if 0.5 mM NaCl uptake was studied in the presence of 50 mM KCl, choline Cl or LiCl, no effect of potassium removal was observed on sodium uptake (Fig. 6). Additionally, the inhibitory potency of furosemide was identical in the presence of NaCl plus potassium, choline or lithium. Thus, it is unlikely that the Na-Cl cotransport system is in reality a $Na^+-K^+-Cl^-$ cotransport system.

Discussion

The presence of a sodium-chloride cotransport system in the medullary TALH is supported by the fact that chloride had a stimulatory effect on sodium uptake into plasma membrane vesicles prepared from isolated TALH cells. This chloride-stimulated sodium uptake is observed under conditions where the possiblity of an electrical diffusion potential created by unequal anion permeabilities is minimized by equilibrating the anions across the vesicle in the presence of potassium plus valinomycin, a condition which effectively causes the electrical potential to be zero. Sodium movement was also greater in the presence of chloride in isotope exchange experiments, where not only the electrical potential gradient is nullified but also no net chemical fluxes occur.

The loop diuretic furosemide inhibited sodium movement across the plasma membrane of the TALH only in the presence of chloride. There was no inhibitory response when chloride was substituted by nitrate, gluconate or sulfate suggesting that furosemide inhibits directly the sodium-chloride cotransporter in the TALH. It is to be noted that furosemide is a nonspecific inhibitor of sodium chloride transport systems; it has been reported to inhibit not only sodium-chloride cotransport systems, but also Cl^-/OH^- (HCO_3^-) exchange systems in the cortical TALH (Andreoli et al., 1981), anion exchange systems in red blood cells (Wiley & Cooper, 1974; Brazy & Gunn, 1976) and $Na^+-K^+-Cl^-$ cotransport systems (Geck, Heinz, Pietrzyk & Pfeiffer, 1978; Palfrey, Feit & Greengard, 1980).

The was no evidence to support the presence of additional sodium chloride transport mecha-

nisms in the plasma membranes of the medullary TALH. Amiloride, at 2 mM, had no effect on 0.5 mM sodium uptake, suggesting that Na^+/H^+ and Cl^-/OH^- exchange mechanisms were not working in synchrony to effect a 'loose' coupling of sodium and chloride transport. Amiloride is an inhibitor of the Na^+/H^+ exchanger with a K_i of 3×10^{-5} M (Kinsella & Aronson, 1981). Also in the rabbit cortical TALH, a $Na^+ - K^+ - Cl^-$ cotransport system has been reported (Greger & Schlatter, 1981) similar to the system found in Ehrlich ascites tumor cells (Geck et al., 1980) and erythrocytes (McManus & Schmidt, 1978; Dunham, Stewart & Ellory, 1980; Palfrey et al, 1980). We could find no effect of potassium removal on the chloride-dependent sodium movement and furosemide inhibited sodium-chloride transport as effectively when potassium chloride, choline chloride or lithium chloride were the accompanying salts. This lack of potassium dependence on the sodium-chloride cotransport system is supported by the recent report of Work, Troutman and Schafer (1981), which found no effect of chloride or furosemide on rubidium movement (and presumably potassium movements) in the isolated perfused medullary TALH.

The plasma membrane preparation used in this study consisted of both luminal and basal-lateral membranes as deduced from the presence of the membrane-bound enzymes Na,K-ATPase a basal-lateral membrane enzyme, and filipin-sensitive Mg-ATPase. There are yet no known luminal marker enzymes in the medullary TALH; however, from preliminary investigations into the separation of luminal and basal-lateral membrane vesicles, the filipin-sensitive Mg-ATPase may be located in the luminal membranes. Thus, its location would be similar to the proximal tubules where it is found in the luminal membranes (Kinne-Saffran & Kinne, 1979). The presence of both luminal and basal-lateral membranes in the plasma membrane vesicle preparation was probably the reason for the large 'nonspecific' residual sodium flux, that is the chloride- and furosemide-insensitive sodium flux. It is presumed that in a pure luminal membrane preparation, these nonspecific sodium conductive pathways would be much smaller. However, it is nevertheless postulated that the coupled sodium-chloride cotransporter is located in the luminal membranes of the TALH. This is concluded from the fact that furosemide and other loop diuretics exert their inhibitory effect only when applied to the luminal side of the TALH tubule and that the electrochemical potential barrier for active chloride movement is across the luminal membrane of the cell (Burg et al., 1973; Rocha & Kokko, 1973; Greger, 1981).

The apparent affinity, K_m, of the sodium-chloride cotransport system for sodium is around 2 mM (from Fig. 5). This is similar to the affinity constants reported for the sodium-chloride cotransporter in the cortical TALH and basal-lateral membranes from the rectal gland of the spiny dogfish (Eveloff et al., 1978; Greger, 1981). However, it is different from the apparent 'K_m' of 70 mM for sodium and chloride, which was reported for half-maximal stimulation of respiration in the medullary TALH cells from which the plasma membrane vesicles were prepared in this study (Eveloff et al., 1981). The discrepancy may be due to the fact that in the plasma membrane vesicles, the ionic requirements of the membrane carrier are investigated directly and the apparent 'K_m' of 2 mM for sodium measures the sodium requirement of the cotransport system alone. In the intact cell preparation, the 'K_m' for sodium and chloride of 70 mM found in the respiration studies is composed of the sum of the cellular sodium and chloride transport processes found in the cell, e.g. the sodium-chloride cotransport system, the Na,K-ATPase, the chloride exit step from the cell and sodium chloride diffusion pathways. Additionally, ion replacement studies in the intact cells may alter the membrane electrochemical potential difference across the cell and thus, the driving forces for sodium and chloride accumulation, which would alter the value of the apparent 'K_m'. We believe that an apparent K_m of 2 mM for sodium is probably a closer reflection of the true K_m of the sodium-chloride cotransporter than that measured in the respiration studies (Eveloff et al., 1981).

It is of interest that the medullary and cortical TALH appear to have different sodium chloride transport mechanisms. Both segments absorb chloride against an electrochemical potential gradient, in a sodium-dependent manner, have very low water permeabilities and lumen-positive transepithelial potential differences (Burg & Green, 1973; Rocha & Kokko, 1973; Greger, 1981; Hebert et al., 1981). Thus, they both act as diluting segments in the countercurrent multiplier system. However, whereas a directly coupled sodium-chloride cotransport system in the luminal membranes of the medullary TALH appears to be the primary mechanism for NaCl transport in this segment, the cortical TALH luminal membranes may contain several different transport systems. Chloride transport in the mouse cortical TALH is sensitive to bicarbonate and CO_2. Their removal from the bath and perfusion medium in isolated perfused tubules

reduces chloride transport by 50% (Friedman & Andreoli, 1981; Hebert et al., 1981). Also, the chloride reabsorption is inhibited by the carbonic anhydrase inhibitor ethoxzolamide, the anion exchange inhibitor SITS and amiloride, as well as furosemide (Andreoli et al., 1981; Friedman & Andreoli, 1981). In addition, chloride reabsorption in the isolated perfused rabbit cortical TALH appears to be potassium sensitive (Greger & Schlatter, 1981). The removal of K^+ from the luminal perfusion fluid reduced the short-circuit current to 66% of the control. Barium (1 mM), a competitive inhibitor of K^+ conductive pathways, further reduced the short-circuit current to 27% of the the control. Thus, it was proposed that an electroneutral $1Na^+ - 1K^+ - 2Cl^-$ cotransport system accounted for active chloride transport across the cortical TALH, analogous to the systems found in erythrocytes (Wiley & Cooper, 1974; McManus & Schmidt, 1978; Dunham et al., 1980; Palfrey et al., 1980) and Ehrlich ascites tumor cells (Geck et al., 1980). It is not yet known if the inhibitory effect of furosemide requires luminal K^+. In contrast, chloride transport in the medullary TALH is insensitive to bicarbonate removal, is potassium-independent and is not inhibited by the diuretics amiloride or ethoxzolamide, or SITS (Andreoli et al., 1981; Friedman & Andreoli, 1981). Thus, three different transport systems for sodium and chloride reabsorption in the cortical TALH can be considered. Since NaCl transport is bicarbonate-dependent, and inhibited by ethoxzolamide, amiloride and SITS, Friedman and Andreoli (1981) have suggested that two parallel exchangers, a Na^+/H^+ and Cl^-/HCO_3^- (OH^-), would effect a net NaCl cotransport across the apical membrane of the mouse cortical TALH. The intracellular hydrolysis of CO_2, catalyzed by the cytoplasmic carbonic anhydrase, would provide a continual supply of substrates (H^+ and HCO_3^-) for the two exchangers. In addition, an independent sodium-chloride cotransport system is also present in the luminal membrane of the mouse cortical TALH. This system would be furosemide-inhibitable and bicarbonate-independent. It is uncertain whether this sodium-chloride cotransport is the same as the $1Na^+ - 1K^+ - 2Cl^-$ cotransport system purportedly located in the rabbit cortical TALH (Greger & Schlatter, 1981) or whether the rabbit cortical TALH possesses the Na^+/H^+ and Cl^-/HCO_3^- antiport systems in addition to the directly coupled NaCl cotransport. The driving force for sodium chloride accumulation in both segments would be the favorable electrochemical potential gradient for sodium which exists across the luminal membrane as is maintained by the basal-laterally located Na,K-ATPase.

In conclusion, we have shown a direct coupling of sodium and chloride fluxes via a cotransport system which is sensitive to furosemide in the plasma membranes of the medullary TALH. Further experiments are necessary to clarify all the mechanisms of transcellular sodium-chloride cotransport in the medullary TALH, especially the questions of the origin of the lumen positive transepithelial potential difference. It is unknown if the sodium-chloride cotransport system is electroneutral or electrogenic. Further, the mechanisms of chloride exit from the basal-lateral membranes are unknown.

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After submission for publication of this article, experiments using a slightly modified isolation procedure and higher chloride concentrations have revealed that the bumetanide and chloride-sensitive sodium uptake into TALH plasma membrane vesicles may increase with increasing potassium concentrations (Koenig, B., Kinne, R., 1982: The role of potassium in chloride transport of the thick ascending limb of Henle's loop. *Pflueger's Arch.* **394**:R23). Thus, it is not excluded that a certain portion of sodium chloride cotransport in the medullary TALH plasma membrane vesicles proceeds via the $Na-K-Cl$ cotransport system.