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R C Ng, … , D Rouse, W N Suki

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Research Article

Calcium transport was studied in isolated S2 segments of rabbit superficial proximal convoluted tubules. 45Ca was added to the perfusate for measurement of lumen-to-bath flux (JlbCa), to the bath for bath-to-lumen flux (JblCa), and to both perfusate and bath for net flux (JnetCa). In these studies, the perfusate consisted of an equilibrium solution that was designed to minimize water flux or electrochemical potential differences (PD). Under these conditions, JlbCa (9.1 +/- 1.0 peq/mm X min) was not different from JblCa (7.3 +/- 1.3 peq/mm X min), and JnetCa was not different from zero, which suggests that calcium transport in the superficial proximal convoluted tubule is due primarily to passive transport. The efflux coefficient was 9.5 +/- 1.2 X 10(-5) cm/s, which was not significantly different from the influx coefficient, 7.0 +/- 1.3 X 10(-5) cm/s. When the PD was made positive or negative with use of different perfusates, net calcium absorption or secretion was demonstrated, respectively, which supports a major role for passive transport. These results indicate that in the superficial proximal convoluted tubule of the rabbit, passive driving forces are the major determinants of calcium transport.

Calcium Transport in the Rabbit Superficial Proximal Convoluted Tubule

Roland C. K. Ng, Diane Rouse, and Wadi N. Suki
Renal Section, Department of Medicine, The Methodist Hospital and Baylor College of Medicine, Houston, Texas 77030

Abstract. Calcium transport was studied in isolated S_2 segments of rabbit superficial proximal convoluted tubules. ^{45}Ca was added to the perfusate for measurement of lumen-to-bath flux (J_{lb}^{Ca}), to the bath for bath-to-lumen flux (J_{bl}^{Ca}), and to both perfusate and bath for net flux (J_{net}^{Ca}). In these studies, the perfusate consisted of an equilibrium solution that was designed to minimize water flux or electrochemical potential differences (PD). Under these conditions, J_{lb}^{Ca} (9.1±1.0 peq/mm·min) was not different from J_{bl}^{Ca} (7.3±1.3 peq/mm·min), and J_{net}^{Ca} was not different from zero, which suggests that calcium transport in the superficial proximal convoluted tubule is due primarily to passive transport. The efflux coefficient was $9.5\pm1.2 \times 10^{-5}$ cm/s, which was not significantly different from the influx coefficient, $7.0\pm1.3 \times 10^{-5}$ cm/s. When the PD was made positive or negative with use of different perfusates, net calcium absorption or secretion was demonstrated, respectively, which supports a major role for passive transport. These results indicate that in the superficial proximal convoluted tubule of the rabbit, passive driving forces are the major determinants of calcium transport.

Introduction

Approximately 60% of filtered calcium is reabsorbed in the proximal convoluted tubule (1, 2). Controversy exists as to the mechanism of calcium transport in this segment of the nephron. Findings supportive of passive transport have been the very high permeability to calcium (3) and the parallelism between the tubular handling of calcium and of sodium and water, such that the tubular fluid-to-ultrafilterable (TF/UF)[1] calcium to sodium concentration ratio is very nearly 1.0 (1), although values as high as 1.2 have been reported (4).

However, several lines of evidence indicate some degree of active calcium transport. The first is that in free-flow micropuncture experiments in the presence of an osmotic diuresis, the $[TF/UF]_{Ca}$ concentration ratio falls to <1.0, which suggests transport of calcium against a concentration gradient (1, 2). A mitigating factor, however, is that a lumen-positive potential difference (PD) exists beyond the very early proximal convolutions (5), which could provide a driving force for calcium transport. A second line of evidence is that Ullrich et al. (6), in an in situ tubule microperfusion study with simultaneous capillary perfusion, found that more calcium was transported than could be accounted for by the calculated electrochemical gradient. When choline or lithium was substituted for sodium, or ouabain was added, in tubular lumen and capillary, the active component of transport was abolished, which suggests a sodium-calcium antiport mechanism such as that found in the squid axon (7).

The purpose of this study was to examine the mechanism of calcium transport in the superficial proximal convoluted tubule under conditions in which passive driving forces are absent or minimal. Any remaining transport should be active. Under these circumstances, we find virtually no net calcium transport. Furthermore, when different perfusion solutions are used to generate either a lumen-positive or lumen-negative potential, net calcium absorption or secretion, respectively, can be demonstrated.

Methods

Segments of rabbit proximal convoluted tubules were perfused in vitro using methods described previously (8–11). Briefly, female New Zealand

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1. *Abbreviations used in this paper:* J_{bl}^{Ca}, bath-to-lumen flux; J^{Ca}, calcium flux; C_i, C_o, disintegrations per minute per nanoliter of ^{45}Ca in the perfusate and collected sample, respectively; K_e^{Ca}, K_i^{Ca}, efflux and influx coefficients for calcium, respectively; J_{lb}^{Ca}, lumen-to-bath flux; J_{net}^{Ca}, net calcium flux; PTH, parathyroid hormone; PD, potential difference; σ, reflection coefficient; TF/UF, tubular fluid-to-ultrafilterable concentration ratio; J_v, water absorption.

white rabbits that weighed between 1.5 and 2.5 kg were killed by decapitation. Immediately after exsanguination, the left kidney was removed and decapsulated, and 1-mm transverse slices were obtained. A second cut parallel to and within 2 mm of the cortical surface was made, and the medullary portion was discarded. This cortical slice was then placed in a Petri dish that contained chilled rabbit serum (type 2-UC rabbit serum from Irvine Scientific, Santa Ana, CA). Single segments of proximal convoluted tubules, identified morphologically as S_2 segments, were dissected with the aid of a dissection microscope, transferred to a thermostatically controlled perfusion chamber, and perfused within 30 min of killing.

All solutions were equilibrated with 5% CO_2 and 95% O_2 gas mixture at 37°C before use. Except for tubules studied at 23°C, the bath was maintained at 37°C throughout the experiment. To ensure constant osmolality, the bath solution was changed at a rate of 0.5 cm^3/min. For tubules studied at 23°C, cooled bath was added to the bath chamber every 2–3 min to maintain the appropriate temperature. Transtubular PD was measured using agarose-Ringer's bridges and calomel half-cells (Beckman Instruments, Inc., Fullerton, CA) as described by Kokko (12).

Before any tubule was studied, it was allowed to equilibrate for 20–30 min. Water absorption (J_v) was measured using exhaustively dialyzed (13) methoxy-^{3}H-inulin, that was added to the perfusate to a final activity of 6–50 dpm/nl. All sample collections exceeded background activity by at least a factor of 50. Calcium flux (J^{Ca}) was measured with $^{45}CaCl_2$ (New England Nuclear, Boston, MA). To either perfusate and/or bath, ^{45}Ca was added to a final activity of 5–10 dpm/nl, and all collected samples exceeded background activity by at least a factor of 30.

Timed fluid collections were made under a layer of oil with calibrated constant-volume constriction pipettes. The samples were expelled into counting vials that contained 1 ml of water and two drops of 12 N HCl. 10 ml of Biofluor (New England Nuclear) was then added and each vial shaken vigorously. Isotopic concentrations were measured in a liquid-scintillation spectrometer (Packard Instruments Co., Inc., Downers Grove, IL). The error in counting averages 1.5%, which would enable the detection of a calcium flux >0.12 peq/mm · min.

Precautions taken to prevent loss of ^{45}Ca-counts included the following: (*a*) To prevent binding of calcium to glass, all pipettes in contact with perfused or collected fluid were siliconized with SC-87 (Pierce Chemical Co., Rockford, IL); (*b*) All oils used were water-equilibrated and bubbled with 100% CO_2 to prevent loss of CO_2, alkalinization of fluid samples, and precipitation of calcium salts; (*c*) Collected samples were expelled from the constant-volume constriction pipette with a 10-mM EGTA solution to ensure complete removal of ^{45}Ca; and (*d*) All counting vials contained two drops of 12 N HCl to acidify the scintillation fluid and prevent loss of counts with time (14). In previous studies, the above maneuvers were sufficient to prevent binding of isotopic calcium from static solutions in the pipettes for at least 2 h. Furthermore, recovery of isotopic calcium in the bath closely matches that which disappears from the perfusate (10, 11).

The compositions of the different perfusates and baths that were used in these studies are listed in Table I. The chemical analyses on all solutions and batches of rabbit serum were performed as follows: sodium and potassium on a flame photometer (Instrumentation Laboratory, Inc., Lexington, MA); calcium and magnesium on an atomic absorption spectrophotometer (Instrumentation Laboratory, Inc.); ionized calcium on an Orion calcium analyzer (Orion Biomedical, Cambridge, MA); bicarbonate on a CO_2 analyzer (Ericsen Instruments, Ossining, NY); chloride on a Corning chloride meter (Corning Medical and Scientific, Corning Glass Works, Medfield, MA); osmolality on an osmometer (Advanced Instruments, Inc., Needham Heights, MA); and glucose and phosphate on a Gilford autoanalyzer (Gilford Instrument Laboratories, Inc., Oberlin, OH). Equilibrium solution was designed to omit those substances that are thought to play a role in volume reabsorption and substitutes nonreabsorbable raffinose for glucose and alanine, and 30 meq sodium cyclamate for 10 meq of sodium acetate and 20 meq of sodium bicarbonate. This perfusion solution, when used with a rabbit serum bath, had previously been shown by Jacobson (15) to abolish water transport in superficial proximal convoluted tubules. To avoid large concentration gradients of calcium, the calcium

Table I. Composition of Perfusate and Bath Solutions

	Equilibrium solution*	Rabbit serum (RS)	RS	RS ultrafiltrate (UF)	A solution*	B solution*
	mM	*mM*	*mM*	*mM*	*mM*	*mM*
Na	144.9	156.1	153.9	142.0	143.0	149.0
K	5.1	5.8	5.5	5.1	5.1	5.1
Cl	115.0	109.8	113.0	115.0	111.0	140.0
HCO_3	5.0	25.5	26.9	25.0	25.7	6.0
Ca	1.4	1.4 (UF)	1.5 (UF)	1.5	1.6	1.6
Ca^{2+}	1.4	1.1	1.3	1.3	1.0	1.4
Cyclamate	30.0	0	0	0	0	10
Raffinose	13.0	0				
Glucose	0				8.0	0
Alanine	0				5.0	0
Osmolality (*mosmol/kg*)	298	298	290	290	290	289
Study groups	I, II, III, IV		VI		V, VII	

* All solutions contained 1.0 mM Mg, 1.0 mM SO_4, and 1.2 mM PO_4.

concentration was selected on the basis of the ultrafilterable calcium of the rabbit serum used in the bath. Solution A simulated an ultrafiltrate of rabbit serum, and solution B simulated proximal tubular fluid beyond the very early portion. When ionized calcium (Ca^{2+}) was measured on these solutions, equilibrium solution Ca^{2+} was 2.8 meq/l while rabbit serum was 2.2 meq/l. For solution B, Ca^{2+} was 2.8 meq/l, and for solution A was 2.0 meq/l. Using the equation $J = K_i^{Ca} \times \Delta C$, where J is flux, K_i^{Ca} the influx coefficient, and ΔC the concentration difference between lumen and bath, and the value for K_i^{Ca} calculated from the unidirectional influx experiments, the contribution of these small concentration gradients is 0.2 peq/mm · min or less.

When rabbit serum was used in the bath, the PD was corrected for the calculated liquid junction potential, which was −1.3 mV when the perfusate was equilibrium solution and −1.1 mV when it was rabbit serum ultrafiltrate. When B solution was used as the perfusate with A solution in the bath, the calculated liquid junction potential was −1.8 mV, and the PD was corrected accordingly. In experiments in which a protein bath was used, no correction was made for the Donnan potential since Berry and Rector (16) and others (17, 18) note insignificant changes in PD when low molarity salt bridges are used. Additionally, Biagi and Giebisch (19) and Laprade and Cardinal (20) have found that the measured liquid junction potentials imposed by artificial serums and solutions that contained no protein were not dissimilar to the values calculated on the basis of ion asymmetries. Using a method similar to Laprade and Cardinal, we have found that the liquid junction potential calculated from ion asymmetries accurately represents the measured potential.

Seven types of experiments were performed: The first type (n = four tubules) was designed to verify the effectiveness of equilibrium solution in eliminating J_v and transepithelial PD. The perfusate consisted of equilibrium solution that contained only the volume marker and the bath was rabbit serum. Low perfusion rates were employed to maximize the perfused-to-collected ^{3}H-inulin concentration difference. The second type (n = six tubules) was designed to measure lumen-to-bath flux of calcium in the absence of water absorption, concentration gradients, or electrical PD. The perfusate consisted of equilibrium solution that contained ^{45}Ca, and the bath was rabbit serum. The third type of experiment (n = six tubules) was designed to measure bath-to-lumen flux of calcium under identical conditions as in the first set of studies, i.e., in the absence of significant J_v, concentration gradients, or electrical potential. ^{45}Ca was added to rabbit serum and allowed to equilibrate overnight. The perfusate was equilibrium solution. The fourth type of experiment consisted of net flux studies in the absence of J_v, concentration gradients, or electrical PD. Equilibrium solution that contained the same specific activity of ^{45}Ca was used in both bath and perfusate. Six tubules were studied using high perfusion rates (>30 nl/min) and five were studied using low (2.0–3.5 nl/min) and moderate (12–17 nl/min) perfusion rates.

The fifth type of experiment (n = six tubules) was designed to measure the reflection coefficient for calcium in the S_2 segment. Tubules were perfused and bathed at 23°C with A solution that contained the identical specific activity of ^{45}Ca. Control collections were made using equiosmolar A solution in the bath and perfusate, and then the bath changed to one of identical composition except for the addition of 25 mosmol/l of raffinose. In the sixth type of experiment (n = seven tubules), net flux studies were performed using rabbit serum ultrafiltrate as the perfusate and rabbit serum as the bath. Under these conditions, luminal PD is negative with respect to the bath (−2.0 mV). ^{45}Ca was added to rabbit serum and allowed to equilibrate over at least 24 h. After bubbling the rabbit serum with a gas mixture of 95% O_2 and 5% CO_2, the ultrafiltrate was prepared by pressure ultrafiltration of the serum through Diaflo PM-30 membranes (Amicon Corp., Lexington, MA). Finally, in the seventh type of experiment (n = five tubules), net flux studies were performed with A solution in the bath and B solution as the perfusate. ^{45}Ca was added to both solutions to obtain identical specific activity (average difference 0.7%). In these studies, fetal calf serum (Gibco Laboratories, Grand Island, NY), 5% vol/vol, was added to the bathing solution. When superficial proximal convoluted tubules are perfused with B solution and ouabain is added, Jacobson (15) has shown that a positive luminal PD will be generated. However, to avoid problems of interpretation arising from the use of ouabain, in these studies the temperature of the bath was instead lowered to 23°C. Under these circumstances, the PD was +1.9 mV.

Calculations. J_v (nl/mm · min) was calculated as follows:

$$J_v = (V_i - V_o)/(L), \tag{1}$$

where V_i is the rate of perfusion (nl/min), V_o is the rate of fluid collection (nl/min), and L is the tubule length (mm).

Unidirectional efflux (J_{lb}^{Ca}) (peq/mm · min) and unidirectional backflux (J_{bl}^{Ca}) (peq/mm · min) were calculated with the following equations:

$$J_{lb}^{Ca} = (V_iC_i - V_oC_o/L)([Ca]_i/C_i), \tag{2}$$

or

$$J_{lb}^{Ca} = K_e^{Ca}[\overline{Ca}]_l, \tag{3}$$

and

$$J_{bl}^{Ca} = (V_oC_o/L)([Ca]_b/C_b), \tag{4}$$

or

$$J_{bl}^{Ca} = K_i^{Ca}[Ca]_b, \tag{5}$$

where C_i, C_o, and C_b are the dpm/nl of ^{45}Ca in the perfusate, collected sample, and bath ultrafiltrate, respectively; $[Ca]_i$ and $[Ca]_b$ are the chemical calcium concentration in the perfusate and bath ultrafiltrate, respectively; $[\overline{Ca}]_l$ is the mean calcium concentration in the lumen; and K_e^{Ca} and K_i^{Ca} the efflux and influx coefficients for calcium, respectively. The efflux values were not different when calculated using Eq. 2 vs. Eq. 3, and the influx values did not differ using Eq. 4 vs. Eq. 5. Net calcium flux (J_{net}^{Ca}) was also calculated using Eqs. 2 and 3. The tubule length was measured with a reticle in the eyepiece of the microscope.

Efflux (K_e^{Ca}) (cm/s) and influx (K_i^{Ca}) (cm/s) coefficients were calculated using the following equations (21):

$$K_e^{Ca} = (V_i/A)\ln(C_i/C_o), \tag{6}$$

and

$$K_i^{Ca} = (V_o/A)\ln(C_b/C_b - C_o), \tag{7}$$

where A is the tubule surface area calculated from the measured length and an assumed internal diameter of 20 μm.

The reflection coefficient (σ) for calcium was calculated using the following equation:

$$J_{net}^{Ca} = J_v(1 - \sigma)\bar{c}, \quad (8)$$

where $\bar{c}$ is the mean calcium concentration in the perfusate and bath solutions, and denotes the change due to the addition of raffinose to the bath. The contribution of solvent drag in experiments where fluid absorption was present was also calculated using this equation.

The transepithelial PD was corrected where appropriate for the liquid junction potential (LJP) as follows (22): LJP = (RT[Up − Vp] − [Ub − Vb]/F[Up + Vp] − [Ub + Vb]) ln(Up + Vp)/(Ub + Vb), where R and F are the gas and Faraday constants, respectively; T the absolute temperature; Up and Ub the sum of the products of the cation concentrations and their conductances in the perfusate and bath, respectively; and Vp and Vb the sum of the products of the anion concentrations and their conductances in the perfusate and bath, respectively. Ion concentrations were corrected for water concentration in protein-containing baths.

Statistical Analysis. A minimum of three to four collection periods were obtained and averaged for each tubule. The results are then expressed as mean±SEM of number of tubules (*n*) studied. The *t* test for either paired or unpaired samples was used when appropriate. Differences were considered significant at a *P*-value < 0.05.

Results

Verification studies. In order to measure unidirectional ^{45}Ca fluxes across a "leaky" epithelium, such as the proximal convoluted tubule, high perfusion rates must be employed to minimize alterations in the specific activity of ^{45}Ca. These high perfusion rates interfere with the accuracy of measuring water absorption, as the concentration difference of perfused and collected ^{3}H-inulin is minimal. For this reason, J_v was measured in four tubules that were bathed with rabbit serum and perfused at the rate of 1.8–3.2 nl/min·mm with equilibrium solution. PD averaged −0.3±0.09 and water absorption −0.07±0.04. Neither value is significantly different from zero. Mean tubule length was 0.7±0.1 mm.

Unidirectional efflux studies. The results for six tubules are summarized in Table II. To minimize alterations in the specific activity of ^{45}Ca, high perfusion rates were deliberately chosen (mean of 37.3±1.9 nl/min). J_v was 0.03±0.16 nl/mm·min, and PD was +0.3±1.0 mV. Both values were not significantly different from zero. Unidirectional calcium flux from lumen-to-bath, corrected for flux due to the concentration gradient, was 9.1±1.0 peq/mm·min, with a mean tubule length of 1.1±0.1 mm. The calculated K_e^{Ca} was 9.5±1.2 × 10^{-5} cm/s (6.0 × 10^{-8} cm^2/s), a value that is roughly an order of magnitude lower than that obtained by Ullrich in rats (14 × 10^{-7} cm^2/s) (6). The ratio of Co/Ci for these experiments averaged 0.90±0.02. Using the mean luminal concentration of calcium and K_e^{Ca} to calculate the J_{lb}^{Ca} did not change the value significantly.

Unidirectional backflux studies. The results for six tubules are summarized in Table II. Again, high perfusion rates were maintained to minimize changes in specific activity of ^{45}Ca (mean of 38.5±4.2 nl/min). J_v was 0.20±0.17 nl/mm·min, and PD was −0.2±0.4 mV. Both values were not significantly different from zero. Unidirectional J^{Ca} from bath-to-lumen, corrected for flux due to the concentration gradient, was 7.3±1.3 peq/mm·min, with a mean tubule length of 0.9±0.1 mm. When the results of calcium backflux were compared with the results of calcium efflux, there was no significant difference ($P > 0.20$). Thus, in the absence of any significant water transport, concentration gradient, or electrical PD, calcium backflux is virtually identical with calcium efflux. The K_i^{Ca} was 7.0±1.3 × 10^{-5} cm/s (4.3 × 10^{-8} cm^2/s), and was not significantly different from K_e^{Ca}, which suggests a purely passive transport for calcium.

Net flux studies-equilibrium solutions. The results of six tubules perfused at high rates are summarized in Table II. Perfusion rates were comparable with those used in the second and third groups of tubules (mean of 35.7±2.3 nl/min). J_v was −0.08±0.15 nl/mm·min, and J_{net}^{Ca} was 0.43±0.82 peq/mm·min. Both values were not significantly different from zero. PD was −0.5±0.2 mV and, although small, was signifi-

Table II. Calcium Fluxes in the Superficial Proximal Convoluted Tubule

Number of tubules	Flux measured	Perfusate	Bath	J^{Ca}	J_v	PD
				peq/mm·min	*nl/mm·min*	*mV*
6	lb	Equil soln	RS	9.1±1.0*	0.03±0.16‡	+0.3±1.0‡
6	bl	Equil soln	RS	7.3±1.3*	0.20±0.17‡	−0.2±0.4‡
6	Net	Equil soln	Equil soln	0.43±0.82‡	−0.08±0.15‡	−0.5±0.2§
7	Net	RSUF	RS	−1.2±0.5§	0.79±0.13§	−2.0±0.6§
5	Net	B soln	A soln	3.1±0.5§	0.44±0.19§	+1.9±0.6§

Last group was studied at 23°C. Corrections for flux due to solvent drag and concentration gradient have been made where appropriate. Bl, bath-to-lumen flux; lb, lumen-to-bath flux; net, net flux; RS, rabbit serum; and RSUF, rabbit serum ultrafiltrate.

* Not significant (NS), I vs. II.

‡ NS vs. zero.

§ $P < 0.05$ vs. zero.

cantly different from zero ($P < 0.05$). Mean tubule length was 0.8±0.1 mm. These studies are consistent with the first two groups of studies and show virtually no net calcium transport in the absence of water transport or significant electrochemical differences. The ratio of Co/Ci was 1.00±0.01 for these experiments. For five tubules studied at low and moderate perfusion rates, the mean length was 0.7±0.1 mm. With a mean perfusion rate of 2.8±0.3 nl/min, J_v was −0.02±0.1 nl/min, and J_{net}^{Ca} was 0.2±0.2 peq/mm · min. When perfusion rate was increased (mean of 13.5±0.9 nl/min), J_v was −0.09±0.13 nl/mm · min, and J_{net}^{Ca} was 0.4±0.4 peq/mm · min. None of the values for these later five tubules was significantly different from zero. PD was not detectable from zero by our methods. The ratio of Co/Ci averaged 0.99±0.01 for these experiments.

Reflection coefficient measurements. The mean perfusion rates in these six tubules were 14.7±0.9 nl/min with no osmotic gradient and 15.1±0.6 in the presence of a 25 mosmol/l-gradient, bath greater than perfusate. The mean J_v was 0.38±0.02 nl/mm · min before and 0.83±0.07 after the imposition of the gradient. PD was not significantly different from zero and the mean reflection coefficient was 0.89±0.38 (Table III). The ratio of Co/Ci was 1.00±0.01 for these experiments.

Net flux studies-rabbit serum and ultrafiltrate. The results for seven tubules are summarized in Table II. Perfusion rate was 21.4±4.2 nl/min. J_v was 0.79±0.13 nl/mm · min, and PD was −2.0±0.6 mV. Mean tubule length was 0.9±0.1 mm. There was net calcium secretion of −1.04±0.45 peq/mm · min in the presence of a lumen-negative PD despite the presence of J_v. When corrected for the contribution of solvent drag, this net secretory flux was −1.2±0.5 peq/mm · min, which is significantly different from zero ($P < 0.02$). The ratio of Co/Ci for these experiments averaged 1.07±0.02.

Net flux studies-A and B solutions at 23°C. The results for five tubules are summarized in Table II. Perfusion rate was 23.6±5.1 nl/min. J_v was 0.44±0.19 nl/mm · min, and PD was +1.9±0.6 mV. Mean tubule length was 1.0±0.1 mm. In the presence of a lumen-positive PD, there was net calcium absorption of 3.5±0.5 peq/mm · min. When corrected for the contribution of the concentration gradient and solvent drag, net calcium absorption was 3.1±0.5 peq/mm · min. The ratio of Co/Ci averaged 0.97±0.01.

Thus, when all contributions to passive transport, i.e., solvent drag, electrical potential, and concentration differences, were minimized, backflux of calcium was nearly equal to efflux. Net flux studies under similar circumstances demonstrated no net transport. When PD was altered via a chemical voltage clamp, there was net calcium secretion in the presence of a negative PD and net absorption in the presence of a positive PD. To further evaluate the relationship between J_{net}^{Ca} and PD, the values for net flux from individual tubules ($n = 18$) were plotted as a function of PD. The regression line is $y = 0.67 \times +0.80$, $r = 0.579$ ($P < 0.02$) (Fig. 1). While the slope of this latter regression line is significantly >0 ($P < 0.01$), the y-intercept is not ($0.05 < P < 0.10$), which indicates the absence of net transport when the PD is zero.

Discussion

The present results demonstrate that calcium transport in the S_2 segments of the superficial proximal convoluted tubule of the rabbit is primarily passive. Under conditions in which solvent drag and electrochemical differences are minimized, there is virtually no net transport of calcium. When either a lumen-negative or lumen-positive PD is generated, net calcium secretion or absorption occurs.

Free-flow micropuncture studies in the rat had earlier suggested active transport of calcium when the $[TF/UF]_{Ca}$ concentration ratio fell below 1.0 under conditions of an osmotic diuresis (1, 2). However, since electrical PD was not measured, the mechanism of transport remained unclear. Subsequently, studies by Fromter et al. (5) demonstrated a lumen-positive potential beyond the very early proximal convolutions, and that, coupled with the findings by Murayama (3) of very high permeability to calcium, cast further doubt on an active transport mechanism.

Table III. Calcium Reflection Coefficient Determinations

	V_i		J_v		J_{Ca}^{net}		σ_{Ca}
Experiment #	N	H	N	H	N	H	
	nl/min	*nl/min*	*nl/mm · min*	*nl/mm · min*	*peq/mm · min*	*peq/mm · min*	
1	14.92	16.00	0.35	0.86	2.82	2.93	0.94
2	17.50	17.15	0.33	0.71	2.41	2.46	0.96
3	12.93	13.63	0.37	0.78	3.06	3.32	0.82
4	15.36	14.18	0.42	0.70	1.09	1.32	0.77
5	12.66	14.68	0.44	1.10	1.89	2.02	0.94
$\bar{M}$	14.67	15.13	0.38	0.83	2.25	2.41	0.89
±SEM	0.88	0.64	0.02	0.07	0.35	0.35	0.38

N, equiosmolar bath and perfusate; H, 25-mosmol/l gradient, with bath greater than perfusate.

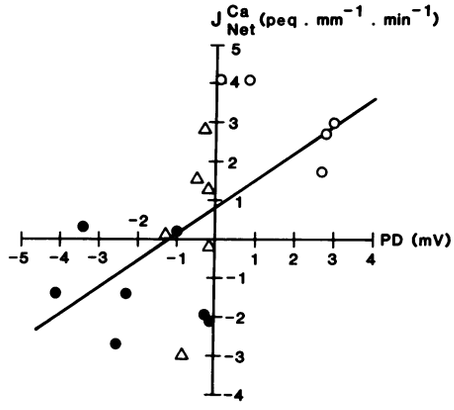

Figure 1. Voltage dependence of J_{net}^{Ca} in the superficial proximal convoluted tubule. The mean J_{net}^{Ca} of individual tubules (n = 18) are plotted against the mean PD. The PD of these tubules was altered with the use of different perfusates and baths. With the use of rabbit serum and rabbit serum ultrafiltrate (●), a negative potential difference resulted in net secretion of calcium. With B solution (simulating late proximal tubular fluid) as perfusate and A solution (simulating rabbit serum ultrafiltrate) as bath (○), a positive PD resulted in net absorption of calcium. When equilibrium solution was used as both bath and perfusate (△), no net transport could be demonstrated. The linear regression line was y = 0.67 × +0.80, r = 0.579 ($P < 0.02$). The y-intercept is not different from zero ($P < 0.1$).

More recently, Ullrich et al. (6), using stopflow microperfusion with simultaneous capillary perfusion, were able to demonstrate an active component of calcium transport in the proximal tubule of the rat. This demonstration was based on transport in excess of the calculated electrochemical gradient. When sodium in the tubular and capillary perfusates was replaced with either choline or lithium, the calculated component of active transport was abolished. Furthermore, when ouabain was added to the perfusates in studies of golden hamsters, the component of active transport was again inhibited, which suggests a link between sodium and calcium as in a Na^+-Ca^{++} antiport mechanism in the basolateral aspect of the membrane (7, 23, 24). Although these investigators did not measure the total transport rate of calcium in the proximal tubule under control conditions, the active component was calculated as 3.35×10^{-13} mol/cm·s (4.0 peq/mm·min), or roughly one-third the net transport rate of 12.0 peq/mm·min that Murayama et al. (3) obtained from microperfusion experiments in the same species of rats. Thus, a significant percentage of total net transport could conceivably be due to active mechanisms.

In contrast to Ullrich's results in the rat, we find no strong evidence for active transport of calcium in the S_2 segment of the rabbit proximal tubule; backflux of calcium was virtually identical with efflux under conditions of negligible PD, concentration gradient, or water flux. Similarly, under such conditions, J_{net}^{Ca} was not different from zero. When J_{net}^{Ca} was plotted as a function of luminal potential difference, there was a highly significant correlation with absorption occurring in the presence of a positive PD and secretion in the presence of a negative PD. Coupled with the observation that the K_e^{Ca} was not statistically different from the K_i^{Ca}, this suggests primarily passive, voltage-dependent transport of calcium in this tubule segment. The coexistence of an active transport mechanism in a segment of the nephron that normally transports calcium passively in a voltage-dependent fashion is not without precedent. Bourdeau and Burg (14) and Shareghi and Agus (26) have well demonstrated the voltage-dependence of presumably passive, net calcium transport in the rabbit cortical thick ascending limb of Henle, yet parathyroid hormone (PTH) is able to enhance net calcium absorption without a change in PD (26, 27). Similarly, Suki et al. (10) have also demonstrated apparently passive, voltage-dependent calcium transport in the medullary thick ascending limb, which is enhanced by calcitonin in the absence of any change in PD (11). One explanation for such findings is the presence of an active transport mechanism of low basal activity, which is enhanced or uncovered in the presence of a hormone such as PTH or calcitonin (11). Certainly, the enzymes necessary for such active transport are present in this segment of the nephron, i.e., PTH-sensitive adenyl cyclase (28) and high affinity Ca-Mg-ATPase (29).

There are additional possible explanations for the discrepancy between our findings and that of Ullrich. The most obvious is a species difference. For example, in comparison with rats or mice, rabbits may have different strengths of activities or distribution of enzymes such as Na-K-ATPase (30) or PTH-sensitive adenyl cyclase (28). Similarly, species differences may also apply to Ca-Mg-ATPase, although comparative studies have not yet been done (29). A second possible difference is the methodology—one in vivo and the other in vitro. Lastly, some component of the equilibrium solution that was used in the present study may have abolished active transport of calcium independently of its effect on the passive driving forces. However, the evidence for such an effect is lacking in this and other studies which have used this solution.

Recent preliminary studies by Bomsztyk and Wright (31) in micropuncture studies in rats support our observations that calcium transport in the proximal convoluted tubule is primarily passive. In the absence of water flux, there was net calcium absorption with a positive electrochemical gradient and net secretion with a negative electrochemical gradient. In contrast to our findings, however, in the absence of an electrochemical gradient, there was residual net transport of calcium, which suggests the presence of a small component of active transport.

Although our study is the first to directly examine the mechanism of calcium transport in the rabbit proximal convoluted tubule in vitro, there are two other reports of calcium transport in proximal convoluted tubules in vitro. In a preliminary report, 25-hydroxycholecalciferol was noted to enhance unidirectional calcium efflux from 15 to 26 peq/mm·min (32). In another recent paper that examined the effects of

lowering peritubular sodium concentration on water transport in the proximal convoluted tubule, net calcium transport was measured in six tubules (33). Substitution of lithium or choline for sodium in the bath fluid lowered the J_{net}^{Ca} of 6.7 peq/mm · min by 60%. It is difficult to compare these results with ours, however, since no information is given regarding the location of the tubules used, whether superficial or juxtamedullary. Since calcium transport generally follows sodium transport, it is also conceivable that calcium handling would be primarily active in juxtamedullary proximal convoluted tubules and primarily passive in superficial proximal convoluted tubules, as demonstrated for sodium by Jacobson (15). Because the relative permeabilities of sodium and chloride differ in superficial and juxtamedullary nephrons (34), the PD profile would be lumen-positive in superficial nephrons enhancing passive cation transport, whereas it would be lumen-negative in juxtamedullary nephrons, which require, under normal in vivo conditions, transport of cations against an electrical gradient. Another difficulty in comparing our results with the information provided by the work of Friedman et al. (33) is that the contributions of changes in J_v and PD on changes in J_{net}^{Ca} are not readily apparent in their study.

Extrapolations from data obtained in in vitro microperfusion experiments to in vivo occurrences are limited by the functional and morphological heterogeneities found along the nephron. Such extrapolations, however, can be helpful in comparing transport rates of a single solute among various nephron segments, as well as the transport rates of several solutes in the same nephron segment. The magnitude of net calcium transport that we find (3.10 peq/mm · min) with an artificial perfusate simulating late proximal tubular fluid is threefold the magnitude of net transport in the thick ascending limb of Henle (10). Assuming that there is a filtered load of calcium of 73.1 peq/min (ultrafilterable calcium = 4.3 peq/nl × 17 nl/min) (35, 36) and a length of the accessible proximal convoluted tubule of 5.4 mm (36), the observed net transport rate can account for absorption of 23% of the filtered load of calcium in the accessible proximal convoluted tubule. This is 40% of the filtered fraction of calcium (60%) that is normally reabsorbed in the proximal convoluted tubule. There are several possible explanations for this lower rate of transport. Firstly, the studies were done with artificial solutions in the absence of any significant amounts of protein in the bath. Secondly, this may be a limitation of the in vitro method. For example, in clearance and micropuncture experiments in rabbits by Chonko et al. (36), the J_v rate in the proximal convoluted tubule was 1.9 nl/mm · min. However, most studies of J_v in the proximal tubule in vitro find 50% of this rate, i.e., 0.8–1.0 nl/mm · min (15, 18, 33, 37). Thus, if one can extrapolate from these findings, namely, that calcium follows sodium and water transport in the proximal convoluted tubule, then the estimated in vitro transport rate is probably 50% the true in vivo rate. Finally, this lower transport rate could be artifactual, but a number of precautions were taken to avoid ^{45}Ca loss. Furthermore, loss of ^{45}Ca in our technique would tend to overestimate the net transport rate rather than the reverse, since net transport is measured by disappearance of the isotope. It appears more likely that the apparently low transport rate is probably due to inherent limitations of the in vitro microperfusion technique, possibly combined with the use of artificial solutions.

The finding of passive calcium transport in the S_2 segment of the superficial proximal convoluted tubule of the rabbit is consonant with the published literature that suggests that there is an interdependence of sodium and calcium (38, 39). Those maneuvers, which depress proximal tubular reabsorption of sodium and water, such as volume expansion (38), PTH, (40), insulin (41), renal vasodilators (42), and acetazolamide (43), also similarly depress proximal tubular reabsorption of calcium, which results in parallel excretion of sodium and calcium in the final urine. In contrast, those factors which dissociate sodium from calcium in the final urine enhance either distal active sodium reabsorption, such as mineralocorticoids (44, 45), or distal active calcium reabsorption, such as PTH (40) or thiazide diuretics (46). The high permeability of the proximal convoluted tubule to calcium that we and others (3) have demonstrated suggests that passive driving forces would more than adequately explain the parallel handling of sodium and calcium in this segment of the nephron. In this regard, it is interesting to note that our calculated permeability (flux coefficient) for calcium in the superficial proximal convoluted tubule closely approximates the values for the permeability of sodium that were obtained by others in the proximal convoluted tubule of the rabbit (47).

In rats, rabbits, and dogs, the S_2 segment of the proximal tubule normally displays a lumen-positive PD. The present results imply that calcium would be reabsorbed in this segment under normal physiological conditions. Should calcium transport in the early convolutions (S_1) also be passive, the lumen-negative PD, which is normally found in this segment, would cause secretion of calcium. However, one cannot assume that the mechanism for calcium transport in the early convolutions is the same as in the later convolutions. There may well exist an active component for calcium reabsorption early in the proximal tubule.

In summary, we have demonstrated that calcium transport in the superficial proximal convoluted tubule of the rabbit is due primarily to passive driving forces, i.e., solvent drag and electrochemical differences. In the absence of any water flux or electrochemical gradients, backflux of calcium is nearly equal to efflux, and net transport is negligible. Under conditions of a lumen-positive or lumen-negative potential, either absorption or secretion can be demonstrated. Although the presence of active transport cannot be totally excluded, under basal conditions this mode of transport does not appear to play a major role in calcium absorption in this segment of the nephron. Finally, the interdependence of sodium and calcium in clearance studies can probably be attributed to alterations

in the parallel handling of sodium and calcium in the proximal convoluted tubule.

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References

1. Lassiter, W. E., C. W. Gottschalk, and M. Mylle. 1963. Micropuncture study of renal tubular reabsorption of calcium in normal rodents. *Am. J. Physiol.* 204:771–775.

2. Duarte, C. G., and J. F. Watson. 1967. Calcium absorption in the proximal tubule of the dog nephron. *Am. J. Physiol.* 212:1355–1360.

3. Murayama, Y., F. Morel, and C. LeGrimellec. 1972. Phosphate, calcium and magnesium transfers in proximal tubules and loops of Henle, as measured by single nephron microperfusion experiments in the rat. *Pfluegers Arch. Eur. J. Physiol.* 333:1–16.

4. Le Grimellec, C. 1975. Micropuncture study along the proximal convoluted tubule. Electrolyte reabsorption in first convolutions. *Pfluegers Arch. Eur. J. Physiol.* 354:133–150.

5. Fromter, E., and K. Gessner. 1974. Free flow potential profile along rat proximal tubule. *Pfluegers Arch. Eur. J. Physiol.* 351:69–83.

6. Ullrich, K. J., G. Rumrich, and S. Kloss. 1976. Active Ca^{2+} reabsorption in the proximal tubule of the rat kidney. Dependence on sodium and buffer transport. *Pfluegers Arch. Eur. J. Physiol.* 364:223–228.

7. Blaustein, M. P. 1974. The interrelationship between sodium and calcium fluxes across cell membranes. *Rev. Physiol. Biochem. Pharmacol.* 70:33–82.

8. Burg, M. B., J. Grantham, M. Abramow, and J. Orloff. 1966. Preparation and study of fragments of single rabbit nephrons. *Am. J. Physiol.* 210:1293–1298.

9. Rouse, D., R. C. K. Ng, and W. N. Suki. 1980. Calcium transport in the pars recta and thin descending limb of Henle of the rabbit, perfused in vitro. *J. Clin. Invest.* 65:37–42.

10. Suki, W. N., D. Rouse, R. C. K. Ng, and J. P. Kokko. 1980. Calcium transport in the thick ascending limb of Henle. Heterogeneity of function in the medullary and cortical segments. *J. Clin. Invest.* 66:1004–1009.

11. Suki, W. N., and D. Rouse. 1981. Hormonal regulation of calcium transport in thick ascending limb renal tubules. *Am. J. Physiol.* 241:F171–F174.

12. Kokko, J. P. 1973. Proximal tubule potential difference. Dependence on glucose, HCO_3, and amino acids. *J. Clin. Invest.* 52:1362–1367.

13. Schafer, J. A., S. L. Troutman, and T. E. Andreoli. 1974. Volume reabsorption, transepithelial potential differences, and ionic permeability properties in mammalian superficial proximal straight tubules. *J. Gen. Physiol.* 64:582–607.

14. Bourdeau, J. E., and M. B. Burg. 1979. Voltage dependence of calcium transport in the thick ascending limb of Henle's loop. *Am. J. Physiol.* 236:F357–F364.

15. Jacobson, H. R. 1979. Characteristics of volume reabsorption in rabbit superficial and juxtamedullary proximal convoluted tubules. *J. Clin. Invest.* 63:410–418.

16. Berry, C. A., and F. C. Rector. 1978. Relative sodium-to-chloride permeability in the proximal convoluted tubule. *Am. J. Physiol.* 235:F592–F604.

17. Burg, M., C. Patlak, N. Green, and D. Villey. 1976. Organic solutes in fluid absorption by renal proximal convoluted tubules. *Am. J. Physiol.* 231:627–637.

18. Imai, M., and J. P. Kokko. 1972. Effect of peritubular protein concentration of reabsorption of sodium and water in isolated perfused proximal tubules. *J. Clin. Invest.* 51:314–325.

19. Biagi, B. A., and G. Giebisch. 1979. Temperature dependence of transepithelial potential in isolated perfused rabbit proximal tubules. *Am. J. Physiol.* 236:F302–F310.

20. Laprade, R., and J. Cardinal. 1983. Liquid junctions and isolated proximal tubule transepithelial potentials. *Am. J. Physiol.* 244:F304–F319.

21. Imai, M., and J. P. Kokko. 1974. Sodium chloride, urea, and water transport in the thin ascending limb of Henle. Generation of osmotic gradients by passive diffusion of solutes. *J. Clin. Invest.* 53:393–402.

22. MacInnes, D. A. 1961. The Principles of Electrochemistry. Dover Publications, Inc., New York. 220–245.

23. Gmaj, P., H. Murer, and R. Kinne. 1979. Calcium ion transport across plasma membranes isolated from rat kidney cortex. *Biochem. J.* 178:549–557.

24. Taylor, A., and E. E. Windhager. 1979. Possible role of cytosolic calcium and Na-Ca exchange in regulation of transepithelial sodium transport. *Am. J. Physiol.* 236:F505–F512.

25. Deleted in press.

26. Shareghi, G. R., and Z. S. Agus. 1982. Magnesium transport in the cortical thick ascending limb of Henle's loop of the rabbit. *J. Clin. Invest.* 69:759–769.

27. Bourdeau, J. E., and M. B. Burg. 1980. Effect of PTH on calcium transport across the cortical thick ascending limb of Henle's loop. *Am. J. Physiol.* 239:F121–F126.

28. Morel, F. 1981. Sites of hormone action in the mammalian nephron. *Am. J. Physiol.* 240:F159–F164.

29. Doucet, A., and A. I. Katz. 1982. High affinity Ca-Mg-ATPase along the rabbit nephron. *Am. J. Physiol.* 242:F346–F352.

30. Katz, A. 1982. Renal Na-K-ATPase: its role in tubular sodium and potassium transport. *Am. J. Physiol.* 242:F207–F219.

31. Bomsztyk, K., and F. S. Wright. 1982. Contribution of diffusion to transport of calcium by renal proximal tubule. *Clin. Res.* 30:443A. (Abstr.)

32. Brennan, J. P., and J. P. Kokko. 1973. Effect of 25-hydroxycholecalciferol on the transport of Ca and Na in the proximal tubule. *Clin. Res.* 21:678. (Abstr.)

33. Friedman, P. A., J. F. Figueiredo, T. Maack, and E. E. Windhager. 1981. Sodium-calcium interactions in the renal proximal convoluted tubule of the rabbit. *Am. J. Physiol.* 240:F558–F568.

34. Jacobson, H. R., and J. P. Kokko. 1976. Intrinsic differences in various segments of the proximal convoluted tubule. *J. Clin. Invest.* 57:818–825.

35. Burg, M. 1972. Perfusion of isolated renal tubules. *Yale J. Biol. Med.* 45:321–326.

36. Chonko, A. M., R. W. Osgood, A. E. Nickel, T. F. Ferris, and J. H. Stein. 1975. The measurement of nephron filtration rate and

absolute reabsorption in the proximal tubule of the rabbit kidney. *J. Clin. Invest.* 56:232–235.

37. Burg, M. B., and N. Green. 1976. Role of monovalent ions in the reabsorption of fluid by isolated perfused proximal renal tubules of the rabbit. *Kidney Int.* 10:221–228.

38. Walser, M. 1961. Calcium clearance as a function of sodium clearance in the dog. *Am. J. Physiol.* 200:1099–1104.

39. Sutton, R. A. L., and J. H. Dirks. 1975. The renal excretion of calcium: a review of micropuncture data. *Can. J. Physiol. Pharmacol.* 53:979–988.

40. Agus, Z. S., L. B. Gardner, L. H. Beck, and M. Goldberg. 1973. Effects of parathyroid hormone on renal tubular reabsorption of calcium, sodium, and phosphate. *Am. J. Physiol.* 224:1143–1148.

41. DeFronzo, R. A., M. Goldberg, and Z. S. Agus. 1976. The effects of glucose and insulin on renal electrolyte transport. *J. Clin. Invest.* 58:83–90.

42. Gonda, A., N. Wong, J. F. Seely, and J. H. Dirks. 1971. The role of hemodynamic factors on urinary calcium and magnesium excretion. *Can. J. Physiol. Pharmacol.* 47:619–626.

43. Beck, L. H., and M. Goldberg. 1973. Effects of acetazolamide and parathyroidectomy on renal transport of sodium, calcium, and phosphate. *Am. J. Physiol.* 224:1136–1142.

44. Massry, S. G., J. W. Coburn, L. W. Chapman, and C. R. Kleeman. 1968. The effect of long-term desoxycorticosterone acetate administration on the renal excretion of calcium and magnesium. *J. Lab. Clin. Med.* 71:212–219.

45. Suki, W. N., R. S. Schwettman, F. C. Rector, and D. W. Seldin. 1968. Effect of chronic mineralocorticoid administration on calcium excretion in the rat. *Am. J. Physiol.* 215:71–74.

46. Costanzo, L. S., and E. E. Windhager. 1978. Calcium and sodium transport by the distal convoluted tubule of the rat. *Am. J. Physiol.* 235:F492–F506.

47. Jacobson, H. R. 1981. Functional segmentation of the mammalian nephron. *Am. J. Physiol.* 241:F203–F218.