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Effect of Sodium on Cellular Calcium Transport in Rat Kidney

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Summary. The influence of extracellular Na (Na_o) on cellular Ca transport and distribution was studied in rat kidney slices. Calcium efflux from prelabeled slices was depressed when Na_o was completely replaced by choline or tetraethylammonium (TEA) ions and it was markedly stimulated when Na was reintroduced in a Na-free medium. However, reducing Na_o (with choline or TEA as substituting ions) did not increase the total slice ^{40}Ca, their total exchangeable Ca pool, or the ^{40}Ca or ^{45}Ca of mitochondria isolated from these slices. Kinetic analyses of steady-state ^{45}Ca desaturation curves showed that reducing Na_o depressed the exchange of Ca across the plasma membrane, slightly decreased the cytosolic Ca pool, but did not significantly affect the mitochondrial Ca pool and Ca cycling. Ouabain (10^{-3} M) which should reduce the Na gradient across the plasma membrane had no effect on calcium distribution and transport. These results suggest that in kidney cells low Na_o depresses Ca influx as well as Ca efflux; there may be an interaction between Na and Ca at a possible carrier located in the plasma membrane, but there is no Na/Ca exchange as described in several excitable tissues.

Key words Na/Ca exchange · kidney cells · cellular Ca · mitochondrial Ca · Ca transport

Introduction

A Na/Ca antiporter has been described in heart, squid axon, and other excitable tissues [3, 21]. Several recent articles have reviewed this counter-transport mechanism by which calcium can be transported out of the cells in exchange for extracellular Na (Na_o) against a large electrochemical gradient without direct metabolic energy coupling [2, 3, 7, 21]. Na−Ca exchange is characterized by: (i) a drop in calcium efflux when Na_o is lowered, (ii) a drop in calcium efflux when the concentration of intracellular Na (Na_i) is increased, and (iii) a gain in cell or tissue calcium when Na_o is decreased or completely replaced by substituting ions such as choline, lithium, or tetraethylammonium.

It is not clear whether such Na/Ca antiporters exist in nonexcitable tissues. Judah and Ahmed [16, 17] and Wallach [29] observed that liver slices incubated in choline chloride have a higher calcium content and that calcium extrusion is inhibited in low sodium media. However, Van Rossum [27] showed that the net extrusion of calcium from liver is not inhibited by high intracellular sodium and is not affected by the sodium gradient. Furthermore, ouabain has no effect on liver calcium content or fluxes [27, 28]. Cittadini and Van Rossum [14] also reported that the unidirectional efflux of ^{45}Ca and the net extrusion of calcium from liver cells are not prevented by ouabain or by a Na-free medium. However, they are reversibly inhibited by anaerobiosis and respiratory inhibitors and depend on external magnesium; this suggests that calcium extrusion is metabolically driven and independent of sodium. Similarly, Höfer and Kleinzeller [15] observed that the total calcium of kidney slices barely rises in Na-free media. In many cells or tissues, such as Ehrlich ascites cell, fibroblasts, adrenal medulla, pancreas, and salivary gland, the existence of a Na/Ca exchange mechanism is doubtful, even when some effects of Na_o on calcium movements can be observed [7]. The present studies were undertaken to see whether sodium has any effect on calcium metabolism and transport in kidney cells and to look for evidence for or against a Na/Ca antiporter. I found that extracellular sodium influences calcium fluxes across the plasma membrane of kidney cells, but the effects suggest a cooperative activation of calcium transport by sodium rather than a Na/Ca countertransport mechanism.

Materials and Methods

Male Sprague-Dawley rats weighing between 150 and 200 g were sacrificed by decapitation. The abdominal cavity was opened and both kidneys were removed, decapsulated and placed in a Krebs Henseleit medium buffered with bicarbonate (KHB) or Hepes (KHH) at 0 °C. The outer surface of the cortex opposite the papilla was cut tangentially with a Stadie-Riggs tissue slicer into a 3-mm thick piece. This slice was placed flat on a Mickle microtome and cut into 0.3-mm thick slices. The slices were preincubated in their respective buffers for 1 hr during which the buffer was

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changed twice. During the incubation, the slices were gently stirred at 37 °C with a magnetic stirrer.

The technique for the steady state kinetic analysis of ^{45}Ca desaturation from kidney slices and the equations for the calculation of pools and fluxes have been published in detail [25]. The slices were preincubated in control or experimental media for 1 hr; ^{45}Ca (1 μCi/ml) was then added, and the slices were labeled for exactly 60 min. They were rapidly washed in nonradioactive media, placed in a perfusion chamber, and perfused with nonradioactive buffer for 4 hr.

Kidney mitochondria were isolated from the slices after 1 hr preincubation plus 1 hr labeling with ^{45}Ca. The slices were homogenized in 250 mM sucrose containing 5 μM ruthenium red and 0.1 mM EDTA to prevent mitochondrial uptake of calcium during their separation. The homogenate was centrifuged at 0 °C for 20 min at $800 \times g$ and the supernatant at $8{,}000 \times g$ for another 20 min.

The radioactivity of the labeling medium, cells, slices, and mitochondria was measured by liquid scintillation spectrophotometry, their ^{40}Ca content by the method of Borle and Briggs [8] and the protein concentration by the method of Lowry [18].

The composition of the medium used for incubating or for perfusing the control slices was as follows (in mM): Na^+, 145; K^+, 5; Ca^{2+}, 1; Mg^{2+}, 1; P_i, 1; SO_4^{2-}, 1; glucose, 10, with Cl^- as the main balancing anion; the medium was buffered with 5 mM Hepes with a gas phase of 100% O_2 (KHH) or with 24 mM HCO_3^- and 5% CO_2 + 95% O_2 (KHB). In experimental conditions, NaCl was replaced mole for mole by choline chloride or by tetraethylammonium chloride (TEA), or, osmole for osmole by sucrose.

The 60-min Ca uptake by slices or mitochondria was calculated as follows: cpm/(mg protein × medium specific activity). This 60 min uptake value indicates the amount of calcium (in the slice or in the mitochondria) which has exchanged with the medium at that particular time. It is smaller than the total exchangeable pool measured by kinetic analysis because isotopic steady state is not achieved in 1 hr. The calculations of the exchangeable calcium pools (S_1, S_2, S_3) and calcium fluxes (ρ_{10}, ρ_{21}, ρ_{32}) in kidney slices were done according to Uchikawa and Borle [25]. The slices were labeled for 1 hr in media of known radioactivity and then desaturated in flow-through chambers by perfusion of nonradioactive medium for 3 hr. The desaturation curve relating the radioactivity of cells with time was analyzed by the weighted nonlinear least squares method. The coefficients and exponential constants obtained were used to calculate the pools, the fluxes, and rate constants [25]. These kinetic analyses reveal three distinct compartments of exchangeable calcium which have been identified with: (i) the glycocalyx and binding sites external to the plasma membrane (S_1), (ii) the cytosolic pool which consists of the ionized calcium plus all the calcium bound to cytosolic ligands (S_2), and (iii) the mitochondrial pool (S_3) [5, 9, 10]. It should be emphasized that these exchangeable pools are not a measure of ionized calcium. The exchange of calcium with the glycocalyx (ρ_{10}), the calcium transport across the plasma membrane (ρ_{21}), and calcium exchange between the cytosolic and mitochondrial pools (ρ_{32}) are steady-state fluxes where influx equals efflux.

The nonexchangeable calcium of the kidney slices was calculated by subtracting the sum of the three exchangeable pools obtained by kinetic analysis from the total ^{40}Ca of the slices. Similarly, the nonexchangeable calcium of mitochondria was calculated by subtracting the third kinetic compartment from the total mitochondrial ^{40}Ca.

The immediate effects of changing Na_o on ^{45}Ca efflux were measured in some experiments by substituting choline or TEA for Na_o 60 min after the initiation of the ^{45}Ca desaturation. The changes in fractional efflux were calculated and plotted against time according to Borle [6]. The fractional efflux (FE) is the radioactivity appearing in the medium during a given time divided by the radioactivity present in the slices during the same period. The fractional efflux ratio (FER) is the FE of an experimental group subjected to an alteration in desaturation conditions divided by the FE of a control group whose desaturation proceeds unperturbed.

For the ^{45}Ca uptake experiments, renal tubule cells were dissociated by a modification of the method of Burg and Orloff [13, 24]. Male Sprague-Dawley rats, 140–200 g, were decapitated and their kidneys perfused *in situ* with cold saline and cold dissociating medium (KHB with 1 g/100 ml bovine serum albumin and collagenase, Sigma type IV at a concentration equivalent to 60 U/ml). The kidneys were removed, the medulla was discarded, and the cortex minced into 3-mm pieces. They were incubated for 50 min in the collagenase solution at 37 °C with constant stirring, and every 15 min the tissue was dispersed with a wide-mouthed pipette. It was then passed through a 212 μm stainless steel sieve and centrifuged 2 min at $50 \times g$. The pellet was resuspended and washed three times with ice-cold KHB. Under phase-contrast microscopy, the preparation consisted of isolated cells and short tubular fragments. The cells were incubated at 37 °C at a concentration of 1–3 mg cell protein/ml under the selected conditions with paired experimental and control studies run simultaneously with cells derived from the same kidney preparation. Cell viability was assessed by Trypan blue exclusion and by the lack of stimulation of O_2 consumption after the addition of succinate to the cell suspension. Only cell preparations showing 85% trypan blue exclusion and stimulation of O_2 consumption by succinate of no more than 10% were used. Preparations which did not meet these criteria were discarded.

The cells were first incubated for 1 hr in nonradioactive media (KHB) to achieve steady state. In the experimental groups, Na_o was lowered to 50 mM and choline was used as substituting cation. After 1 hr of preincubation, 1 μCi ^{45}Ca/ml was added and the radioactivity accumulated by the cells was measured at 1, 5, 10, 15, 20 and every 15 min thereafter for 2 hr by the method previously described [11, 26]. The relative radioactivity of the cells plotted against time can be fitted with a double exponential equation when analyzed by computer with a weighted nonlinear least squares method. The calculation of the pools and rates of exchange was done according to Uchikawa and Borle [11, 26]. The third kinetic pool S_3 obtained by the kinetic analysis of ^{45}Ca desaturation curves cannot be detected by ^{45}Ca uptake; this third kinetic phase is less than the standard deviation of the points in the tail end of the uptake curve. Consequently, only one extracellular calcium pool (S_1) and one intracellular (S_2) can be measured by this technique where S_2 represents all the intracellular exchangeable calcium pools and subpools.

Statistical analyses were made by first determining the equality of variances between groups by the F test criterion and the significance of the results were calculated accordingly either by F statistics or by Student's t test [23]. All the values presented in the tables are expressed as the mean ± the standard error of the mean.

Results

Effect of Na_o Substitution on ^{45}Ca Efflux from Kidney Slices

In a first series of experiments, kidney slices were labeled with ^{45}Ca for 60 min. The tracer was washed out by perfusing the labeled slices with unlabeled media. When choline or TEA were substituted for Na_o during the desaturation, ^{45}Ca FER was first depressed slightly, then it slowly rose above the control level. When extracellular choline or TEA were then

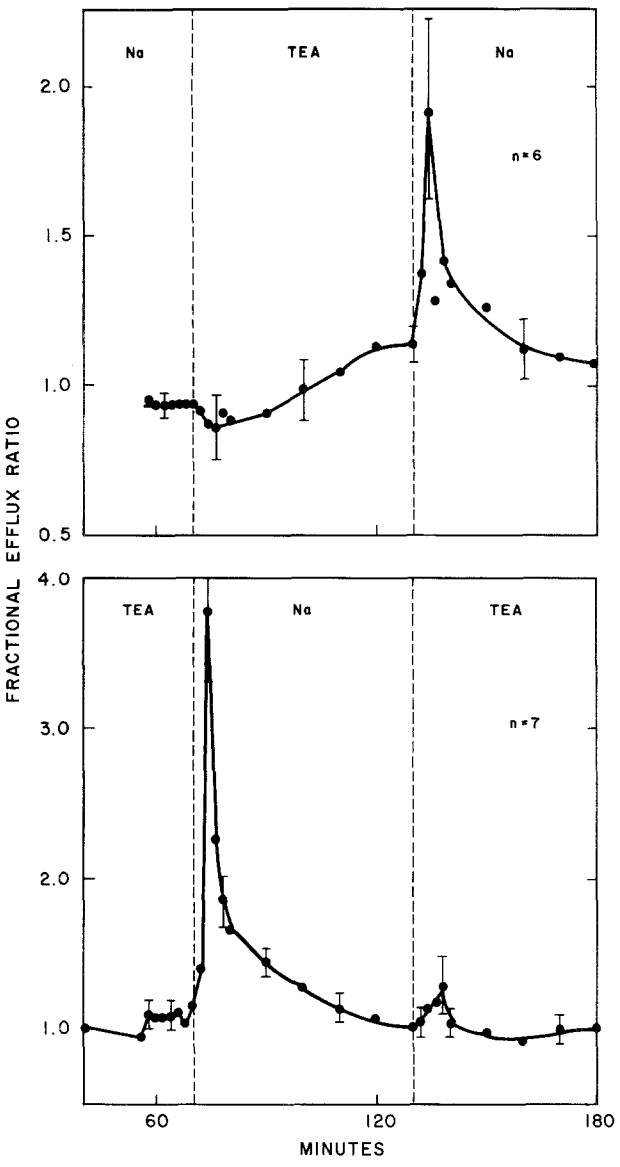

Fig. 1. Effect of removal of extracellular Na and TEA substitution on the fractional efflux of ^{45}Ca from rat kidney slices. *Upper panel:* substitution of Na_o with 145 mM tetraethylammonium (TEA) chloride at min 70, and replacement of TEA with 145 mM NaCl at min 130. *Lower panel:* substitution of TEA with 145 mM NaCl at min 70, and replacement of Na_o with TEA at min 130

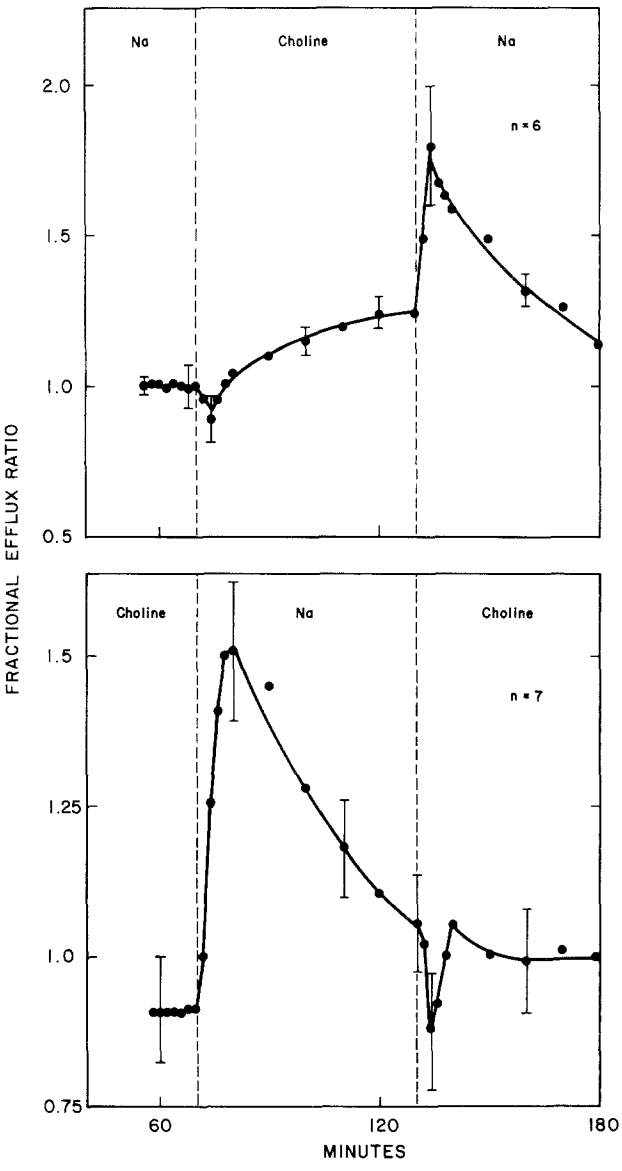

Fig. 2. Effect of removal of extracellular Na and choline substitution on the fractional efflux of ^{45}Ca from rat kidney slices. *Upper panel:* substitution of Na_o with 145 mM choline chloride at min 70, and replacement of choline with 145 mM NaCl at min 130. *Lower panel:* substitution of 145 mM choline with NaCl at min 70, and replacement of Na_o with choline at min 130

replaced by Na, ^{45}Ca efflux was markedly stimulated and almost doubled before returning toward the control rate (Figs. 1 and 2, upper panels).

In a second series of experiments, kidney slices were incubated, labeled, and desaturated in Na_o-free media, choline or TEA being the substituting ions. When Na was reintroduced during the desaturation, ^{45}Ca efflux was markedly increased. The FER peaked within a few minutes before returning to the control levels. The peak reached 150% when Na replaced choline and 375% when Na replaced TEA (Figs. 1 and 2, lower panels). When Na_o was replaced again by choline there was a small inhibition of ^{45}Ca efflux, but when TEA was substituted, another small stimulation was observed.

Effects of Na_o Substitution on Slice and Mitochondrial Calcium

Table 1 shows the effects of reducing the extracellular Na concentration on kidney calcium measured with two different buffers at four different Na_o concentra-

Table 1. Effects of decreasing Na_o and of ouabain on the total calcium and on ^{45}Ca uptake in rat kidney slices and isolated mitochondria

Medium	(mM)	Slices				Mitochondria			
		Total Ca^{40}	Total Ca pools S_{1+2+3}	Non-exchangeable Ca	60′ ^{45}Ca uptake	Total Ca_{40}	Pool S_3	Non exchangeable Ca	60′ ^{45}Ca uptake
		(nmol mg^{-1} slice protein)				(nmol mg^{-1} slice protein)			
1. HCO_3 buffer									
Na	145	33.7	21.6	12.1	14.8	5.22	2.27	2.95	2.56
Choline	0	±1.5	±1.8	±2.3	±0.8	±0.38	±0.23	±0.44	±0.14
		(10)	(19)	–	(8)	(13)	(19)	–	(12)
Na	100	31.4	24.1	7.30	13.3	4.10	3.08	1.02	2.12
Choline	45	±3.6	±1.0	±3.7	±1.9	±0.28	±0.28	±0.40	±0.18
		(8)	(8)	–	(3)	(5)	(8)	–	(6)
Na	50	29.1	21.4	7.70	13.2	3.84	2.48	1.36	2.26
Choline	95	±1.7	±2.1	±2.7	±0.9	±0.44	±0.48	±0.65	±0.12
		(9)	(6)	–	(3)	(5)	(6)	–	(6)
Na	0	28.8	25.5	3.30	14.8	5.04	2.29	3.75	2.36
Choline	145	±1.5	±1.6	±2.2	±1.2	±0.48	±0.13	±0.50	±0.10
		(6)	(6)	–	(2)	(3)	(6)	–	(3)
2. Hepes buffer									
Na	145	32.7	29.6	3.10	16.8	5.20	3.89	1.31	2.56
Choline	0	±1.2	±1.6	±2.0	±1.0	±0.24	±0.28	±0.37	±0.41
		(23)	(17)	–	(18)	(14)	(17)	–	(14)
Na	100	43.5[a]	28.7	14.8	19.8	4.22	2.85	1.37	2.24
Choline	45	±4.4	±2.0	±4.8	±3.9	±0.24	±0.34	±0.42	±0.16
		(6)	(6)	–	(2)	(4)	(6)	–	(4)
Na	50	31.6	26.6	5.00	15.8	4.80	2.91	1.89	2.16
Choline	95	±1.1	±1.4	±1.8	±0.9	±0.26	±0.31	±0.40	±0.18
		(8)	(6)	–	(3)	(6)	(6)	–	(6)
Na	0	33.5	27.9	5.60	19.2	7.02	2.82	4.20	2.22
Choline	145	±1.3	±2.3	±2.6	±2.5	±0.16	±0.50	±0.52	±0.08
		(13)	(6)	–	(5)	(3)	(6)	–	(3)
Na	0	41.5[a]	26.0	15.5	15.5	–	5.30	–	–
TEA	145	±3.7	±6.2	±7.2	±0.8		±3.6		
		(8)	(4)	–	(5)		(4)		
Na	0	64.4[a]	71.6[a]	0	47.8[a]	–	10.3[a]	–	–
Sucrose	290	±6.5	±4.6		±5.3		±0.84		
		(7)	(3)		(4)		(3)		
Na	145	30.3	25.7	4.6	19.4	–	4.58	–	–
Ouabain	1	±2.3	±1.5	±3.9	±1.4		±0.50		
		(6)	(6)	–	(2)		(6)		
Mean of controls (KHB + KHH at 145 Na_o)		33.2	25.6	7.60	15.8	5.21	3.08	2.13	2.56

Values are mean ±standard error: number of experiments in parentheses. [a] $P<0.05$.

tions and with two different substituting ions, choline and TEA; 290 mM sucrose was also substituted for 145 mM Na_o.

In bicarbonate buffer, decreasing the extracellular Na from 145 mM to 100, 50 or 0 mM using choline as substituting ion does not increase the total tissue calcium. If anything, total kidney slice calcium slightly decreases with decreasing Na_o. The 60-min calcium uptake, which reflects the amount of cell calcium that has exchanged with extracellular calcium, is also unaffected by lowering Na_o. The nonexchangeable calcium of the slice appears to decrease at low Na_o, which would suggest a shift of calcium within the slice from a nonexchangeable to an exchangeable pool. However, since this derived measurement contains the analytical errors of two other determinations, the significance of this finding is questionable. The total exchangeable pools ($S_1+S_2+S_3$) is not significantly increased by lowering Na_o. Similarly, no change is observed in mitochondria: decreasing extra-

Table 2. Effect of decreasing Na_o and of ouabain on calcium exchange and calcium pools of rat kidney slices determined by kinetic analysis of ^{45}Ca desaturation curves

Medium	(mM)	n	Calcium fluxes			Calcium pools		
			Surface exchange (ρ_{10})	Plasmalemmal transport (ρ_{21})	Mitochondrial exchange (ρ_{32})	Surface glycocalyx (S_1)	Cytosol (S_2)	Mitochondria (S_3)
			(pmol min^{-1} mg^{-1} slice protein)			(nmol mg^{-1} slice protein)		
1. Bicarbonate buffer								
Na	145	19	2,910	498	34.8	13.4	5.95	2.27
Choline	0		±416	±87	±4.1	±1.6	±0.75	±0.23
Na	100	8	3,340	369	22[a]	15.3	5.67	3.08
Choline	45		±201	±29	±2.2	±1.4	±0.80	±0.28
Na	50	6	3,190	455	18.1	12.6	6.30	2.48
Choline	95		±402	±45	±3.7	±1.9	±0.62	±0.46
Na	0	6	4,550[a]	578	17.0[a]	17.2	6.00	2.29
Choline	145		±302	±113	±1.3	±1.4	±0.80	±0.13
2. Hepes buffer								
Na	145	17	3,740	802	33.2	15.6	9.83	3.89
Choline	0		±415	±94	±2.6	±1.3	±0.84	±0.28
Na	100	6	4,400	381[a]	24.7	19.4	6.41[a]	2.85
Choline	45		±249	±98	±4.7	±1.9	±0.65	±0.34
Na	50	6	4,130[a]	426[a]	31.4	17.0	6.72[a]	2.91
Choline	95		±328	±65	±3.0	±1.2	±0.73	±0.31
Na	0	6	4,350	554	25.7	17.9	7.14	2.82
Choline	145		±710	±94	±6.1	±2.1	±0.73	±0.50
Na	0	4	2,617	459	51.5	12.8	7.87	5.30
TEA	145		±552	±207	±37.2	±3.8	±3.3	±3.6
Na	0	3	5,101	1,490[a]	1,570[a]	32.9[a]	28.4[a]	10.3[a]
Sucrose	290		±802	±238	±33.5	±1.1	±4.4	±0.84
Na	145	6	3,960	640	43.3	13.5	7.68	4.58
Ouabain	1		±361	±93	±4.5	±1.2	±0.88	±0.50

Values are the mean ±standard error. [a] $P<0.05$. n=number of experiments.

cellular Na does not affect the total mitochondrial calcium, their uptake of ^{45}Ca, or the distribution between exchangeable and nonexchangeable fractions.

In Hepes buffer the findings are essentially the same. Decreasing Na_o does not increase the tissue calcium or any other parameter, with two exceptions: at 100 Na_o+45 mM choline and at 0 Na_o+145 mM TEA, the total calcium of the slices is significantly elevated; but since all other parameters, total exchangeable pools, calcium uptake, mitochondrial calcium, and uptake are not increased, these two values can be regarded as aberrant. In these two cases, the nonexchangeable calcium is also elevated but only because these values are derived from the elevated total calcium measurements. The results obtained by kinetic analyses of ^{45}Ca desaturation (Table 2) support the conclusion that in these two conditions there is no increase of calcium in kidney slices.

On the other hand, substituting sucrose for Na_o produces a large and significant increase in all the parameters measured. There is a two- to threefold increase in total calcium, exchangeable calcium, and calcium uptake, and this effect is in agreement with the results obtained by the kinetic measurements (Table 2).

Finally, as an attempt to decrease the Na concentration gradient across the plasmalemma by increasing the intracellular Na concentration, the kidney slices were exposed to 10^{-3} M ouabain for 2 hr. No change was observed in any parameter.

Kinetic Analyses of ^{45}Ca Desaturation from Kidney Slices (Table 2)

In bicarbonate buffer, lowering the extracellular Na concentration from 145 mM to 100, 50 and 0 mM, with choline as the substituting ion, does not alter the size of the three exchangeable pools: glycocalyx, cytosol, and mitochondria. Surface exchange with extracellular ligands of the glycocalyx is significantly in-

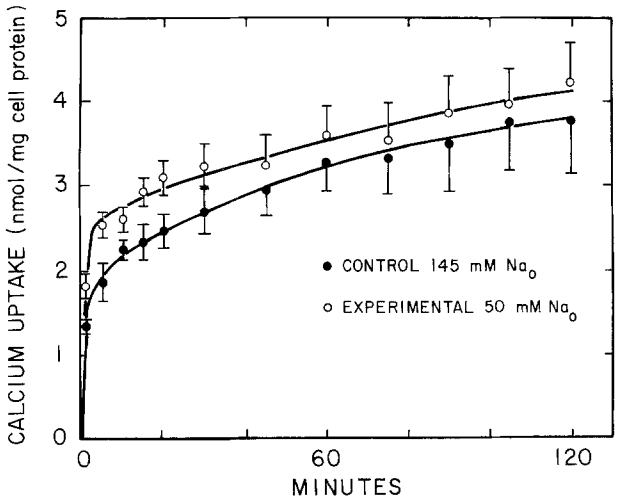

Fig. 3. Effect of low Na_o (50 mM) on calcium uptake by isolated renal cells. The data points are the mean ±SE of five separate experiments in each group. The lines represent the best fit obtained by nonlinear least squares analysis of the data. The control curve is drawn from the following equation: $R(t)=1.75$ (1-exp(−1.36 t)+ 2.31 (1-exp(−0.0174 t)); the sum of squares of residuals between the data and the curve=0.042, $P<0.0001$. The experimental curve is drawn from the following equation: $R(t)=2.54$ (1-exp(1.22 t))+ 2.48 (1-exp(−0.00857 t)); the sum of squares of residuals between the data and the curve=0.149, $P<0.0001$

Table 3. Effect of low medium Na (50 mM) on the exchangeable calcium pools and calcium fluxes of isolated renal cells

	Control $Na_o=145$ mM	Low Na_o $Na_o=50$ mM
Calcium pools (nmol/mg cell protein)		
Glycocalyx (S_1)	1.81	2.58
Intracellular (S_2)	2.25	2.45
Calcium exchange (pmol min^{-1} mg^{-1} cell protein)		
Medium-glycocalyx (ρ_{10})	2.42	3.13
Cytosol-glycocalyx (ρ_{21})	0.040	0.021

creased by 56% in Na-free media. However, calcium transport across the plasmalemma is not affected while calcium exchange between mitochondria and cytosol is depressed up to 50%. In other words, in KHB, kidney calcium metabolism is minimally affected when Na_o is reduced or absent, and no increase in cell calcium can be observed.

In Hepes buffer, there is a small (−30%) but significant fall in the cytosolic pool of exchangeable calcium while the glycocalyx and mitochondrial calcium pools are unaffected. There is also a significant drop in steady-state calcium fluxes across the plasma membrane ranging from 30 to 50%, while glycocalyx and mitochondrial calcium fluxes are not significantly affected. When TEA is the substituting ion, the plasmalemmal calcium fluxes are also depressed but there is no increase in any exchangeable calcium pools. On the other hand, when sucrose is substituted for Na_o, there is a significant twofold rise in the glycocalyx calcium and a significant threefold increase in the cytosolic and mitochondrial calcium pools; plasmalemmal calcium transport is almost doubled, while the mitochondrial exchange increases almost 50-fold. These results agree well with the chemical measurements of total calcium and of calcium uptake obtained with sucrose (Table 1). Ouabain has no significant effect on any kinetic parameters in agreement with the chemical measurements.

Kinetic Analysis of ^{45}Ca Uptake by Isolated Renal Cells

Figure 3 shows that lowering Na_o from 145 to 50 mM, with choline as substituting ion, very slightly increases the overall calcium uptake by isolated renal cells. Table 3 shows that this small stimulation of uptake is mostly due to a 42% increase in the size of the extracellular glycocalyx (S_1) and a 30% increase in exchange rate between this pool and the medium (ρ_{10}). The intracellular exchangeable calcium pool (S_2) is not significantly changed, but calcium transport across the plasma membrane (ρ_{21}) between the intracellular and extracellular compartment is reduced by half. The results obtained with cells and with slices are not directly comparable since the protein concentration of kidney slices is several times smaller than the protein concentration of isolated cells on a wet weight basis. Nevertheless, there is a good qualitative agreement between the two sets of kinetic data: a low extracellular Na increases the glycocalyx calcium pool and exchange in both preparations. However, a low Na_o inhibits both calcium influx and efflux across the plasma membrane without significantly affecting the intracellular calcium pools.

Discussion

The cellular calcium transport and distribution of rat kidney slices incubated in KHB or KHH with 145 mM Na_o were similar to that published previously [9]. There was no significant difference between the slices incubated in bicarbonate or in Hepes buffers, and an average of the two control groups was made in the last row of Table 1. The total calcium of these kidney slices was 33 nmol/mg protein of which 78% or 26 nmol/mg protein was exchangeable. The calcium uptake of 16 nmol/mg protein indicates that, after 1 hr incubation with ^{45}Ca, 50% of the total slice calcium had exchanged with the medium or 62% of the total exchangeable pools had turned over. The

total calcium of mitochondria was 5.20 nmol/mg slice protein or 16% of the total slice calcium. Since the major fraction of the slice calcium is extracellular and associated with the glycocalyx, the mitochondrial calcium is certainly a much larger fraction of the intracellular calcium. The mitochondrial pool of exchangeable calcium, S_3 obtained by kinetics (3.1 nmol/mg slice protein) agreed very well with the value of mitochondrial calcium uptake (2.56 nmol/mg slice protein) which measures the fraction of mitochondrial calcium that has exchanged after 1 hr incubation with ^{45}Ca. The nonexchangeable calcium fraction of the mitochondria was 2.13 nmol/mg slice protein or 30% of the nonexchangeable calcium of the slice (7.6 nmol/mg protein).

Figures 1 and 2 show that the sudden substitution of Na_o by choline or TEA inhibits calcium efflux, while re-addition of Na to the medium produces a marked stimulation of calcium efflux.

The interpretation of these FER profiles is not easy since the appearance of ^{45}Ca in the perfusing medium depends on three independent parameters: (i) the rate of ^{40}Ca efflux across the plasma membrane, (ii) the specific activity of the cytosolic pool which decreases constantly with time, and (iii) the redistribution of calcium among intracellular compartments with different specific activities. For instance, the "peak" pattern of ^{45}Ca FER shown in Figs. 1 and 2 does not necessarily mean that the stimulation of calcium efflux is a transient event, because a rapid fall in cytosolic radioactivity and specific activity can be expected during such a stimulation and this alone could account for the return to baseline of the fractional efflux. Thus when the FER returns towards its control value of 1, this could be due to a fall in cellular specific activity and not to a decrease in the stimulated rate of calcium efflux. Regardless of the validity of these considerations, it appears that removal of Na from the extracellular fluids inhibits calcium efflux from kidney slices, while the replacement of a substituting ion with Na stimulates calcium efflux. The only exception was observed when TEA replaced Na (Fig. 2, lower panel). There is no obvious explanation for this observation. If one assumes that fractional efflux reflects calcium transport out of the cell across the plasma membrane, the results would support the idea that calcium efflux is Na_o dependent and suggest the existence of a Na/Ca exchange mechanism in renal cells. This interpretation, however, is not compatible with the data obtained in steady-state experiments which show that, with low Na_o or in its total absence, calcium influx is depressed, as well, and cell calcium is not increased (Tables 1–3). Indeed, in all tissues where a Na/Ca exchange mechanism has been convincingly demonstrated, there is an increased calcium influx and a net gain in tissue calcium when Na_o is decreased [7].

The results presented in Tables 2 and 3 clearly demonstrate that lowering Na_o or removing it entirely does not increase the kidney slice calcium, the mitochondrial calcium, or any of the intracellular pools of exchangeable calcium. If anything, there is a tendency for some of these parameters to decrease with decreasing Na_o. Except for two aberrant values, this is true with both substituting ions, TEA and choline, and with both buffer systems, bicarbonate or hepes. In addition, calcium fluxes across the plasma membrane are also decreased in low Na_o, whether these fluxes are measured by ^{45}Ca desaturation from slices (Table 2) or by ^{45}Ca uptake by isolated cells (Table 3). This indicates that low Na_o inhibits both calcium influx and efflux across the cell membrane. On the other hand, in both slices and isolated cells, the extracellular exchangeable calcium pool of the glycocalyx and calcium exchange between this pool and the medium tend to increase in low Na_o (Table 2 and 3). This may represent a Na/Ca competition for nonspecific binding sites in the glycocalyx or at the external surface of the plasma membrane.

Only when the medium NaCl was replaced by 290 mM sucrose did the slices accumulate significant amounts of calcium. In this case, the concentration of ^{40}Ca and ^{45}Ca in both slices and mitochondria, and cell calcium fluxes and exchangeable pools increased two- to threefold. Sucrose seems to be a poor osmotic substitute for medium electrolytes, and it may have deleterious effects on the cell membrane permeability to calcium.

Finally, ouabain which increases the intracellular concentration of Na (Na_i) has been shown to increase calcium influx and the total cell calcium in many tissues in which a Na/Ca exchange has been postulated [7]. In liver, however, ouabain has no effect on calcium fluxes or cell calcium [27, 28]. In rat kidney, 1 mM ouabain has been shown to completely inhibit Na transport without at all affecting calcium influx or efflux from slices [19]. Similarly, in the present experiments, 1 mM ouabain has no effect on calcium fluxes across the plasma membrane, and it does not increase the calcium concentration of the slices or of their intracellular compartments (Tables 1 and 2). These results bring another argument against the existence of a Na/Ca exchange in kidney cells. It is true that the rat is relatively insensitive to ouabain. However, the concentration of 1 mM used in these experiments is well above the I_{50} obtained in rat kidney [1, 19, 20]. Indeed, Allen and Schwartz have shown that the I_{50} of ouabain on rat kidney Na–K-ATPase was 100 μM; at 1 mM, ouabain reduces the enzyme activity 80% within 30 sec [1]. More recently,

Periyasami et al. obtained still a lower I_{50} of 0.7 to 1.0×10^{-4} M in a highly active Na−K-ATPase of rat kidney [20]. Finally, Bowman et al. have shown that in rat kidney a concentration of ouabain of 0.8 mM is very effective since it increases the fractional excretion of Na fivefold and reduces the fractional excretion of K by 50% [12], and Matsushima and Gemba have shown that 1 mM ouabain totally supress Na transport in rat kidney slices [19]. Therefore it is unlikely that the lack of effect of 1 mM ouabain on calcium metabolism in rat kidney is due to a lack of action on Na−K-ATPase and on Na_i.

In this series of experiments, the only findings which could support the existence of a Na/Ca exchange mechanism in kidney cells, are: (i) the inhibition of calcium efflux by substituting Na_o with TEA or choline, and (ii) its stimulation when Na_o is restored to normal. All other findings conflict with this idea. First, calcium influx is inhibited when Na_o is reduced whereas one would expect a stimulation. Second, the calcium concentration of the slices, the mitochondria, and of all intracellular exchangeable pools is not increased with decreasing Na_o; on the contrary, some of these parameters are significantly reduced. If a Na/Ca exchange were present, one would expect a gain in cell calcium. Third, ouabain has no effect on calcium fluxes and on the concentration of cell calcium, whereas one would expect a change in both in the presence of high Na_i. Nevertheless, the fact that low Na_o reduces calcium fluxes remains to be explained. The simplest explanation could be that the calcium carrier complex is activated by Na. Perhaps, like sugars and amino acid transport in kidney and intestine, a site of the calcium carrier must be occupied by Na in order to achieve the maximal velocity of the calcium carrier complex across the plasma membrane.

In conclusion, these experiments suggest that, although Na may activate calcium influx and efflux across the kidney cell membrane, there is no Na/Ca exchange mechanism as postulated in several excitable tissues.

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