J. Membrane Biol. 48, 325–341 (1979)

Effect of pH on the Calcium Metabolism of Isolated Rat Kidney Cells

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Received 9 October 1978; revised 6 March 1979

Summary. The effects of metabolic and respiratory acidosis and alkalosis on cellular calcium metabolism were studied in rat kidney cells dispersed with collagenase. In both types of acidosis, the intracellular pH, total cell calcium, and the cell relative radioactivity after 60 min of labeling are significantly depressed. Kinetic analysis of ^{45}Ca desaturation curves shows that acidosis decreases all three cellular calcium pools and depresses calcium fluxes between the superficial and cytosolic pools and between the cytosolic and mitochondrial pools. In alkalosis the intracelluar pH, the total cell calcium, and the cell relative radioactivity are significantly increased. Kinetic studies show that in alkalosis, only the mitochondrial pool is consistently increased. Calcium exchange between the mitochondrial and cytosolic pool is increased in metabolic alkalosis only. These results suggest that hydrogen ion is an important modulator of calcium metabolism, and that the intracellular pH rather than extracellular pH is the critical factor in determining the calcium status of cells during altered acid-base conditions.

The factors which control cellular calcium homeostasis remain incompletely delineated in spite of the increased awareness in recent years of the importance of calcium in the initiation and control of many cellular functions. Hydrogen ion concentration has often been implicated in the control of calcium metabolism at the systemic, cellular, and subcellular level. Farquharson *et al.* have shown that ingestion of acid-producing substances by humans causes an increased renal excretion of calcium [10]. Lemann *et al.* [12] demonstrated that the calciuria of chronic metabolic acidosis in humans is the result of a decreased renal reabsorption of calcium. Sutton and Dirks [21] have concluded from renal micropuncture studies in acidotic dogs that acidosis causes a specific defect in calcium transport by the distal tubule. Langer and Poole-Wilson [11] have reported a decrease in calcium influx and efflux from a slowly exchanging calcium pool in rabbit interventricular septa perfused with

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0022-2631/79/0048-0325 $03.40

media equilibrated with 30% CO_2 (pH 6.67). At the subcellular level, Bygrave [8] has enumerated several mitochondrial sites where hydrogen ions might be expected to alter calcium transport; however, direct experimental evidence for these intracellular effects is lacking.

These studies report the effects of extracellular and intracellular alkalosis and acidosis on the calcium metabolism of isolated renal cells. Freshly isolated rat kidney cells were studied under conditions of respiratory acidosis (high CO_2), metabolic acidosis (low HCO_3^-), respiratory alkalosis (low CO_2), metabolic alkalosis, (high HCO_3^-) and in extracellular isohydric conditions with an intracellular acidosis or alkalosis (parallel changes of HCO_3^- and CO_2 with extracellular pH maintained at 7.4). We found that acidosis causes a depletion of cell calcium and a decrease in calcium fluxes across the plasma membrane and between the intracellular calcium pools. Alkalosis causes an increase in total cell calcium, predominantly in the mitochondrial compartment, and an increase in the superficial and mitochondrial calcium fluxes. Changing the intracellular pH alone while keeping the extracellular pH at 7.4 produces effects which are similar to those obtained with extracellular acidosis or alkalosis. These results suggest that hydrogen ion is an important modulator of intracellular calcium metabolism. Intracellular pH rather than the extracellular pH appears to be the determinant factor.

Materials and Methods

Cell Preparation and Incubation Media

The composition of the standard Krebs Henseleit bicarbonate incubation medium (KHB) and its modifications for the experimental conditions studied are given in Table 1. The renal tubule cells were dissociated by a modification of the method of Burg and Orloff [7]. Male Sprague-Dawley rats, 140–200 g, were decapitated and their kidneys perfused in situ with cold saline and cold dissociating medium (KHB with 100 mg/100 ml bovine serum albumin and collagenase, Worthington type I or III 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 27 °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 being run simultaneously with cells derived from the same kidney preparation. Cell viability was assessed by evaluating cell potassium content and the rate of ^3H leucine incorporation into the TCA precipitable cell fraction [1].

Table 1. Effects of changing medium bicarbonate and CO_2 on renal tubule cell intracellular pH

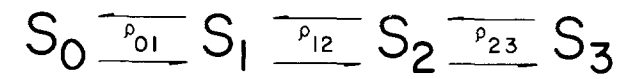
Condition	Extracellular pH	Intracellular pH	Medium Composition		
			CO_2 (%)	$NaHCO_3$ (mM)	NaCl (mM)
Control	7.42 ± 0.027 (9)	7.44 ± 0.013 (38)	5	24	118
Metabolic acidosis	6.84 ± 0.016 (6)	7.20 ± 0.020 (24)	5	6	136
Metabolic acidosis	7.13 ± 0.013 (9)	7.25 ± 0.010 (33)	5	12	130
Respiratory acidosis	6.84 ± 0.007 (8)	7.11 ± 0.011 (21)	20	24	118
Metabolic alkalosis	7.74 ± 0.010 (9)	7.66 ± 0.023 (33)	5	48	94
Metabolic alkalosis	8.04 ± 0.008 (6)	7.92 ± 0.016 (20)	5	96	46
Respiratory alkalosis	7.98 ± 0.008 (6)	7.90 ± 0.012 (23)	1.25	24	118
Isohydric, high CO_2 and HCO_3^-	7.41 ± 0.012 (6)	7.24 ± 0.029 (24)	20	96	46
Isohydric, low CO_2 and HCO_3^-	7.42 ± 0.019 (7)	7.56 ± 0.018 (20)	1.25	6	136

Values are mean ± SE. All pH_i are significantly different from pH_e ($P < 0.05$) except in the control and metabolic alkalosis, pH_e 7.7 groups. All pH_i are significantly different from the control pH_i. All media contained 5 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 1 mM $NaHPO_4$, 11 mM glucose and were in equilibrium with a gas phase of O_2 and CO_2 as listed.

Cell Calcium

Total cell calcium was determined by the method of Borle and Briggs [5] on cells taken at 70, 80, 100 and 120 min incubation and at the end of the ^{45}Ca desaturation (320 min) [3]. After 60 min preincubation in all experimental media, there were no additional changes in cell calcium with time, thus the results obtained between 70 and 320 min were pooled. Cell protein content was determined by a modification of the Lowry method [13] and the results expressed as nmol Ca/mg cell protein.

After 1 hr preincubation in nonradioactive media, the cells were labeled for 60 min with 1 μCi ^{45}Ca/ml (New England Nuclear). The radioactivity of the cells at 60 min was measured as previously described [4]. The cells' relative radioactivity is defined as their radioactivity divided by the labeling medium specific activity and has the units of nmol/mg protein. This value represents neither the rate of calcium exchange nor the total exchangeable calcium pool of the cell. It merely indicates the cell calcium which has ex-



S_0 medium

S_1 glycocalyx

S_2 cytosolic pool

S_3 mitochondrial pool

ρ_{12} exchange across the plasma membrane

ρ_{23} exchange across the mitochondrial membrane

$\rho_{ij} = \rho_{ji}$ at steady state

Fig. 1. Symbolic representation of the open three-compartment catenary system used to describe renal cell calcium metabolism

changed with extracellular calcium at the beginning of the desaturation period after 60 min of loading. It is smaller than the sum of the pools determined by desaturation and is much less than the total cell calcium, for a large fraction of cell calcium is non-exchangeable. The cellular exchangeable calcium pool sizes and calcium fluxes were determined by ^{45}Ca desaturation for 200 min by the method of Uchikawa and Borle [22, 23]. The desaturation curve relating the relative radioactivity of the cells with time was integrated as follows: the sum of the radioactivity in each wash plus the radioactivity of the cells at the end of the wash-out period is taken as their radioactivity at the beginning of the desaturation. The radioactivity remaining in the cells at each time point is obtained by sequentially subtracting the radioactivity of each wash period. The integrated points relating the radioactivity of the cells with time are divided by the specific activity of the labeling medium to give the cell relative radioactivity. The coefficients and exponents of the equations fitting the resulting curves were determined by the weighted nonlinear least squares method. The data was analyzed as an open 3-compartment catenary system, represented by Fig. 1, in which S_0 is the medium compartment, S_1 the most rapidly exchanging compartment (extracellular), and S_2 and S_3 the two intracellular compartments. ρ_{ij} is the flux between compartments i and j, and since the system is at steady state $\rho_{ij}=\rho_{ji}$. The justification of this model, the assumptions, and equations for calculating all the kinetic parameters have been published [3, 4, 6, 22, 23].

The identification of the second pool as cytosolic calcium is based primarily on the identity of its transmembrane rate of calcium exchange of about 40 fmol cm^{-2} sec^{-1} with that of transmembrane fluxes measured in nerve, muscle, and other cellular systems [3]. This pool (S_2) includes not only the free calcium of the cytosol but also that calcium bound to intracellular ligands but still freely exchangeable. The identification of the third compartment is based on its characteristics being comparable to those of isolated mitochondria: (i) the pool size is increased by raising extracellular phosphate; (ii) antimycin A and Warfarin abolish calcium uptake into the compartment [3]; (iii) and there is agreement between the kinetic measurements and the chemical determination of calcium in mitochondria [6]. The total exchangeable calcium is defined as the sum of the kinetically

determined pools; the nonexchangeable cell calcium is the difference between this value and the chemically determined total cell calcium.

Intracellular pH

Intracellular pH was determined by the weak acid distribution method of Waddell and Butler [24]. In these experiments the media contained 20 mg/100 ml carrier inulin in addition to the usual components. The pH of the medium was monitored with a Beckman combination electrode and an Orion pH meter (model 801A). Kidney cells were isolated, preincubated, and the intracellular pH determined at 90, 180 and 300 min. Thirty minutes before the pH measurements 1 μCi ^{14}C-DMO and 2.6 μCi ^{3}H-inulin were added to 24 ml of the cell suspension. After 30 min equilibration, six samples were placed into tarred centrifuge tubes gassed with the same O_2-CO_2 mixture as the cells. The tubes were rapidly sealed and centrifuged 10 min at 20,000 × *g*. The decanted medium from the samples was pooled, and the tubes were drained, wiped dry, and reweighed. Two of the samples were dried overnight at 80 °C and used for determination of pellet total water. The remaining four were sonicated in distilled water and the ^{14}C and ^{3}H activities of the cell samples and medium were determined by liquid scintillation spectrophotometry on a dual channel Beckman LS-300. Intracellular pH was calculated by the equations published by Waddell and Butler [24]. Since there was little drift in cellular pH with time, the values obtained at different times were pooled to give an average cell pH for a given experimental condition.

Values are reported as the mean ± SE. The statistical significance of the results was determined by student's *t* test. In the case where groups with unequal variance are compared, the method of Snedecor and Cochran was used [18].

Results

Cell Viability

Data on parameters which may be used to verify the viability of an isolated cell preparation are given in Tables 2 and 3. In cells incubated under control conditions, potassium content did not decrease from the beginning to the end of the desaturation 320 min after dissociation, verifying the ability of the cells to maintain a constant intracellular ionic environment during the course of the kinetic studies. This constancy is also seen in the calcium content of cells incubated in control medium at pH 7.4 (Table 2). The rate of ^{3}H-leucine incorporation into protein by control cells measured for 60 min was linear and was the same at the beginning and at the end of the experiments: 150 ± 15 and 160 ± 13 ng/mg protein/hr. The rates determined from cells incubated in experimental media (values from 1–3 preparations) were similar (Table 3). The potassium content of cells incubated under conditions of acidosis and alkalosis is also reported in Table 3. These values are identical with the potassium content of control cells, thus experimental acidosis

Table 2. Potassium and calcium content of cells sampled from 65 to 320 min post dissociation

Time (min)	Cell potassium (meq/100 g protein)	Cell calcium (nmol/mg protein)
65	24.30 ± 0.64 (23)	21.21 ± 0.79 (24)
80	23.74 ± 0.70 (23)	22.04 ± 0.81 (22)
100	25.00 ± 0.72 (20)	22.62 ± 0.75 (21)
120	24.47 ± 0.58 (45)	22.58 ± 0.48 (40)
320	24.00 ± 1.00 (26)	21.60 ± 0.80 (25)

Values are mean ± SE.

Table 3. Cell potassium and ^{3}H-leucine incorporation during incubation in control and experimental conditions

	Cell potassium (meq/100 g protein)	Rate of ^{3}H-leucine incorporation into TCA precipitable protein (ng/mg/hr)	
		1–2 hr	4–5 hr
Control pH_e 7.4	24.88 ± 0.47 (30)	150 ± 15 (6)	160 ± 13 (6)
Metabolic acidosis pH_e 6.8	25.25 ± 0.53 (34)	130	140
Respiratory acidosis pH_e 6.8	24.09 ± 0.74 (26)	140	140
Metabolic alkalosis pH_e 8.0	25.96 ± 0.76 (24)	140	200
Respiratory alkalosis pH_e 8.0	24.28 ± 0.60 (23)	140	170

and alkalosis did not alter the ability of the cells to maintain their principal ion concentration at control levels. The reversibility of the effects of acidosis and alkalosis on cell calcium fluxes was tested in preliminary experiments. The fractional efflux of ^{45}Ca from the cells always returned to control levels after being perturbed by an acidosis (pH 6.8) or alkalosis (pH 8.0) of 60 min duration.

Intracellular pH

The intracellular pH (pH_i) obtained in the 8 different experimental conditions is given in Table 1. When the cells are incubated in control buffer (5% CO_2, 24 mM HCO_3^-, pH 7.42) the intracellular pH is 7.44. These results are similar to those of Struyvenberg *et al.* [19] who reported a cellular pH of 7.32 in dog kidney cells with an extracellular pH (pH_e) of 7.39. These investigators suggested that the relative alkalinity of the kidney cells was due to their high rate of hydrogen ion secretion. The equivalence of intra and extracellular pH in our rat kidney cells is probably not due to deterioration of the cell preparation: first, the cells maintain a pH significantly different from that of the medium in acidosis or alkalosis; second, their pH is significantly different from 7.4 when the CO_2 and HCO_3^- are altered simultaneously, keeping the medium pH at 7.4.

When the extracellular pH is decreased to 6.84 by increasing CO_2 or decreasing HCO_3^-, the cell pH drops to 7.11 or 7.20. When medium HCO_3^- is lowered to provide a less severe acidosis of pH 7.13, the cell pH is still significantly decreased to 7.25. Kidney cell pH appears to be buffered less effectively in alkalosis than in acidosis. The cellular pH is 7.90 in respiratory and 7.92 in metabolic alkalosis of pH_e 8.0, and is 7.66 in metabolic alkalosis with a medium pH of 7.74. These are larger shifts in intracellular pH than in acidosis for equivalent changes in extracellular pH. These results also agree with Struyvenberg's data.

When the cells are incubated in a high CO_2 and high HCO_3^- medium, the intracellular pH decreases to 7.24 in spite of a normal medium pH. Conversely, in a low CO_2 and low HCO_3^- medium with a pH of 7.4, the cells have an alkaline intracellular pH of 7.56. These experimental conditions can thus be used to distinguish effects due to changes in intracellular pH alone from those dependent on both intra and extracellular pH changes.

Cellular Calcium in Acidosis

The effects of acidosis on cell calcium are given in Table 4. Total cell calcium is equally decreased in severe metabolic and respiratory acidosis, while it is diminished to a lesser degree by the more moderate metabolic acidosis of pH_e 7.1. Cell relative radioactivity after 60 min of labelling is depressed in both moderate and severe acidoses. In respira-

Table 4. Effect of acidosis on cell calcium metabolism

	Control	Metabolic acidosis: pH_e 7.13, pH_i 7.25	Control	Metabolic acidosis: pH_e 6.84, pH_i 7.20	Control	Respiratory acidosis: pH_e 6.84, pH_i 7.11
Total cell calcium (nmol/mg protein)	22.29 ± 0.42 (21)	19.43 ± 0.64[b] (20)	25.35 ± 0.55 (27)	20.35 ± 0.46[b] (23)	21.43 ± 0.58 (23)	17.28 ± 0.47[b] (25)
Cell relative radioactivity (nmol/mg protein)	8.12 ± 0.10 (15)	7.03 ± 0.22[b] (15)	8.76 ± 0.26 (10)	5.41 ± 0.27[b] (10)	8.52 ± 0.27 (10)	4.51 ± 0.08[b] (10)
Calcium fluxes [pmol/(mg protein) (min)]						
Glycocalyx exchange (ρ_{01})	1888 ± 164	1656 ± 122	1577 ± 58	1423 ± 44	1678 ± 111	1382 ± 113
Plasma membrane exchange (ρ_{12})	144 ± 7.1	114 ± 5.3[b]	130 ± 5.4	77 ± 4.9[b]	138 ± 4.1	86 ± 5.3[b]
Mitochondrial exchange (ρ_{23})	28.0 ± 1.4	22.4 ± 0.44[b]	25.7 ± 2.1	16.9 ± 1.6[b]	27.2 ± 1.0	21.3 ± 1.4[b]
Calcium pools (nmol/mg protein)						
Glycocalyx (S_1)	4.29 ± 0.24	3.54 ± 0.18[a]	5.24 ± 0.36	3.50 ± 0.38[b]	5.07 ± 0.22	2.59 ± 0.08[b]
Cytosolic pool (S_2)	3.91 ± 0.22	3.02 ± 0.11[b]	3.56 ± 0.15	2.12 ± 0.11[b]	3.98 ± 0.24	1.82 ± 0.08[b]
Mitochondrial pool (S_3)	2.99 ± 0.40	2.54 ± 0.20	2.37 ± 0.19	1.48 ± 0.09[b]	2.86 ± 0.16	1.90 ± 0.07[b]
Total exchangeable calcium ($S_1+S_2+S_3$)	11.19 ± 0.52	9.10 ± 0.29[b]	11.17 ± 0.43	7.10 ± 0.41[b]	11.91 ± 0.36	6.31 ± 0.13[b]
Nonexchangeable cell calcium	11.10 ± 0.67	10.33 ± 0.70	14.18 ± 0.70	13.25 ± 0.61	9.52 ± 0.84	10.97 ± 0.59

Values are mean ± SE of 5–8 determinations except where noted in parenthesis.
[a] $P<0.05$. [b] $P<0.01$.

Table 5. Effect of alkalosis on cell calcium metabolism

	Control	Metabolic alkalosis: pH_e 7.74, pH_i 7.67	Control	Metabolic alkalosis: pH_e 8.04, pH_i 7.92	Control	Respiratory alkalosis: pH_e 7.98, pH_i 7.90
Total cell calcium (nmol/mg protein)	22.05 ± 0.45 (19)	26.63 ± 0.76[b] (16)	19.62 ± 0.66 (16)	26.65 ± 1.4[b] (17)	22.29 ± 0.46 (24)	29.57 ± 1.1[b] (23)
Cell relative radioactivity (nmol/mg protein)	8.87 ± 0.13 (19)	9.63 ± 0.20[a] (19)	8.50 ± 0.33 (8)	9.61 ± 0.46 (11)	6.98 ± 0.36 (10)	8.22 ± 0.16[a] (11)
Calcium fluxes [pmol/(mg protein) (min)]						
Glycocalyx exchange (ρ_{01})	2314 ± 68	2590 ± 85[a]	1773 ± 229	2048 ± 239[b]	1621 ± 194	2389 ± 109[b]
Plasma membrane exchange (ρ_{12})	160 ± 4.2	175 ± 4.2[a]	116 ± 9.4	125 ± 5.6	147 ± 9.8	141 ± 8.2
Mitochondrial exchange (ρ_{23})	32.5 ± 1.4	39.0 ± 1.5[b]	20.6 ± 3.1	36.1 ± 4.7[b]	35.4 ± 3.6	32.5 ± 1.0
Calcium pools (nmol/mg protein)						
Glycocalyx (S_1)	4.60 ± 0.07	5.29 ± 0.24[a]	5.69 ± 0.30	6.38 ± 0.44	4.42 ± 0.73	4.92 ± 0.12
Cytosolic pool (S_2)	4.28 ± 0.10	4.42 ± 0.11	3.82 ± 0.34	3.90 ± 0.16	3.03 ± 0.41	3.16 ± 0.11
Mitochondrial pool (S_3)	3.25 ± 0.16	4.11 ± 0.18[a]	1.62 ± 0.18	3.69 ± 0.21[b]	2.54 ± 0.27	3.55 ± 0.15[b]
Total exchangeable calcium ($S_1+S_2+S_3$)	12.13 ± 0.20	13.82 ± 0.32[b]	11.13 ± 0.49	13.97 ± 0.51[b]	9.99 ± 0.88	11.63 ± 0.22
Nonexchangeable cell calcium	9.92 ± 0.64	12.81 ± 0.95[a]	8.49 ± 0.96	12.68 ± 1.57	12.30 ± 1.04	17.94 ± 1.20[b]

Values are mean ± SE of 5–8 determinations except where noted in parentheses.
[a] $P < 0.05$. [b] $P < 0.01$.

tory acidosis all exchangeable calcium pools are depressed. During metabolic acidosis of pH_e 6.8, the glycocalyx (S_1), cytosolic (S_2), and mitochondrial pools (S_3) are depressed while only the glycocalyx and cytosolic pools are decreased when the extracellular pH is decreased to 7.1. Thus the total exchangeable calcium is significantly decreased by both metabolic and respiratory acidosis. However, there is no significant influence of acidosis on the nonexchangeable fraction of cell calcium. Calcium exchange with the extracellular glycocalyx (ρ_{01}) is not changed; however, the plasma membrane (ρ_{12}) and mitochondrial (ρ_{23}) exchanges are depressed in the three types of acidosis.

Cellular Calcium in Alkalosis

The data from cells incubated under alkalotic conditions is given in Table 5. Total cell calcium is increased by low CO_2 and by high medium HCO_3^-; however, the cells' relative radioactivity at 60 min is increased significantly only in the case of respiratory alkalosis and metabolic alkalosis pH_e 7.7. In all types of alkalosis the mitochondrial pool (S_3) is increased, while cytosolic calcium (S_2) remains at control levels. The increase in glycocalyx calcium (S_1) is significant only with the moderate metabolic alkalosis. While there are significant increases in both the total exchangeable and nonexchangeable fractions of cell calcium in alkalosis, the average percent increase in nonexchangeable calcium (41%) is much greater than the average increase in the exchangeable fraction (19%). The most rapid calcium exchange (ρ_{01}) is significantly increased in all alkaloses, and there is a small increase in plasma membrane exchange (ρ_{12}) at pH_e 7.7. The mitochondrial exchange (ρ_{23}) is significantly increased in metabolic alkalosis but is unchanged with low CO_2.

Cell Calcium in ECF Isohydric Conditions

The effects of changing both CO_2 and HCO_3^- while keeping the medium pH at 7.4 are given in Table 6. When both are increased there is an intracellular acidosis (Table 1). Total cell calcium falls, the cells' relative radioactivity at 60 min is depressed, and all calcium pools are decreased, as are the plasma membrane and mitochondrial calcium ex-

Table 6. Effect of constant extracellular pH (7.4) with changes in HCO_3^- and CO_2 on cell calcium metabolism

	Control (5% CO_2, 24 mM HCO_3^-)	High CO_2, high HCO_3^- (20% CO_2, 96 mM HCO_3^- pH_e 7.41, pH_i 7.24)	Low CO_2, low HCO_3^- (1.25% CO_2, 6 mM HCO_3^- pH_e 7.42, pH_i 7.56)
Total cell calcium (nmol/mg protein)	21.00±0.46 (37)	18.12±0.48[b] (25)	19.65±0.46 (23)
Cell relative radioactivity (nmol/mg protein)	7.51±0.24 (14)	5.56±0.26[b] (8)	8.25±0.23 (6)
Calcium fluxes [pmol/(mg protein) (min)]			
Glycocalyx exchange (ρ_{01})	1418 ±149	1156 ±53	1544 ±75
Plasma membrane exchange (ρ_{12})	112 ± 8.2	88 ± 3.4[a]	130 ± 4.5
Mitochondrial exchange (ρ_{23})	24.7 ± 2.9	16.7 ± 0.94[a]	31.2 ± 1.1
Calcium pools (nmol/mg protein)			
Glycocalyx (S_1)	4.52±0.24	3.28±0.13[a]	4.12±0.11
Cytosolic pool (S_2)	3.47±0.18	2.15±0.03[b]	3.61±0.05
Mitochondrial pool (S_3)	2.30±0.17	1.51±0.08[a]	2.85±0.05[a]
Total exchangeable calcium ($S_1+S_2+S_3$)	10.29±0.34	6.94±0.16[b]	10.58±0.13
Nonexchangeable cell calcium	10.71±0.75	11.18±0.62	9.07±0.59

Values are mean ±SE of 6–7 determinations except where noted in parentheses.
[a] $P<0.05$. [b] $P<0.01$.

change. The total exchangeable calcium is decreased, while there is no change in the nonexchangeable fraction. In spite of a normal extracellular pH of 7.4, the pattern of cell calcium metabolism is identical to that seen in extracellular acidosis. Thus, alterations in intracellular pH alone in the presence of normal extracellular pH alter calcium pool sizes and fluxes.

The effects of an intracellular alkalosis with normal extracellular pH produced by low CO_2 and low HCO_3^- are comparable to those obtained in both intra and extracellular alkalosis: the intracellular pH is elevated (Table 1), the mitochondrial pool (S_3) is increased, while the glycocalyx and cytosolic pools (S_1 and S_2) remain constant. In this case, however, there is no change in total cell calcium, in exchangeable and nonexchangeable calcium, in the cell relative radioactivity, or in any of the fluxes.

Changes in Cell Calcium as a Function of Intracellular pH

These alterations in cell calcium metabolism due to acidosis or alkalosis can perhaps be more readily visualized by expressing the data as % change from control. The results are consistent with the idea that changes in cellular calcium metabolism are the result of intracellular changes in pH. In Figs. 2 and 3 the data is plotted as a % change *vs.* intracellular pH for the eight experimental conditions studied. The pattern of changes would be similar if the data were plotted as a function of extracellular pH, but the values from the isohydric groups would be aberrant. With the exception of the isohydric low $CO_2 - HCO_3^-$ group there is a decrease in cell calcium with decreased pH_i and an increase with an increased pH_i. The pattern of cell relative radioactivity is similar; however, the depression in acidosis is more marked and the increase in alkalosis less than that of total cell calcium. The changes in pool sizes and calcium fluxes are shown in Fig. 3. All three pools are decreased and show a linear relationship with pH_i in acidosis. However, in alkalosis, the glycocalyx and cytosolic pools are changed very little while the mitochondrial pool is markedly increased. Cell calcium fluxes also show

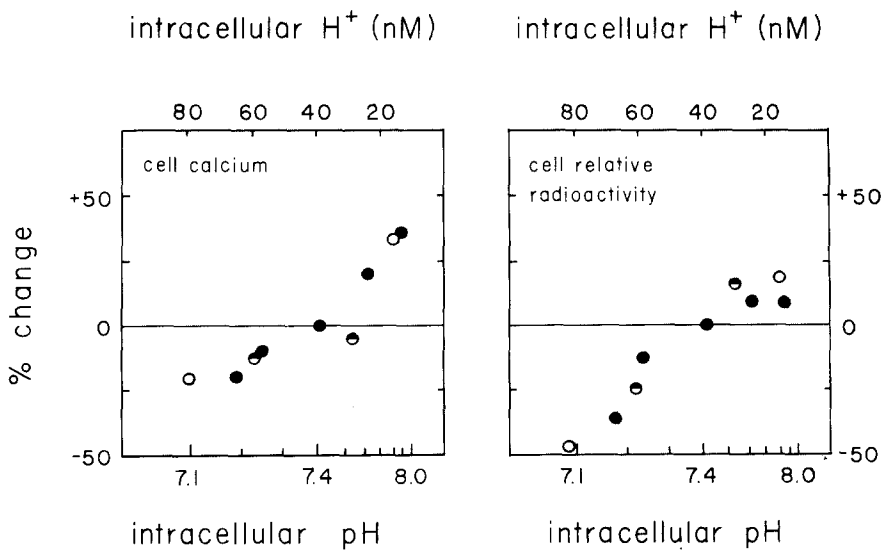


Fig. 2. Effect of intracellular H^+ on cell calcium and cell relative radioactivity. Data expressed as % change of experimental mean values from their respective controls. ○ = respiratory acidosis and alkalosis; ● = metabolic acidosis and alkalosis; ◓ = extracellular isohydric conditions

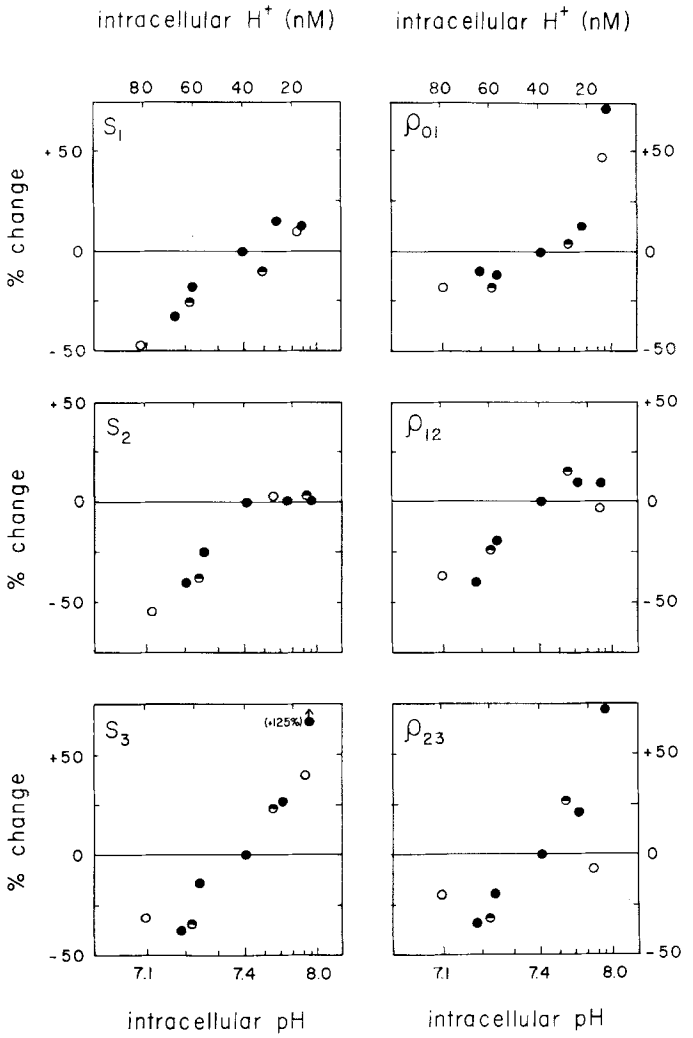


Fig. 3. Effect of intracellular pH on the kinetically determined cell calcium pools (S_1, S_2, S_3) and calcium fluxes (ρ_{01}, ρ_{12}, ρ_{23}). Data expressed as % change of experimental mean values from their respective controls. ○ = respiratory acidosis and alkalosis; ● = metabolic acidosis and alkalosis; ◓ = extracellular isohydric conditions

a strong correlation with pH_i. Glycocalyx and mitochondrial exchange are increased with an increase in pH_i and decreased when the intracellular hydrogen ion concentration is increased. Plasma membrane calcium exchange is depressed by cellular acidosis; however, alkalosis does not affect this flux between the cytosolic and rapidly exchanging cell calcium pool.

Discussion

While total cell calcium exhibits a superficially simple inverse relationship with intracellular hydrogen ion concentration, an analysis of the kinetically discernable cell pools and fluxes reveals a more complex interrelation between hydrogen ion and cell calcium metabolism.

Acidosis causes a decrease in total cell calcium and all three exchangeable pools. The loss in cell calcium appears to be predominantly from the exchangeable pools since there is little change in the nonexchangeable component. Alkalosis results in an increase in total cell calcium but the sum of cell exchangeable pools is barely increased. The only significant change seen consistently is in the mitochondrial pool. However, since this increase is much less than the gain in cell calcium, the bulk of the additional calcium must be sequestered in a nonexchangeable fraction, perhaps as a precipitation of calcium phosphate within the mitochondria.

In our experiments, acidosis decreases calcium fluxes between the kidney cell compartments. Langer and Poole-Wilson [11] have shown that decreasing extracellular pH to 6.67 inhibited ^{45}Ca efflux from perfused rabbit interventricular septa. They also followed the uptake of ^{45}Ca in this preparation and found a decreased influx during acidosis. They concluded that respiratory acidosis decreases calcium exchange between the medium and an intracellular pool. These results agree with the decreased plasma membrane exchange (ρ_{12}) which we observed in both metabolic and respiratory acidosis. In liver slices, Wallach *et al.* [25] have found that a medium pH of 6.8 decreased the ^{45}Ca influx transfer coefficient by 10%, and that a pH of 8.2 increased the influx transfer coefficient 60% and the efflux coefficient 25% with a net increase in cell calcium. These results showing a decrease in calcium exchange in acidosis and an increase in total cell calcium in alkalosis are in agreement with our data.

A steady-state analysis of calcium pools and fluxes yields no information on the primary cell site affected by a given perturbation or on the initial step in the sequence of events which leads to the new steady state. However, the mitochondrial pools and mitochondrial calcium exchange were altered by both acidosis and alkalosis. While the transmitochondrial membrane potential difference is the immediate source of energy for mitochondrial calcium transport [8] the mitochondrial proton gradient may be capable of modifying the rate and capacity of this

transport in several ways. It could affect calcium transport by changing the calcium carrier conformation, by affecting the release of calcium from the carrier inside the mitochondria, or by changing the internal alkalinity of mitochondria which could affect the internal binding sites for calcium. Recently Nichols [15] has studied the regulation of extramitochondrial free calcium by rat liver mitochondria *in vitro*. He found that the concentration of medium calcium was influenced by the incubation pH; when incubated at pH 7.4 the mitochondria established a steady-state free calcium of 0.32 μM, but if the pH was decreased to 6.8 the medium calcium was maintained at 0.8 μM. This suggests that hydrogen ion does have a direct influence on mitochondrial calcium transport.

More pertinent to the current studies with kidney cells, an influence of pH on both the kinetics of the initial rate and steady-state calcium transport of *in vitro* kidney mitochondria has been observed [20]. Acidosis increased the K_m and decreased the V_{max} of the initial rate of mitochondrial calcium uptake, while alkalosis decreased the K_m. When the kidney mitochondria were incubated in steady-state conditions with a free calcium of 0.75 μM, acidosis depressed the total calcium, the exchangeable calcium, and the rate of calcium exchange. Conversely, alkalosis increased the mitochondrial calcium pool and rate of exchange [20]. These results are in agreement with the effects of acidosis and alkalosis on mitochondrial calcium metabolism studied in intact renal cells.

However, these combined results do not necessarily indicate that the mitochondria are the primary or only site of action of hydrogen ion on cell calcium metabolism. In alkalosis, the increase in mitochondrial exchangeable calcium without changes in cytosolic calcium and with little change in calcium transport across the plasma membrane observed in isolated cells could be accounted for by the decreased K_m of calcium transport and the increased capacity for calcium accumulation measured in isolated mitochondria. However, there is no explanation for the increased rate of glycocalyx calcium exchange.

On the other hand, acidosis decreases the total cell calcium, all exchangeable pools, and all cellular calcium fluxes. Since acidosis also decreases the calcium content and calcium exchange in isolated mitochondria, the changes occurring in the mitochondrial pool are probably not secondary to the perturbations observed in cytosolic calcium. However, inhibition of mitochondrial calcium transport alone does not provide an adequate explanation for all the effects of acidosis on cell calcium metabolism. It cannot explain the lower cytosolic calcium and the depressed calcium transport across the plasma membrane. Other effects of acidosis on cal-

cium binding to cellular ligands or on calcium transport across the plasma membrane must be assumed.

For example, extrapolation of data from studies of the effect of pH on calcium binding to serum proteins [14] or cellular membranes [9] shows that when cell pH is decreased from 7.4 to 7.1, calcium-protein-H^+ interactions could be expected to decrease cell calcium by 0.65 nmol/mg protein. Such effects could depress the total and exchangeable cell calcium and calcium transport in general.

The influence of hydrogen ion on active calcium extrusion across the plasma membrane has been studied in erythrocytes by Plishker and Gitelman [16] and Romero and Whittam [17]. They concluded that there is no major effect of medium pH on the plasma membrane calcium pump in these cells. However, the same investigators did find that acidosis decreases the calcium permeability of the erythrocyte plasma membrane. This effect, if present in renal cells, could also contribute to the observed changes in cell calcium metabolism seen in acidosis.

Regardless of the specific cellular sites affected by hydrogen ion, the changes in cell calcium metabolism in these isolated renal cells could explain the calciuria of acidosis observed *in vivo* [2, 12, 21]. While it is not certain that *in vivo* transtubular calcium fluxes are directly related to *in vitro* cellular calcium transport, our observations of decreased cellular calcium fluxes in acidosis are consistent with these physiological studies demonstrating the inhibition of renal calcium reabsorption in acidosis. But the most important conclusion supported by these studies is that the cellular H^+ concentration is an important modulator of intracellular calcium metabolism.

This work was supported by a grant from the National Institutes of Health, U.S. Public Health Service, AM 07867 and Biomedical Research Support Grant RR 05416.

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