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Research Article

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Abnormal Cation Transport in Uremia

Mechanisms in Adipocytes and Skeletal Muscle from Uremic Rats

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Abstract

The cause of the abnormal active cation transport in erythrocytes of some uremic patients is unknown. In isolated adipocytes and skeletal muscle from chronically uremic chronic renal failure rats, basal sodium pump activity was decreased by 36 and 30%, and intracellular sodium was increased by 90 and 50%, respectively, compared with pair-fed control rats; insulin-stimulated sodium pump activity was preserved in both tissues. Lower basal NaK-ATPase activity in adipocytes was due to a proportionate decline in [^{3}H]ouabain binding, while in muscle, [^{3}H]ouabain binding was not changed, indicating that the NaK-ATPase turnover rate was decreased. Normal muscle, but not normal adipocytes, acquired defective Na pump activity when incubated in uremic sera. Thus, the mechanism for defective active cation transport in CRF is multifactorial and tissue specific. Sodium-dependent amino acid transport in adipocytes closely paralleled diminished Na pump activity (r = 0.91), indicating the importance of this defect to abnormal cellular metabolism in uremia.

Introduction

Defective regulation of transmembrane ion transport due to changes in the activity of cation transportors and/or altered cation permeability of cell membranes could initiate or participate in many abnormalities of cellular function. The accessibility of the erythrocyte has made it an obvious choice for studying ion transport in humans. However, it is unclear whether depressed erythrocyte sodium pump activity in uremia results from fewer functionally active NaK-ATPase enzyme units, from a change in the rate of ion pumping (turnover rate) of each enzyme, or both (1–6). It is also unclear whether the defect in active cation transport can be acquired by normal erythrocytes upon exposure to uremic sera (7, 8). Finally, it is uncertain whether defects in the regulation of ion transport in erythrocytes can be generalized to nucleated cells.

In a limited number of other tissues (9–12), there is evidence that sodium pump activity may be abnormal in uremia, and in skeletal muscle of dialysis patients, there is an abnormally low resting membrane potential and increased intracellular sodium, consistent with a defect in sodium pump function (13). Despite these reports, it cannot be concluded that in uremia, all tissues will exhibit the same pathophysiology for defective basal and/or hormonally stimulated active sodium transport. For example, insulin-mediated potassium uptake is normal in uremic humans (14, 15), implying that a hormonally responsive subset of NaK-ATPase units is not impaired by chronic renal failure (CRF).[1]

We have studied partially nephrectomized rats with metabolic and acid-base disturbances of chronic uremia similar to those seen in humans with chronic uremia (15). Using this model, we examined active and passive cation transport in skeletal muscle and adipocytes from CRF rats and from pair-fed, sham-operated (SO) control rats. These tissues were studied because they contribute substantially to total protein and energy metabolism, and because each has a basal NaK-ATPase activity contributing to the resting membrane potential, as well as an insulin-responsive subset of NaK-ATPase isoenzymes (16, 17). In each tissue, basal and insulin-stimulated ouabain-sensitive and -insensitive ^{86}rubidium-chloride (^{86}Rb) flux and intracellular sodium were quantified, and [^{3}H]-ouabain binding was measured to assess the number of sodium pump sites. In adipocytes, changes in ^{86}Rb transport were compared with changes in sodium-dependent amino acid transport. Finally, the effect of uremic serum on basal rates of active ^{86}Rb influx was studied in both the adipocyte and in skeletal muscle.

Methods

Animals. Male Sprague-Dawley rats weighing 75–100 g (Charles River Breeding Laboratory, Inc., Wilmington, MA), housed in a temperature-controlled room with a 12-h light/dark cycle, were used in all studies. To induce CRF, rats were anesthetized (pentobarbital, 50 mg/kg i.p.) and branches of the left renal artery were ligated to infarct about five-sixths of the kidney. One week later, a right nephrectomy was performed through a flank incision; the right kidney of control (SO) rats was manipulated through a laparotomy, but not injured. Subsequently, rats were paired by weight, housed in individual metabolic cages, and pair fed for at least 21 d with a diet yielding normal growth in CRF rats and a consistent degree of uremia (18). The diet contained 40% protein and consisted of casein, chow (RMH 3000; Agway Country Foods, Syracuse, NY); and dextrin (40, 35, and 25% wt/wt/wt). Pair feeding was accomplished by giving control rats the

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1. *Abbreviations used in this paper:* CRF, chronic renal failure; KH, Krebs-Henseleit bicarbonate; MeAIB, methylaminoisobutyric acid; NEFA, nonesterified fatty acid; ^{86}Rb, ^{86}rubidium-chloride; SO, sham operated.

same amount of diet as eaten by CRF rats the previous day. Except for experiments in which normal tissues were incubated in serum (see below), all experiments were analyzed by comparing data from a CRF animal with its pair-fed control.

Materials. ^{86}Rb, 2-deoxy[^{3}H]glucose, [^{14}C]methylaminoisobutyric acid (MeAIB), D[U-^{14}C]-sorbitol, ^3H_2O, and [^{3}H]ouabain were purchased from New England Nuclear (Boston, MA). Collagenase was obtained from Cooper Biomedical, Inc. (Malvern, PA), and bovine serum albumin was obtained from Armour Biochemical (Kankakee, IL). Only those lots that allowed a significant insulin-induced increase in ouabain-sensitive ^{86}Rb influx in adipocytes were used (16, 17, 19–21). All other chemicals were analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO).

Preparation of adipocytes. Adipocytes were isolated from epididymal fat pads by a slight modification of the method of Rodbell (19, 20). All procedures were carried out in polypropylene tubes. Fat pads were incubated at 37°C for 1 h with 1 mg/ml collagenase in buffer containing millimolar: 140, NaCl; 5, KCl; 2.4, $CaCl_2$; 1, $MgSO_4$; 1, $Na\,H_2PO_4$; 10, Hepes; and 4 g/dl albumin (H-A buffer) at pH 7.4. The cells were filtered through 12× mesh silk, washed three times in H-A buffer, and adjusted to a "lipocrit" of ~ 25%. To assess insulin responsiveness, 0.5 μCi 2-deoxy[^{3}H]glucose were added for 3 min, and transport was terminated by centrifuging 100 μl of adipocytes through dinonylphthalate. Radioactivity in the solubilized cell pellet and supernatant was measured by scintillation counting with correction for quenching using an external standard.

The cell number and size were determined in each experiment using a cell analyzer and channelizer (Coulter Electronics, Inc., Hialeah, FL) calibrated with latex particles of known size.

^{86}Rb transport in adipocytes. ^{86}Rb transport was measured using the method of Resh (21). Briefly, adipocytes (0.5 ml, final volume) were preincubated at 37°C for 20 min in the presence or absence of 8.5 nM insulin, 1 mM ouabain, and 1 mM furosemide. After 0.5 μCi ^{86}Rb was added, the mixture was incubated for 20 min in a gently shaking water bath at 37°C and the reaction was stopped by centrifugation of 100-μl aliquots through dinonylphthalate oil as described by Gliemann (22). Quadruplicate measurements were made. The tubes were cut through the oil layer to separate the cell pellet, which was solubilized by incubating with 0.4 ml 5% sodium lauryl sulfate, scintillation fluid was added, and radioactivity was measured. ^{86}Rb uptake was calculated as picomoles per 10^6 cells per minute. In addition to ouabain-sensitive flux (NaK-ATPase activity), ouabain-resistant and furosemide-sensitive ^{86}Rb uptake (Na, K, Cl co-transport), and the ouabain- and furosemide-resistant ^{86}Rb uptake (passive permeability) were determined.

Influence of uremic sera on ^{86}Rb uptake in adipocytes. The effect of pooled sera from 12 CRF rats on Rb flux in tissues from normal rats was compared with the effects of sera from 12 SO rats. Blood was obtained in nonheparinized syringes, the sera were separated, pooled into CRF and SO batches, and 0.3 mg Hepes/ml was added, the pH titrated to 7.4 and potassium to 8 meq/liter (23). Adipocytes from six normal rats were preincubated for 100 min with 0.4 ml (80%) of uremic or control sera, and then in the presence or absence of 10^{-3} M ouabain for 20 min. After this 100-min preincubation, 0.5 μCi ^{86}Rb were added and ouabain-sensitive and -insensitive uptake were evaluated as described. In parallel incubations, the influence of uremic sera on amino acid transport was investigated.

Amino acid uptake. To determine whether abnormalities in sodium pump activity affected a sodium-dependent, co-transport mechanism, amino acid uptake through system A was measured as the accumulation of the nonmetabolizable substrate specific for system A, MeAIB (24). 3 μM [^{14}C]MeAIB was added to 1 ml of an adipocyte suspension in Hepes albumin buffer, and after a 4-min incubation, 100-μl aliquots were centrifuged through dinonylphthlate oil (25). Uptake was linear for 5 min.

[^{3}H]Ouabain binding. Specific ouabain binding was measured by incubating adipocytes for 60 min at 37°C with 5.5×10^{-7} M [^{3}H]-ouabain (22.1 Ci/mmol) in the presence or absence of 10^{-3} M nonradioactive ouabain (21). Radioactivity in the cell layer was then determined. To examine the effect of insulin, [^{3}H]ouabain binding was also determined in a separate study in the presence or absence of 1.5×10^{-6} M insulin. The presence of insulin did not change [^{3}H]ouabain binding (data not shown). Results were corrected for nonspecific binding and are presented as [^{3}H]ouabain bound per cell.

Intracellular sodium content. The intracellular sodium concentration was determined after washing adipocytes in Hepes–albumin buffer in which NaCl was replaced by choline-Cl. 200-μl aliquots of adipocytes were centrifuged through oil, the cell pellet was solubilized with Acationox (American Scientific Products, McGaw Park, IL) and the sodium content of the solubilized pellet and infranatent was determined by atomic adsorption spectrophotometry. In parallel incubations, the intra- and extracellular water space were determined from the distribution volume of ^3H_2O and D[U-^{14}C]sorbitol, respectively. After correcting for sodium in the extracellular space, the intracellular sodium concentration was calculated.

^{86}Rb transport in skeletal muscle. Intact rat epitrochlearis muscles were incubated in Krebs-Henseleit bicarbonate (KH) buffer containing (millimolar) 118, NaCl; 6, KCl; 1, $CaCl_2$; 1, $MgSO_4$; 1, NaH_2PO_4; 25, $NaHCO_3$, and 2, glucose; and were gassed with 95% O_2, 5% CO_2 (pH 7.4) for 20 min in the presence or absence of 850 nM insulin and/or 1 mM ouabain, with or without 1 mM furosemide. The flasks were regassed, and 0.5 μCi ^{86}Rb/ml was added and incubated for another 30 min. The reaction was terminated by transferring the muscle into iced KH buffer, the muscles were washed four times in cold buffer, blotted, weighed, and dissolved in scintillation vials containing 1 N NaOH. After 30 min, scintillation fluid (dimilume; Packard Instrument Co., Downers Grove, IL) was added, the vials were capped, vortexed, and scintillation was determined with correction for quenching using an external standard. ^{86}Rb uptake was calculated after correction for values of tissue water and extracellular space. Ouabain-sensitive uptake (NaK-ATPase activity) was determined, as well as ouabain-insensitive, furosemide-sensitive uptake (Na, K, Cl co-transport).

Influence of uremic sera on ^{86}Rb transport in skeletal muscle. Epitrochlearis muscles of 12 SO rats were preincubated for 120 min in 2.5 ml (83%) pooled sera from either CRF or SO rats. To determine ouabain-insensitive transport, 10^{-3} M ouabain was added to duplicate flasks for the final 20 min of the preincubation. 0.5 μCi ^{86}Rb/ml was added, the flasks were regassed, incubated for 30 min, and ^{86}Rb uptake was determined.

[^{3}H]Ouabain binding in skeletal muscle. Specific [^{3}H]ouabain binding was determined by incubating epitrochlearis muscles for 120 min at 37°C in potassium-free KH buffer containing 5 mM glucose buffer with 10^{-6} M [^{3}H]ouabain in the presence or absence of 1 mM nonradioactive ouabain (26–29). Subsequently, the muscles were washed four times for 30 min each in iced buffer and processed as detailed.

Intracellular sodium. The intracellular sodium content was determined as described by Clausen (26). Muscles were washed in buffer in which NaCl was replaced by choline Cl, blotted, weighed, homogenized in 1.5% TCA, and centrifuged. The sodium content of the supernatant was determined by atomic absorption spectrophotometry. A correction for extracellular water space was evaluated in parallel incubations using D[U-^{14}C]sorbitol and [^{3}H]water.

Other analytical procedures. The pH of aortic blood was measured using a blood gas analyzer (Instrumentation Laboratory, Inc., Lexington, MA), serum sodium and potassium concentrations by flame photometry, and serum urea nitrogen fluorometrically (18). Plasma nonesterified fatty acids (NEFA) were quantitated by an HPLC technique using derivatization of plasma NEFA with the fluorescent compound, 4-bromomethyl, 7-acetoxy coumarin (30–32). To 15-μl aliquots of plasma, 3 nmol of heptadecanoic acid (Sigma Chemical Co.) in water were added as an internal standard (final volume, 500 μl); 3 ml of chloroform containing 5 mg/dl butylated hydroxytoluene were added and the samples were vortexed for 1 min and centrifuged. NEFA were separated from neutral and other polar lipids using disposable aminopropyl columns (Bond Elut; Analytichem Intl., Harbor City, CA) as described by Kaluzny et al. (32). 4-Bromomethyl, 7-acetoxy coumarin

derivatized NEFA was separated on a radial compression column (4 μm particle size, Nova Pak C_{18}; Waters Assoc., Div. of Millipore Corp., Milford, MA) and eluted by increasing the ratio of methanol to acetonitrile/water. The coefficient of variation was < 5% with derivatized samples containing at least 1 pmol NEFA/μl of plasma; the detection limit was ~ 10 fmol (33).

Calculations. Because the rats were pair fed, a paired Student's *t* test was used for most calculations. For other comparisons, an unpaired *t* test was used. As a limit for significance, a $P < 0.05$ was assumed.

Results

After surgery and a 2-d recovery period, CRF and SO rats were pair fed an average of 26.6 d before experiments were performed. CRF rats were azotemic and had a metabolic acidosis, and despite the same food intake, gained slightly less weight compared with SO rats (Table I), although their final body weights were not different. Despite similar body weights, the weight of epididymal fat pads expressed either as a percent of body weight or absolute values were lower in CRF rats ($P < 0.001$; Table I). There was evidence of enhanced lipolysis in CRF rats, since the plasma concentration of NEFA was 23% higher in these animals ($P < 0.025$; Table II). Although the concentration of total saturated NEFA was higher due to a significant increase in palmitic (16:0) and stearic (18:0) acids, the concentration of total unsaturated NEFA was unchanged, resulting in a significant increase in the saturated-to-unsaturated NEFA ratio in CRF animals.

^{86}Rb flux in adipocytes. Ouabain-sensitive ^{86}Rb uptake by uremic adipocytes was decreased 36% ($P < 0.02$) from 7.13±1.02 (SO) to 4.58±0.59 nmol/10^6 cells/min (CRF) (Table III). However, the stimulatory effect of insulin on NaK-ATPase was preserved; in CRF and in SO animals, insulin caused a 20.4 and 19.9% increase, respectively, in ouabain-sensitive ^{86}Rb transport. The ouabain-resistant, furosemide-sensitive fraction of ^{86}Rb uptake (Na, K, Cl co-transport) was decreased 24% by CRF ($P < 0.05$), but passive permeability (ouabain- and furosemide-insensitive flux) was unchanged (Table III). Insulin did not affect ouabain-resistant ^{86}Rb transport or passive permeability (data not shown).

[^{3}H]Ouabain binding to isolated adipocytes was decreased by 46% in CRF animals ($P < 0.025$; Table III). Consistent with these results, the adipocyte intracellular sodium concentration was increased 93% in uremic animals ($P < 0.001$).

Table I. Characteristics of Experimental and Control Groups

	CRF	Controls	*P*
Duration of renal failure (*d*)	28.7±2.6		
Duration of pair feeding (*d*)	26.6±2.6		
Average food intake (*g/d*)	13.2±0.5		
Final body weight (*g*)	202.6±7.8	211.0±10.0	NS
Weight gain/day	3.6±0.3	3.9±0.24	NS
Serum urea nitrogen (*mg/dl*)	131.9±6.5	36.8±1.6	<0.001
Sodium (*meq/liter*)	130.5±2.2	140.3±3.1	<0.02
Potassium (*meq/liter*)	5.9±0.4	4.7±0.3	<0.05
Bicarbonate (*mM*)	14.1±0.7	19.9±0.5	<0.001
pH	7.21±0.02	7.34±0.01	<0.001
Weight of epididymal fat pad (*μg*)	585.1±68.1	896.1±77.5	<0.001
Fat pad/body weight (%)	0.27±0.02	0.40±0.02	<0.001

Values of 21 pairs of rats are given as means±SEM.

Table II. Plasma NEFA

	CRF	Controls	*P*
	μM		
12:0	73±0.9	77±2.1	NS
14:0	51.2±2.0	48.9±1.7	NS
16:0	289.2±14.1	235.5±11.1	<0.02
18:0	340.4±32.2	238.4±24.7	<0.05
Total saturated NEFA	688.2±41.6	530.5±89.1	<0.05
14:1	32.0±2.7	33.7±3.0	NS
18:3	3.4±0.2	4.7±0.6	<0.05
16:1	15.4±4.5	13.7±3.0	NS
20:4	12.0±2.4	13.6±2.1	NS
18:2	21.6±2.0	21.9±0.8	NS
20:3	12.7±1.7	12.6±2.1	NS
18:1	131.6±7.5	112.3±5.8	<0.05
Total unsaturated NEFA	228.7±11.2	212.5±12.6	NS
Total NEFA	916.9±45.4	743.0±36.1	<0.02
Ratio saturated/unsaturated	2.92±0.20	2.50±0.17	NS

Values from six pairs of rats are given in μmol/liter as mean±SEM.

To determine if these abnormalities in sodium pump activity affected transport mechanisms dependent upon the transmembrane sodium gradient, sodium-dependent amino acid transport by system A was measured. CRF decreased uptake of MeAIB, a nonmetabolizable probe specific for system A, into adipocytes by 43%, from 12.06±2.19, SO to 6.84±0.69 nmol/10^6 cells/min CRF; n = six pairs; $P < 0.001$). In these experiments, ^{86}Rb influx was also measured in another aliquot from the same adipocyte preparation. Ouabain-sensitive ^{86}Rb influx was decreased by 50% ($P < 0.001$), and there was a high correlation between the rate of active ^{86}Rb transport and amino acid uptake ($r = 0.9116$, $P < 0.001$) (Fig. 1).

To determine if the abnormality in NaK-ATPase activity could be transferred by uremic serum, adipocytes from normal rats were incubated with sera from CRF rats. The 2-h preincubation with uremic sera did not decrease ^{86}Rb uptake compared with incubation of normal adipocytes with control sera (Table IV). Incubation with uremic sera also did not affect the rate of MeAIB uptake by normal adipocytes.

^{86}Rb flux in skeletal muscle. Similarly to the data obtained in adipocytes, ouabain-sensitive ^{86}Rb transport in epitrochlearis muscle was decreased 30% in CRF rats compared with that in SO animals ($P < 0.01$; Table V). The stimulatory effect of insulin on ^{86}Rb transport was preserved, as in adipocytes; insulin caused a 21 and 28% rise of ouabain-sensitive ^{86}Rb uptake in CRF and SO rats, respectively, but did not alter ouabain-resistant ^{86}Rb transport. Furosemide-sensitive ^{86}Rb flux was decreased by 77% in CRF muscles ($P < 0.001$; Table V). Although passive permeability (ouabain- and furosemide-insensitive ^{86}Rb influx) was lower in CRF than in SO muscles, the difference was not quite statistically significant ($P < 0.10$).

In contrast to the marked reduction in [^{3}H]ouabain binding in adipocytes, [^{3}H]ouabain binding to epitrochlearis muscles was not significantly decreased (Table V), even though intracellular sodium was 43% higher in muscle from CRF rats

Table III. ^{86}Rb Influx, [^{3}H]Ouabain Binding, and Intracellular Sodium Concentration in Adipocytes

Measurement	CRF	Controls	P
	nmol/10^6 cells/min		
Basal ouabain-sensitive ^{86}Rb uptake	4.58±0.59	7.13±1.02	0.05
Insulin-stimulated ouabain-sensitive ^{86}Rb uptake	5.45±0.66*	8.50±1.20*	0.05
Furosemide-sensitive ^{86}Rb uptake	0.77±0.15	1.18±0.25	0.05
Ouabain and furosemide-resistant ^{86}Rb uptake	0.45±0.66	0.59±0.08	NS
[^{3}H]Ouabain bound × 10^3/cell	182.04±12.64	340.32±64.28	0.025
Intracellular sodium (*meq/liter*)	18.46±3.52	9.58±1.38	0.001

Values from eight pairs of rats are given as mean±SEM.
* $P < 0.001$ by paired analysis vs. basal ouabain-sensitive ^{86}Rb uptake.

($P < 0.025$; Table V). [^{3}H]Ouabain binding in muscle from both control and experimental rats was reduced compared with values reported by others (28, 29). This may be due to differences in the composition of our incubation media (i.e., we used a lower phosphate concentration and no vanadate) or because of a difference in the type of muscle, or in the strain, sex, or age of the rat we studied.

Again, in contrast to adipocytes, incubation of muscle from normal rats in uremic sera decreased ouabain-sensitive ^{86}Rb uptake by 50% ($P < 0.01$; Table IV). Incubation of normal muscle in uremic sera also decreased ouabain-resistant ^{86}Rb transport ($P < 0.001$; Table IV).

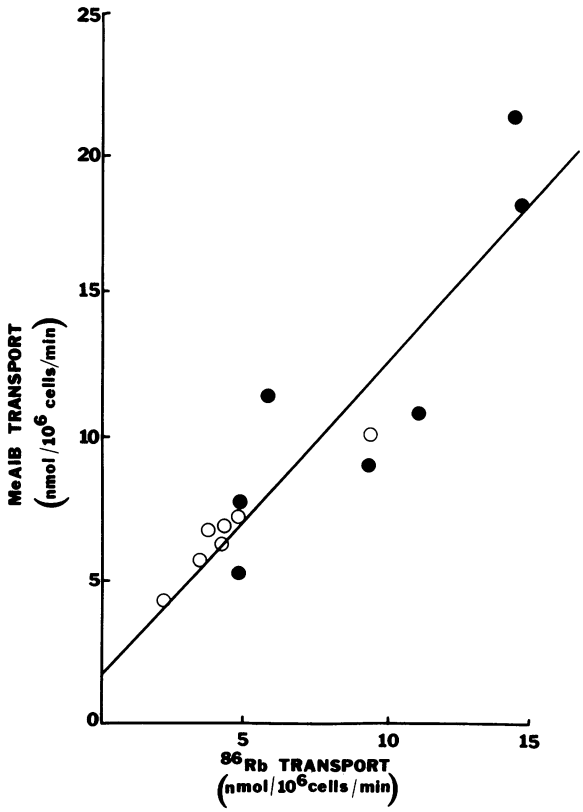

Figure 1. The rate of uptake of a nonmetabolizable amino acid probe specific for the sodium-dependent amino acid uptake pathway system A, MeAIB, is plotted against the ouabain-sensitive influx of ^{86}Rb into adipocytes from both uremic (*open circles*) and pair-fed, SO control (*closed circles*) animals. The correlation between these two parameters is highly significant ($r = 0.91$; $P < 0.001$).

Discussion

The presence of a defect in erythrocyte NaK-ATPase activity, and perhaps other cation flux pathways in uremia, has been extensively studied since the reports of Welt and co-workers (1–8). However, evidence for a similar defect in nucleated, metabolically more active cells, remains controversial. Using an animal model of CRF, we find that ouabain-sensitive influx of the radiolabeled potassium analogue, ^{86}Rb, is diminished in both isolated adipocytes and in incubated skeletal muscle compared with results obtained from SO, pair-fed control rats. Consistent with reduced sodium pump activity, there was an increase in intracellular sodium in both adipocytes and skeletal muscle. It is unlikely that the latter abnormality was due to a generalized increase in membrane cation permeability, as ouabain-insensitive ^{86}Rb influx rates, including both furosemide-sensitive and -insensitive ^{86}Rb influx, were lower in both tissues from uremic animals. The potential adverse consequence of this defect in sodium pump function for normal cellular metabolism is suggested by the high correlation we

Table IV. Effect of Uremic Sera on ^{86}Rb Uptake and Amino Acid Transport in Normal Adipocytes and Skeletal Muscle

	Uremic sera	Control sera	P
	nmol/10^6 cells/min		
Adipocytes			
Ouabain-sensitive ^{86}Rb uptake	6.29±0.18	5.76±0.24	NS
MeAIB uptake	16.31±0.43	18.78±0.94	NS
	Uremic sera	Control sera	P
	nmol/g/min		
Epitrochlearis muscle			
Ouabain-sensitive ^{86}Rb uptake	29.85±5.66	60.22±4.97	<0.005
Ouabain-resistant ^{86}Rb uptake	13.72±0.79	36.17±2.88	<0.001

Values from six pairs of rats are given as mean±SEM.

Table V. ^{86}Rb Influx, [^{3}H]Ouabain Binding, and Intracellular Sodium Content of Epitrochlearis Muscle

	CRF	Controls	P
	nmol/g wet wt/min		
Ouabain-sensitive ^{86}Rb uptake	68.35±13.54	105.0±23.83	<0.025
Insulin-stimulated, ouabain-sensitive ^{86}Rb uptake	82.68±13.1	134.2±17.3	<0.001
Basal furosemide-sensitive ^{86}Rb uptake	2.9±1.53	13.1±2.6	<0.01
Basal ouabain- and furosemide-resistant uptake	13.01±1.9	20.9±4.15	NS
[^{3}H]Ouabain binding (*pmol/g wet wt*)	70.5±8.46	83.51±8.84	NS
Intracellular sodium content (*mmol/g wet wt*)	18.61±2.03	13.05±1.06	<0.025

All values are from at least seven pairs of rats and are given as mean±SEM.

found between ouabain-sensitive ^{86}Rb influx and amino acid uptake by the sodium-dependent transporter, system A.

Recently, the reports of Marks and Seeds (34) and of Sweadner (35), that there are at least two, and probably more, NaK-ATPase isoenzymes, have been confirmed (16, 36). One such isoenzyme, designated "alpha +," is found in several mammalian tissues including neural tissue and adipocytes, but not in erythrocytes. It appears to be responsive to insulin, at least in adipocytes, and is distinct from the "alpha −" isoenzymes that account for basal sodium pump activity. Although basal, ouabain-sensitive ^{86}Rb influx was diminished in both adipocytes and skeletal muscle, the percent rise in insulin-stimulated ^{86}Rb influx was unaffected by uremia. These findings indicate that there cannot be a disproportionate decline in the function or number of the hormonally responsive subset of sodium pumps. The results are consistent with observations in uremic humans that insulin-mediated potassium uptake is intact (15).

Our results clearly indicate that the cause of the defect in ouabain-sensitive cation flux in uremia is multifactorial, and that the cellular response is tissue specific. In the adipocyte, ouabain-sensitive ^{86}Rb was reduced in proportion to the decrease in [^{3}H]ouabain binding. In contrast, ouabain-sensitive ^{86}Rb influx in skeletal muscle was also depressed, but there was no significant decrease in [^{3}H]ouabain binding. We conclude that uremia decreased sodium pump turnover rate only in skeletal muscle even though in both tissues, intracellular sodium was higher and should have stimulated NaK-ATPase activity. Importantly, we could not induce a measurable decline in ouabain-sensitive ^{86}Rb influx in normal adipocytes by incubating in uremic sera (compared with incubating in sera from normal rats adjusted to have similar concentrations of H^+ and K^+). However, uremic sera induced a prompt decline in sodium pump activity in incubated skeletal muscle. This suggests that a factor present in serum acutely influenced the NaK-ATPase turnover rate in skeletal muscle, similar to results obtained when normal erythrocytes were incubated in uremic sera (2, 6–8). It is unlikely that the abrupt decline in ouabain-sensitive ^{86}Rb influx activity in muscle was due to withdrawal of sodium pumps from the cell membrane; a more likely explanation is that there was direct or indirect inhibition of the pump turnover rate. Interestingly, compared with incubation in serum-free media, serum from normal rats caused a decline in ouabain-sensitive ^{86}Rb flux in normal skeletal muscle and in normal adipocytes, although the effect was more marked in skeletal muscle (Table IV). Ouabain-resistant flux rates were not affected by control sera in either tissue. These data suggest that if normal tissue is incubated with normal sera for 2 h, some unidentified factor reduces ouabain-sensitive cation flux rates.

The resistance of cation transport in normal adipocytes to the acute effects of incubating in uremic sera suggests that the mechanism causing reduced sodium pump activity is different from that of muscle. Despite the equal weights and nearly equal growth rates of the pair-fed, CRF, and SO rats, there was a significant decrease in epididymal fat pad weight and an increase in plasma NEFA in uremic rats. These findings are consistent with stimulation of triglyceride lipolysis similar to that occurring in fasted normal rats; adipocytes from fasted, normal rats also exhibit a decline in ouabain-sensitive ^{86}Rb influx and [^{3}H]ouabain binding (data not shown). Thus, the number of NaK-ATPase units in the adipocyte membrane may be closely regulated by insulin and/or other hormonal regulators. Finally, adipocyte NaK-ATPase turnover was relatively unaffected by chronic uremia or by incubation in uremic sera. In contrast, [^{3}H]ouabain binding was unchanged in skeletal muscle obtained from uremic animals, but the turnover rate was markedly reduced. This condition could be rapidly acquired by incubating normal muscle in uremic sera.

Note that maximal ouabain binding may not be achieved using our methods since we used a lower phosphate concentration and did not add vanadate to the incubation media (29). However, we compared [^{3}H]ouabain binding in paired rats using the same technique to demonstrate a difference between uremic and control rats in adipocytes but not muscle. Thus, the mechanism for changes in cation transport in adipocytes in uremia differs from that in muscle.

Welt and associates showed that, at least in a subgroup of uremic patients, the activity of erythrocyte NaK-ATPase was decreased, and, as a consequence, intracellular sodium concentration was elevated (1). Later, they demonstrated that the defect in ion transport can be induced in normal human erythrocytes by incubating in uremic sera (2). Subsequent studies have produced conflicting results: erythrocyte NaK-ATPase activity is reportedly decreased (1, 2, 5, 6), normal (4), or even decreased (3), and intracellular sodium is raised, normal, or decreased, respectively, in CRF. Several authors have been unable to reproduce a transport defect by incubating normal erythrocytes in uremic sera (7, 37), and it is controversial whether the pump site number per erythrocyte is normal (6) or decreased (5) in uremia, or whether any defect is corrected by hemodialysis. Although there seems to be agreement that some

proportion of CRF patients exhibit altered active cation transport, these controversial results may be explained, at least in part, by characteristics inherent to erythrocytes. Mature erythrocytes cannot synthesize NaK-ATPase units, and the pump number is affected by cell age (38). Since their half-life is reduced in CRF and since many CRF patients receive transfusions, heterogeneity in age of erythrocytes makes interpretation of ion flux studies in uremia difficult.

Evidence for abnormalities of cation flux pathways in erythrocytes, other than the sodium pump, also remains controversial. Increased passive permeability of the red cell membrane to sodium ions has been reported (7), as well as normal (4) or increased (39) Na/Li countertransport. Recent reports have described another abnormality in the regulation of cation flux in uremic erythrocytes, reduced Na, K co-transport (4, 39). Our data confirm that ^{86}Rb flux through this pathway in both adipocytes and muscle is substantially reduced in uremia. The mechanism is unknown, but chronic hyperkalemia has been suggested as a potential cause for reduced Na, K co-transport in the erythrocyte (40), and furosemide-sensitive K efflux in erythrocytes is inversely correlated in at least one other disease characterized by abnormal plasma K levels, Bartter's syndrome (41). That we found markedly reduced Na, K co-transport rates in adipocytes and muscle in hyperkalemic, uremic rats (Table I) supports the hypothesis that this pathway is important in K homeostasis.

It is very unlikely that a single mechanism accounts for the changes in active cation transport we observed. Although intracellular sodium is an important regulator of sodium pump turnover rate in vivo, in adipocytes and skeletal muscle from uremic rats, NaK-ATPase turnover rates were either normal or low in spite of an increase in cell sodium. This indicates that the enzyme turnover rate was affected independently of the cell sodium concentration, and/or that the enzyme affinity for sodium was altered by uremia. Since the isolated, reconstituted enzyme has a pH optimum of 7.4 (42), the chronic metabolic acidosis characteristic of uremia might also affect sodium pump activity in vivo, but is unlikely to explain our results, as all and incubations were performed at pH 7.4. Vanadate, acting as a nonspecific inhibitor of several cation transporting ATPases within cells, may accumulate in CRF and could affect active sodium transport (43). However, its pathophysiologic importance remains unproven.

Chronic endocrine abnormalities could affect sodium pump number. Both experimental (44) and human CRF (45) are associated with hypothyroidism, which can affect sodium pump number directly. It seems unlikely that hypothyroidism was a major factor in our study because there was no reduction in [^{3}H]ouabain binding in muscle, a tissue known to be responsive to thyroid hormones (46). Mineralocorticoids can increase sodium pump number in several tissues, most notably ion-secreting epithelia, probably because intracellular sodium is increased (47). Therefore, it is unlikely that changes in circulating mineralocorticoids account for our observations, which were not linked to an increase in [^{3}H]ouabain binding.

In conclusion, uremia induces profound alterations in cellular ion transport in many tissues as well as in erythrocytes (48). The defects cannot be explained by a single mechanism; both a reduction in ouabain binding and basal enzyme turnover rates occur, but the cellular response to uremia appears to be tissue specific. Despite a defect in basal, active cation transport, the insulin-mediated increase in NaK-ATPase activity remains unimpaired while Na, K co-transport rates are markedly depressed in both adipocytes and skeletal muscle. Circulating factors, as well as chronic cellular adaptations to the uremic state, both may play a role in the pathophysiology of ion transport defects in CRF.

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