Effect of Chronic Renal Failure on Na,K-ATPase α1 and α2 mRNA Transcription in Rat Skeletal Muscle

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Abstract

Previous studies have suggested that an alteration in the expression of the Na,K-ATPase of muscle may be an important determinant of enhanced insulin sensitivity in chronic renal failure. Therefore, in the present studies we have examined the effect of uremia on the Na,K-ATPase α isoforms in skeletal muscle, at the level of mRNA expression and enzymatic activity. The activity of the sodium pump, as measured ouabain-sensitive 86Rb/K uptake in soleus muscle, revealed a reduction in the activity in uremia, related to the increment in plasma creatinine values. The decrement in 86Rb uptake by the rat soleus muscle of experimental animals was associated with changes on Na,K-ATPase gene product. Northern analysis of mRNA revealed isoform-specific regulation of Na,K-ATPase by uremia in skeletal muscle: a decrease of ~ 50% in α1 subunit Na,K-ATPase mRNA, as compared to controls. The decrement in α1 mRNA correlates with the decreased activity of the Na,K-ATPase in uremia, under basal conditions and with the almost complete inhibition of the Na,K-ATPase, of uremic tissue by a concentration of 10^-5 M ouabain. Although the activity of the α2 isoform pump was not modified by uremia, the 3.4-kb message for this enzyme was increased 2.2-fold; this discrepancy is discussed. Altogether these findings demonstrate that the defective extrarenal potassium handling in uremia is at least dependent in the expression of α1 subunit of the Na,K-ATPase. (J. Clin. Invest. 1991. 88:2137-2141.) Key words: Na,K-ATPase gene • rat soleus • uremia • potassium homeostasis

Introduction

Skeletal muscle plays an important role in the clearance of a potassium load (1) and a decreased muscle uptake of potassium contributes to the maintained hyperkalemia observed after meals in chronic renal failure. In fact, we and others (2, 3) have shown a defect in extrarenal potassium disposal in atazotemia, due to a less active sodium pump in skeletal muscle (4, 5); whereas an increment in the number of sodium pumps of the secretory epithelia has been described in uremia (6).

As it is well established, cell potassium is mainly controlled by the Na,K-ATPase. The Na,K-ATPase is the intrinsic membrane protein responsible for pumping Na+ and K+ against their concentration gradients. The enzyme is composed of a catalytic subunit, α, and a smaller, glycosylated subunit, β, whose function is unknown. Two isoforms of the α subunit of the enzyme, known as α1 and α2 (+), were first discovered by Sweadner (7). Recently, a third, brain-specific, form designated α3 was discovered using a rat brain cDNA library. Even though all three forms are ~ 85% homologous, they possess important differences (8). The α2 and α3 forms have a high affinity for the cardiac glycoside ouabain (K0.5 = 10^-6 – 10^-7 M) whereas α1 is relatively resistant to the drug (K0.5 = 10^-4 – 10^-5 M). It has also been shown in both rat adipocytes and rat brain synaptosomes that the α1 and α2 isoforms have different sodium affinities, with K0.5 values of 12-17 mM and 36-52 mM, respectively. Insulin activation is mediated by lowering the K0.5 for sodium of the α2 isoform (9-10).

Little is known about the regulation of Na,K-ATPase isoforms in skeletal muscle in advanced chronic renal failure. We have recently shown that the addition of oral glucose to a potassium load was more effective in the translocation of potassium to the intracellular compartment in uremic animals (4). Further, in vitro studies indicated that insulin caused a relatively greater stimulation of ouabain-sensitive 86Rb uptake in the uremic tissue as compared to control soleus muscle (4). These results suggest changes in the expression of the Na,K-ATPase. Thus, this study was designed to determine the molecular basis of the enhanced insulin sensitivity in the uremic skeletal muscle. We examined the expression of two isoforms of the Na,K-ATPase, the α1 and α2, in soleus muscle fibers from control and chronic renal failure rats. The results suggest that tissue-specific alterations in the expressions of these two isoforms may be the major mechanism for the decreased potassium uptake in uremic muscle tissue as compared to controls.

Methods

Animals. Male Sprague-Dawley rats (~ 250 g) fed a standard rat chow diet were used. Chronic renal failure was induced by ligating branches of the artery to infarct about ¼ of the kidney; a right nephrectomy was performed after 4 d. The experiments were done 3–4 wk after surgery. Plasma creatinine, blood arterial gases, pH, and plasma potassium were controlled at the beginning of each study.

86Rb transport in skeletal muscle. The muscle was removed from control and uremic animals and then groups of fibers weighing 8–15 mg were incubated in separate vials.

The muscular fibers were washed for 15 min in 2 ml of KRB containing (mM) 4.2 KCl; 1.19 KH₂PO₄; 120 NaCl; 25 NaHCO₃; 1.2 MgSO₄; 1.3 CaCl₂; 5 d-glucose (pH = 7.4). The buffer was gassed with 95% O₂ and 5% CO₂. Thereafter, the muscles were preincubated for 30 min in KRB in the presence or absence of 10^-2 M or 10^-3 M ouabain. Finally, the muscles were incubated for 20 min in KRB containing 86Rb (0.1 μCi/ml) in the presence or absence of 10^-2 M or 10^-3 M ouabain. The reaction was stopped by transferring the muscle into iced KRB; the muscles were washed in cold buffer and blotted. Radioactiv-
ity of the samples was determined by the Cerenkov radiation in a liquid scintillation counter.

**RNA isolation and Northern blot analysis.** Upon killing of rats, soleus muscles were removed and immediately processed. Total cellular RNA was extracted from ~250 mg of soleus skeletal muscle using the guanidinium thiocyanate (11). RNA was electrophoresed on 2.2 M formaldehyde-agarose gels (12), blotted and fixed onto nylon membranes, and then hybridized to cDNA probes. The α1 and α2 cDNA was provided to us by Nelson Ruiz-Opazo, Boston University.

Isoform-specific restriction endonuclease Bam HI fragments were prepared from these probes. The α1 and α2 cDNA probes were labeled with digoxigenin-d UTP, using the nonradioactive DNA labeling and detection kit from Boehringer Mannheim Corp., Indianapolis, IN.

Membranes were hybridized to the corresponding probes for 5–6 h at 42°C in 50% formamide in 5× SSC (0.3 M NaCl, 0.03 M sodium citrate) pH 7.0 with 5% of blocking reagent, according to instructions recommended by the kit. Then the membranes were washed at high stringency twice in 2× SSC with 0.1% SDS for 1 or 2 min each at room temperature, then twice for 15 min each in 0.1× SSC with 0.1% SDS at 68°C to assure the specificity of the Northern. The hybridized membranes were detected immediately by the enzyme-linked immunosassay system. The amount of mRNA in each sample was quantified by computer scanning densitometry analysis, comparing the intensity of the sample to control rat mRNA samples or to the internal standard GAPDH mRNA (glyceraldehyde-3-phosphate dehydrogenase) for each processed skeletal muscle (12). The specific cDNA probe (p RGAPDH-13) was kindly provided by P. Carvallo, NIH.

DNA was determined on 250 mg of muscle using the spectrophotometric method of Labarca and Paigen (14). Total cellular RNA was determined by spectrophotometry (12) and protein content by the Harree method (15).

**Quantification dots on Northern blots.** Color dots were quantified with a scanning densitometer (Geniscan, Fremont, CA) interfaced to an IBM PC 286 computer with a GS-4500 scanner and specially written C program.

Significance was determined with Student's t test (paired) and accepted at the P < 0.01 level.

**Results**

**Characteristics of the experimental rats.** The characteristics of experimental animals, 3–4 wk after partial nephrectomy (3/4), were similar to those previously described (4). The experimental rats have body weights and blood pH values similar to control rats. The nephrectomized rats with plasma creatinine values above 1.0 mg/dl were considered with established renal insufficiency.

To evaluate the influence of azotemia in the Na,K-ATPase activity of the skeletal muscle we measured ouabain sensitive ⁴⁶Rb uptake by the soleus muscle of rats with different degrees of renal insufficiency, as determined by plasma creatinine values. Fig. 1 includes the results on ⁴⁶Rb uptake of the isolated soleus fibers. As shown in the figure, there is an inverse relationship between both parameters: as plasma creatinine increases, the activity of the sodium pump reaches very low values. These results confirm a defect in the Na,K-ATPase activity in chronic renal failure and are indicative that this defect is related to the degree of renal insufficiency.

**Regulation of Na,K-ATPase mRNAs in skeletal muscle.** Next, we examined the expression of the two α isoforms of the Na,K-ATPase known to be present in muscle, from control and uremic rats. In this study, and subsequent experiments, mean plasma creatinine values were 0.4±0.1 mg/dl and 1.6±0.4 mg/dl, respectively. Preliminary results were indicative of dramatic change in both isoforms mRNA with uremia.

Figure 1. Correlation of plasma creatinine with ouabain-sensitive ⁴⁶Rb uptake by isolated soleus muscle fibers. Values represent individual rats, either sham operated animals (n = 3) or rats with different degrees of chronic renal insufficiency as measured by plasma creatinine concentration (n = 11). ⁴⁶Rb uptake was measured with or without 10⁻⁵ M ouabain, as described in Methods, in triplicate samples. The correlation between these two parameters is highly significant (r = 0.83; P < 0.01).

Therefore, we assayed muscle weight, content of DNA, RNA, and protein. Table I shows the results: muscle weight did not vary significantly between both groups, nor did nucleic acids or protein content.

Next, we examined the effect of chronic renal failure on the relative amount of the α1 and α2 isoforms mRNA by comparing the results with GADPH mRNA, used as an mRNA marker (13). As shown in the Northern blots of Fig. 2 there are no significant differences in GADPH mRNA content between control and the experimental groups. Therefore, we used GADPH mRNA as a reference to measure the relative abundance of α1 and α2 isoforms mRNA, as compared to the marker. The 3.4-kb α2 transcript is the major one in muscle, whereas in brain the 5.3-kb species is the predominant form; nevertheless, both transcripts encode the same protein.

Quantification of the levels of Na,K-ATPase α isoforms mRNA was made in separate samples for α1 and for 3.4-kb α2. The results obtained in several experiments are shown in Fig. 3; mean values±SE for each isoform are expressed as the relative color intensity of each α isoform dot blot compared to GADPH. As shown in Fig. 3 A, α1 mRNA levels for the uremic tissue decreased significantly to one-half of the control values. When total RNA isolated from the skeletal muscle of uremic rats was probed with the α2-specific cDNA probe, the amount of the 3.4-kb transcript was found to be increased over those

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<tr>
<td>Soleus weight, mg</td>
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<tr>
<td>Total DNA μg/g tissue</td>
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<td>Total protein μg/g tissue</td>
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Values are mean±SE; n = 6 for all groups.
present in normal rats (Fig. 3 B). Since the size of the probes was different no direct comparison between the amount of a1 vs. a2 was made (a1 = 1,700 bp; a2 = 700 bp).

Data derived from this analysis suggest that the two known a isoforms expressed in skeletal muscle are modified with uremia. At least part of the altered Na,K-ATPase activity in skeletal muscle is at the level of the mRNA. It cannot be determined from these experiments whether the pretranslational regulation of the sodium pump expression is at the level of initiation of transcription or stability of the mRNA. These possibilities are currently under investigation.

**Comparison of ouabain sensitivity in skeletal muscle of uremic versus control rats.** From the $^{86}$Rb/K pumping data it has been established that in the range of 10$^{-6}$--10$^{-3}$ M ouabain, a2 is ~90% saturated with ouabain, whereas a1 has ~10% bound (9). Thus, we used a concentration of 10$^{-3}$ M ouabain to inhibit a2 isoform almost exclusively, while at 10$^{-2}$ M ouabain complete inhibition of both a1 and a2 isoforms should be obtained. Therefore, we have studied the activity of the enzyme as an Rb$^+$ pump, under normal conditions by soleus muscle in the presence of the above indicated concentrations of ouabain (Table II). As shown in Results, the soleus muscle of control rats, incubated in normal buffer, have 43% of a2 mediated uptake; this value is similar to that recently described by Hsu and Guidotti for intact soleus fibers (16). However, in the uremic tissue the pumping activity of a2 isoform is increased as compared to a1 mediated transport. Nevertheless, the absolute values of the a2 isoform were 75 mmol/min per g in both normal and uremic muscles, and the enhanced sensitivity to ouabain is mainly due to the decrement a1 isoform from 90 to 20 mmol/min per g. This last value is consistent with the decrement in a1 mRNA observed in the muscle of chronic renal failure rats.

**Discussion**

One of the most prominent transport proteins in nearly all animal cells is the Na,K-ATPase. Under physiological conditions, this enzyme mediates the transport of three Na ions out of the cell and two K ions into the cell per ATP molecule that is split. By this active transport, transmembranes gradients for Na and K are maintained that are essential for a large variety of cellular functions. In epithelial cells, the pump is involved in potassium secretion and whole body potassium homeostasis. It is known that in chronic renal failure, as renal mass is diminished, there is a remarkable adaptive increase in potassium excretion by the remaining functioning nephrons (6). It has also been reported that in uremia, there is increased gastrointestinal potassium secretion resulting in an elevated stool potassium content. Also, during chronic potassium load there is an increment in the number of Na,K-ATPase units in the kidney and colonic tissue with a concomitant increased secretion of potassium (17, 18). As indicated above (6), in both uremic tissues, there is an adaptive increment in the number of Na pumps in epithelial tissue, whereas intracellular Na is elevated

![Figure 2. Soleus muscle Na,K-ATPase α isoforms mRNA levels and GAPDH mRNA in response to chronic renal failure. Northern blot analysis of skeletal muscle RNA (25 μg/lane) from individual rats was performed as indicated under experimental procedures. These blots are representative of five separate experiments.](image)

**Table II. Effect of Ouabain in the Muscle Na,K-ATPase Activity**

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<th>Ouabain-sensitive $^{86}$Rb uptake</th>
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<td>Control CRF</td>
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<tr>
<td>Basal</td>
<td>161.7±8.0 93.3±7.5*</td>
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<tr>
<td>Plus 10$^{-3}$ M ouabain</td>
<td>91.3±5.8 18.2±4.4</td>
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<tr>
<td>Percent inhibition</td>
<td>43.2±3.3 81.8±4.1*</td>
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Basal activity of the pump was measured as the difference of total activity minus 10$^{-2}$ M ouabain. $^{86}$Rb uptake was measured at 20 min in parallel samples. (n = 7). *P < 0.001.

![Figure 3. Relative α subunits mRNA abundance in rat soleus muscle.](image) The dots obtained, as shown in Fig. 2, were densitometrically scanned and quantified. An arbitrary value of 1 was given to GAPDH mRNA abundance of each animal. The data are presented as the mean±SE of a1 and a2 subunits in control (c) and uremic rats (n = 6 for each mean value).
in erythrocytes, leukocytes, and muscle cells of patients with chronic renal failure (19). In fact, as shown here, and in previous work (4), a 50% reduction of the sodium pump activity was found in the uremic muscle.

Several forms of rat Na,K-ATPase have been described that are distinct with respect to sodium affinity (10) regulation by thyroid hormones (20), sensitivity to cardiac glycosides (9) and insulin (10). These functional differences have been correlated with the presence of two or more isoforms of the α subunit. The tissue distribution studies of the multiple forms of rat Na,K-ATPase indicate that α1 is predominantly present in kidney epithelial tissue, whereas a greater proportion of α2 is present in the skeletal muscle (21).

Specific cDNA probes have been developed for the α isoforms of rat Na,K-ATPase (22). Therefore, the tissue-specific expression of mRNAs encoding the α isoforms in the rat skeletal muscle were assessed in the present work. These studies demonstrated that in uremia there is a change in the proportional expression of the α mRNA isoforms in skeletal muscle. In fact, in the uremic tissue we found a diminished α1 transcript as compared to those present in normal skeletal muscle (approximately one-half). By contrast, as illustrated in Fig. 2B, the 3.4-kb mRNA encoding the α2 subunit in the uremic tissue is more abundant than control: the diminished α1 mRNA is consistent with the reduced pumping activity of the uremic skeletal muscle. In fact, as shown by Lytton et al. (9), while α1 is pumping at about 1/2 its maximum capacity under basal conditions, α2 is pumping at only 1/20 of its capabilities. Therefore a decrement in α1 isoform could account for the decreased uptake of 86Rb/K in the uremic muscle. Nevertheless, there is no such relationship in the case of α2 isoform. Even though we have not measured intracellular electrolytes, it is known that in uremia cell sodium is increased (23, 24); the increment in cellular sodium would favour the α2 isoform activity (10). Recently, Hsu and Guidotti (24) also described a divergency between the amount of the α2 isoform and the transcriptional regulation of the α2 gene in the skeletal muscles of hypokalemic rats. One possibility for the lack of correlation between the amounts of mRNAs and active enzyme is that the transcripts are not translated (25). Another possibility is that the α2 transcripts are translated and the α2 polypeptides do not mature into functional enzyme due to an insufficient amount of the β chain (26, 27). A third possibility is that the α2 transcripts are translated, and the polypeptide is broken down more rapidly than usual. Nevertheless, further studies need to be directed to measure the number of units of each isoform of the Na,K-ATPase by specific antibodies (28), before final conclusions could be drawn.

It should be taken into account that we have measured the pumping activity of the rat soleus muscle under normal conditions; as it has been shown by others (16, 29) the activity of the (Na+, K+) pump in isolated fibers incubated in a normal buffer is a fraction of the total possible activity. Due to the low and variable enzyme recovery (0.2–8.9%) we have not studied the pump activity in isolated sarclemma; such subcellular preparations may give misleading data on the pump activity of skeletal muscle cells, as pointed out by Hansen and Clausen (29).

These results provide evidence that uremia induces genespecific modulation in skeletal sodium pump. Furthermore, the modulation of Na,K-ATPase is isoform-specific and the downregulation of the α1 mRNA may represent a deinduction of gene expression elicited in response to stimuli from some intracellular changes that occurred in chronic renal failure. A simultaneous upregulation of α2 subunit mRNA expression could be a related phenomena, but no evidence exists at the present time to support this hypothesis. Herrera et al. (22) have shown a 2- to 3-fold increase in α1 mRNA and a 3- to 15-fold decrease in α2 mRNA in the aorta and left ventricle of hypertensive animals.

The changes in amounts of mRNA could represent transcriptional regulation, although changes in mRNA stability cannot be ruled out.

Determination of the mechanisms by which specific Na,K-ATPase α subunits genes are modulated in uremia may provide insight into the molecular mechanisms involved in the defective extrarenal handling of potassium.

Acknowledgments

We would like to thank Dr. Juan Olate for critical reading of the manuscript, Ms. Lupe Ferrer who coordinated the laboratory work, and Ms. Rosario Flores for her expert technical assistance.

This work was supported by a grant from Fondecyt.

References


