Skeletal Muscle Na,K-ATPase α and β Subunit Protein Levels Respond to Hypokalemic Challenge with Isoform and Muscle Type Specificity*

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During potassium deprivation, skeletal muscle loses K+ to buffer the fall in extracellular K+. Decreased active K+ uptake via the sodium pump, Na,K-ATPase, contributes to the adjustment. Skeletal muscle expresses α1, α2, β1, and β2 isoforms of the Na,K-ATPase aβ heterodimer. This study was directed at testing the hypothesis that K+ loss from muscle during K+ deprivation is a function of decreased expression of specific isoforms expressed in a muscle type-specific pattern. Isoform abundance was measured in soleus, red and white gastrocnemius, extensor digitorum longus, and diaphragm by immunoblot. α2 expression was uniform across control muscles, whereas α1 and β1 were twice as high in oxidative (soleus and diaphragm) as in fast glycolytic (white gastrocnemius) muscles, and β2 expression was reciprocal: highest in white gastrocnemius and barely detectable in soleus and diaphragm. Following 10 days of potassium deprivation plasma K+ fell from 4.0 to 2.3 mM, and there were distinct responses in glycolytic versus oxidative muscles. In glycolytic white gastrocnemius α2 and β2 fell 94 and 70%, respectively; in mixed red gastrocnemius and extensor digitorum longus both fell 60%, and β1 fell 25%. In oxidative soleus and diaphragm α2 fell 55 and 30%, respectively, with only minor changes in β1. Although decreases in α2 and β2 expression are much greater in glycolytic than oxidative muscles during K+ deprivation, both types of muscle lose tissue K+ to the same extent, a 20% decrease, suggesting that multiple mechanisms are in place to regulate the release of skeletal muscle cell K+.

Mammals closely control extracellular fluid (ECF) and intracellular fluid (ICF) potassium within a very narrow range since the ratio of the ICF potassium (K+) to ECF K+ is the primary determinant of resting membrane potential. This is especially important in excitable tissues such as the heart, where disturbances in resting membrane potential can compromise cardiac contractility and become life threatening (1). ECF K+ is maintained by the interplay of two key organ systems: the kidneys control K+ excretion by actively reabsorbing or secreting K+ (1), while skeletal muscle, which contains the largest intracellular pool of K+ in the body (75% of total intracellular K+), adjusts ECF K+ by regulating K+ transport between the ICF and ECF compartments (2, 3).

During dietary K+ deprivation, K+ output can exceed input, leading to a fall in plasma potassium, termed hypokalemia (1). In response, skeletal muscle selectively loses ICF K+ into the ECF. This loss buffers the fall in ECF K+, minimizing the hyperpolarization of cell membranes due to the increased ratio of ICF K+ to ECF K+. There is a concomitant decrease in the number of active sodium pumps (Na,K-ATPase) in skeletal muscle plasma membranes, postulated to be the primary event leading to the transfer of ICF K+ into the ECF (4).

Na,K-ATPase (EC 3.6.1.37) is an intrinsic membrane-bound enzyme found in eukaryotic cells (5). For each molecule of ATP hydrolyzed, Na,K-ATPase transports two K+ and three Na+ out of the cell, generating electrical and chemical gradients of Na+ and K+ across the plasma membrane (7). Na,K-ATPase is a heterodimer, consisting of a catalytic α subunit (Mα ~ 112,000) and a glycosylated β subunit (Mβ ~ 35,000). To date three isoforms of each subunit have been identified (5, 6): α1, α2, α3, β1, β2, and β3 (β3 detected only in amphibians (5)). A putative fourth α subunit, α4, identified in rats and humans, appears isolated to the testis (8). Subunit isoforms expression is tissue-specific (6); skeletal muscles of adult rats express α1, α2, β1, and β2 mRNA and protein (9, 10). Hundal et al. (9) reported evidence of muscle type specificity for expression of β: β1 predominating in slow oxidative fibers and β2 in fast glycolytic fibers.

Our previous studies provided evidence for isoform-specific regulation in response to hypokalemia: α2 protein and mRNA, but not α1 or β1, decreased in hindlimb skeletal muscle of hypokalemic rats (11). Since skeletal muscle is a heterogeneous mix of skeletal muscle tissue types that vary greatly in both metabolic and contractile characteristics (12), we aimed to investigate whether the response to hypokalemia was both isoform- and muscle type-specific.

EXPERIMENTAL PROCEDURES

Animals and Diets—To establish the relative levels of Na,K-ATPase isoform expression in a panel of distinct skeletal muscles, male Sprague-Dawley rats (300 g) were anesthetized with 0.2 ml of sodium pentobarbital/100 g of body weight. Muscles were removed, frozen in liquid nitrogen, and stored at −80 °C. To provoke hypokalemia, Sprague-Dawley rats, approximately 8 weeks of age, were placed on a K+-deficient diet (Harlan Teklad, TD 88239, Madison, WI) for 10 days and paired to a control group of rats fed a comparable diet with K+ restored (Harlan Teklad, TD 88238).

Preparation of Tissue Homogenates—Skeletal muscle was weighed, minced into small pieces, and homogenized at an approximate 1:20 (w/v) ratio for 45–60 s using a Polytron homogenizer (Brinkmann Instruments) in a buffer containing 5% sorbitol, 25 mM histidine-imi-
diazole (pH 7.4). 0.5 mM Na2EDTA, and proteolytic enzyme inhibitors: 0.5 mM phenylmethylsulfonyl fluoride, 1 mM leupeptin, and 1 mM 4-aminobenzenamidine dichloride. Protein concentration was determined by the method of Lowry et al. (13) after trichloroacetic acid precipitation.

**Immunoblot Analysis**—A constant amount of homogenate protein (100 μg for α subunit analysis, 50 μg for β subunit analysis) was resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described previously (14). The gel was blotted electrophoretically onto nitrocellulose membranes or Immobilon-P membranes and then incubated overnight with one of the following antibodies: McK1 (1:200), a monoclonal specific for α1 (15), provided by K. Sweadner (Harvard Medical School); C466.6 (1:100), a monoclonal specific for α1 (16, 17), provided by M. Kashgarian (Yale Medical School); McE2, a monoclonal specific for α2 (15) provided by K. Sweadner (Harvard Medical School); anti-HERED (1:100), a polyclonal specific for α2, and anti-TED (1:200), a polyclonal specific for α3 (18), both provided by T. Presley (Texas Technical University); anti-β1 FP (1:500), a polyclonal against β1 raised in rabbit according to Shyjan and Levenson (19); anti-α1 (1:1,000), a polyclonal against β2 (Upstate Biotechnology, Lake Placid, NY); or SpETβ2 (1:2,000), a polyclonal against human β2 (20), provided by P. Martín-Vasallo (Universidad De La Laguna, Spain). Blots probed with monoclonal antibodies were incubated for 2 h with rabbit anti-mouse IgG secondary antibodies (1:2,000). To ensure that changes in isoform abundance did not reflect loading artifacts a subset of blots was probed additionally with a mouse monoclonal specific for muscle calcesteuin, VIII D1 (21). All blots were processed as described previously (14), probed with 125I-protein A, and visualized by autoradiography using Kodak XAR-5 film and DuPont Cronex LightChem reagents. Protein was detected using the enhanced chemiluminescence (ECL) detection system (Amer sham) using Kodak XAR-5 film. Multiple exposures of autoradiograms and assaying samples at multiple concentrations confirmed that signals were in the linear range, as established previously (22).

**Immunoprecipitation of αβ Complexes**—Immunoprecipitation of non-denatured αβ heterodimers was accomplished using techniques reported previously (23). Briefly, skeletal muscle homogenate (300 μg of protein from 3–10 g/ml) was incubated for 1 h at 4°C in a lysis buffer, at an approximate 1:3 (v/v) ratio, containing 50 mM Tris-buffered saline at pH 8.0, 1% Nonidet P-40, 1% bovine serum albumin, and proteolytic enzyme inhibitors: 0.2 units/ml aprotinin, and 1.0 mg/ml phenylmeth ylsulfonyl fluoride. The samples were centrifuged at 250 × g for 10 min, and the supernatant lysate was precipitated with mouse serum-agarose beads and nonspecific goat IgG-agarose beads at an approximate 1:5 bead to supernatant (v/v) ratio, or 4°C for 1 h with gentle rocking. Samples were centrifuged at 2,500 × g for 30–45 s, the supernatant incubated with 100 μl of 1:1,000 (24) obtained from A. Quaroni (Cornell University, Ithaca), at a approximate 1:5 antibody to supernatant (v/v) ratio, at 4°C for 4 h with gentle rocking. This monoclonal antibody recognizes underdenatured β, thus it can be used to immunoprecipitate αβ heterodimers. Goat anti-mouse IgG-agarose beads at 1:5 beads were added to supernatant and the samples incubated at 4°C for 1 h with gentle rocking to isolate αβ complexes. After washing, the precipitate was heated to 90°C for 5 min to dissociate antibody-antigen complexes and α and β subunits. Finally, the samples were resolved by SDS-PAGE, subjected to immunoblot analysis, probed with α and β isoform-specific antibodies, and visualized by the CDP-StarChem chemiluminescence detection system (Tropix, Bedford, MA) for α subunits or 125I-protein A for β subunits.

**Plasma and Cellular K**^+** Concentrations**—Plasma K^+ and muscle K^+ were measured by flame photometry. Specific muscles and cardiac ventricles were quickly dissected, flash frozen in liquid nitrogen, and stored until assayed. They were later thawed, blotted lightly to remove adherent ECM or blood, homogenized in approximately 1:50 (w/v) 0.3 M trichloroacetic acid for 1–2 min using a Tissuemizer (Tekmar, Cincinnati, Ohio), and centrifuged at 2,000 rpm for 20 min to remove cell debris. K^+ and Na^+ were measured using a Radiometer FLM 3 flame photometer (Radiometer, Copenhagen, Denmark), with lithium as internal standard (25).

**N-Glycanase Treatment**—Sugars were removed from β subunits with peptide N-glycosidase F (N-glycanase, Genzyme Corp., Cambridge, MA). Crude skeletal muscle homogenate (100 μg/sample) was centri fuged at 48,000 rpm for 60 min. The pelleted crude membranes were incubated with N-glycanase as described in Azuma et al. (22) to yield a final protein/enzyme ratio of 2.5 units of N-glycanase/100 μg of protein.

**Quantitation**—Autoradiograms were scanned and quantitated using the Bio-Rad GS670 Imaging Densitometer and software. All data are expressed as means ± S.E. Significance was assessed by a two-tailed Student's t test for unpaired samples, and differences were regarded as significant at p < 0.05.

**Materials**—Chemicals were reagent grade, spectroquality, or electrophoresis purity reagents. SDS-PAGE reagents were from Bio-Rad. Leupeptin, phenylmethylsulfonyl fluoride, 4-aminobenzenamidine dichloride, SDS-PAGE molecular weight standards, and antibodies coupled to aga rose beads were purchased from Sigma. Nitrocellulose transfer mem brane was from Micron Separations. Immobilon-P transfer membrane was obtained from Millipore. 1-Protein A was from ICN. Rabbit anti-mouse secondary antibody was from Calbiochem. Anti-mouse and anti-rabbit alkaline phosphatases were from Tropix. Anti-calcesteuin antibody (IgG2b) was from Affinity Bioreagents.

**RESULTS**

**Immunoblot Analysis of Na,K-ATPase Subunits in Specific Skeletal Muscle**—Immunoblot analysis was used to determine the pattern of Na,K-ATPase α and β subunit expression in a panel of skeletal muscles: soleus, 87% slow oxidative fibers, with some fast glycolytic-oxidative fibers; red gastrocnemius (RG), a mixed muscle type, approximately 30% slow oxidative fibers, 62% fast glycolytic-oxidative, and 8% fast glycolytic; extensor digitorum longus (EDL), a classically fast muscle type, both fast glycolytic-oxidative (42%) and fast glycolytic (56%), with only 2% slow oxidative fibers; white gastrocnemius (WG), very fast glycolytic muscle (84%) with some fast oxidative fibers; and diaphragm, a mixed muscle type, approximately 40% slow oxidative, 27% fast glycolytic-oxidative, and 34% fast oxidative (26–29).

**Typical autoradiograms of rat skeletal muscle homogenates probed with isoform-specific antibodies against α1, α2, β1, and β2 are shown in Fig. 1.** Whereas α1 and α2 are expressed in all skeletal muscles, β1 was very low in WG and β2 barely detected in soleus and diaphragm. The relative levels of expression of Na,K-ATPase subunits are summarized in Fig. 2. The α1, α2, and β1 subunit abundance values were normalized to abundance in soleus muscle, defined as 1. β2 was normalized to abundance in RG. α1 is 2–4-fold higher in the oxidative rich soleus and diaphragm than in glycolytic WG, EDL, and RG. α2 has much less variability than α1, is lowest in WG, and twice as high in diaphragm. β1 parallels the pattern of α1 expression: higher in both fast and slow oxidative rich fibers (soleus, diaphragm, and RG), and barely detected in WG. β2 expression, reciprocal to that of β1, is highest in muscles containing fast glycolytic fibers (WG, EDL, RG) and is below detection in...
oxidative dominated muscle (soleus and diaphragm). Consistent with previous observations that no α3 mRNA is expressed in mature rat skeletal muscle (6, 10), α3 protein was not detected in any individual skeletal muscle. These result suggest that α2β2 is the predominant heterodimer in fast glycolytic muscle (WG) and that both α1β1 and α2β1 heterodimers are expressed in muscles rich in oxidative fibers (soleus and diaphragm). Whether α2β1 and α2β2 are limited to oxidative fibers and fast glycolytic fibers, respectively, cannot be resolved in mixed muscle homogenates.

Characterization of αβ Heterodimers in Soleus—The detection of α1, α2, and β1 but not β2 in soleus suggested that both α subunits form heterodimers with β1. To test this hypothesis an antibody to β1 was used to immunoprecipitate αβ heterodimers. Immunoblot analysis of the immunoprecipitates was used to determine whether both α1 and α2 were precipitated with the anti-β1 antiseraum; samples of brain and kidney homogenate were assayed directly by immunoblot in parallel as positive controls. Typical autoradiograms of these immunoblots are shown in Fig. 3. As shown in panel A, the IEC 1/48 monoclonal antibody immunoprecipitated β1 from both brain and kidney, but did not immunoprecipitate β2, demonstrating that the antibody is β1-specific. As shown in panel B, the antibody coimmunoprecipitated α2 along with β1 from soleus muscle, evidence that β1 forms heterodimers with α2. α1 was also detected in these immunoprecipitated samples from soleus (not shown), evidence for α1β1. A corollary to the findings that both α1 and α2 form heterodimers with β1 and that β2 is not detected in this muscle is that β1 abundance may provide a measure of total sodium pump αβ heterodimer pool size in this muscle.

Effect of Low K⁺ Diet on Na,K-ATPase Isoform Expression in Specific Skeletal Muscles—In 8-week-old rats placed on a K⁺-deficient diet for 10 days, serum K⁺ fell from 4.0 ± 0.14 mm to 2.3 ± 0.08 mm (control animals, n = 12; low K⁺ animals, n = 11). K⁺-deprived rats gained slightly less weight during the 10 days, attaining a final body weight of 287 ± 0.9 g, which was 7% lower than controls (287 ± 1.9 g). Typical autoradiograms of skeletal muscle homogenates from control and hypokalemia rats are shown in Fig. 4, and the relative changes in Na,K-ATPase α and β subunit isoforms are summarized in Fig. 5. There were no significant changes in α1 abundance. In contrast, α2 decreased significantly in all skeletal muscles and cardiac left ventricular muscle. The decline in α2 was most pronounced in fast glycolytic dominated muscles, falling to 0.06 of control in WG, followed by decreases to 0.39, 0.42, 0.44, and 0.71 of control in RG, EDL, soleus, and diaphragm, respectively. β1 decreased 25% or less in RG, EDL, and diaphragm (muscles containing oxidative fibers) to 0.74, 0.76, and 0.83 of control, respectively. However, β1 did not decrease significantly in soleus, which is puzzling given that no β2 was detected in this muscle, and α2 decreased by 56%. This result suggests a dissociation between pool sizes of α and β in this muscle. β2 decreased 60% or more in all muscles where it was detected, falling in RG, EDL, and WG to 0.27, 0.40, and 0.30 of control, respectively. These results demonstrate distinct muscle-specific responses to K⁺ deprivation: α2β2 decreases more than 50% in fast glycolytic muscle fibers (WG, EDL, and RG), whereas α2 alone decreases in oxidative rich soleus, with only
a minor decrease in $\alpha 2\beta 1$ in mixed oxidative diaphragm. Cardiac ventricle was assayed in parallel, and in agreement with previous findings (11) $\alpha 2$ but not $\alpha 1$ abundance decreased to 0.69 of control.

Since changes in $\alpha 2$ abundance were so pronounced, we considered the possibility that the NH$_2$-terminal epitope recognized by the monoclonal McB2 had been cleaved. Blotting with an additional anti-$\alpha 2$ polyclonal antibody, anti-HERED, which recognizes a sequence of amino acids (491–495) found on the early part of the large cytoplasmic loop, between transmembrane segments H4/H5, and unique to $\alpha 2$ isoforms (12, 18), confirmed that the decrease in $\alpha 2$ signal was not due to cleavage of the epitope (Fig. 6). The pattern of change in $\alpha 2$ in response to K$^+$ deprivation is identical with both antibodies.

**Effect of Low K$^+$ Diet on ICF K$^+$ in Control versus Hypokalemic Rats**—Based on the distinct muscle-specific changes in Na,K-ATPase expression in hypokalemia we predicted that intracellular K$^+$ would fall more in WG, which lost greater than 70% of both $\alpha 2$ and $\beta 2$, than in diaphragm, which lost only 30% of $\alpha 2$ and $\beta 1$. To test this hypothesis, ICF K$^+$ was measured in paired muscles from the same pool of rats used for subunit abundance analysis. Fig. 7 summarizes ICF K$^+$ levels expressed as $\mu$mol/g, wet weight, in skeletal muscles from control and K$^+$-deprived rats. In contrast to our prediction, by day 10 all skeletal muscles lost about 20% of ICF K$^+$. Additionally, ICF K$^+$ measured in left ventricles from the same animals did not change. These results suggest a revised hypothesis, that muscles may have multiple mechanisms in place to regulate intracellular K$^+$ loss besides a decrease in pump number, such as pump translocation to internal stores, as described previously for $\alpha 2\beta 1$ in soleus in response to insulin stimulation (30, 31).

**DISCUSSION**

The existence of isoforms suggests the potential for differential isoform-specific function, tissue-specific expression, and regulation. Functional differences in enzymatic and transport properties between the $\alpha 1$ and $\alpha 2$ isoforms expressed in skeletal muscle are subtle if any (32). This study demonstrates a muscle type-specific pattern of Na,K-ATPase isoform expression and distinct muscle-specific patterns of regulation of Na,K-ATPase isoforms in response to K$^+$ deprivation.

Regarding muscle-specific expression, Hundal et al. (9) reported that pooled membranes from muscles enriched in slow twitch oxidative fibers expressed $\beta 1$, not $\beta 2$; that pooled membranes from muscles composed of fast twitch glycolytic fibers expressed $\beta 2$, not $\beta 1$ isoform; and that $\alpha 1$ and $\alpha 2$ were similar in the two types of muscle membranes. This distinction prompted our examination of $\alpha$ and $\beta$ isoform expression in five distinct skeletal muscles. $\alpha 1$ and $\beta 1$ were detected in all muscles and were at least twice as abundant in oxidative fiber rich soleus and diaphragm than in fast twitch glycolytic WG, whereas $\alpha 2$ expression was equivalent in all five muscles. In agreement with Hundal (9), $\beta 2$ expression was limited to fast glycolytic muscle types. Additionally, we note that $\beta 2$ expression appears directly proportional to the relative percentage of fast glycolytic fibers, that is, $\beta 2$ is most abundant, relative to cellular protein, in WG, which is 84% fast glycolytic; less abundant in EDL, which is 56% fast glycolytic; and below detection in slow oxidative soleus, which has no fast glycolytic fibers. In contrast to the Hundal study, we detected significant levels of $\beta 1$ in EDL, an almost pure fast muscle with 42% fast glycolytic-

![Fig. 4. Detection of Na,K-ATPase $\alpha$ and $\beta$ isoform abundance in skeletal muscles from control and K$^+$-deprived rats.](image)

![Fig. 5. Effect of K$^+$ deprivation on Na,K-ATPase $\alpha$ and $\beta$ isoform abundance in skeletal muscles.](image)
oxidative fibers. This may be a consequence of deglycosylation of EDL samples prior to analysis, allowing for enhanced β1 subunit detection in this study. We conclude that α1, β1, and α2 are expressed ubiquitously in skeletal muscle, α1 and β1 enriched in oxidative fibers and α2 about the relative abundance across muscle, whereas β2 is expressed in only a subset of muscles containing fast twitch glycolytic fibers.

The expression of two α and two β isoforms suggests the potential for four different αβ heterodimer combinations unless constraints prevent an α from combining with a β subunit. We know from both purification and immunoprecipitation studies that α1β1 heterodimers are the active sodium pumps in kidney and epithelial cell lines (23). The immunoprecipitation of both α1β1 and α2β1 from soleus muscle in this study provides in vivo evidence that both α complex with β1. The corollary to this finding is that if nearly all the β is assembled as heterodimers, β1 subunit abundance will provide a relative measure of total sodium pump pool size in soleus, since it lacks β2. The work of Gloor et al. (33) demonstrates that glial β2 forms heterodimers with α2 but not α1 subunits. Whether β2 will be constrained to form heterodimers with only α2 in skeletal muscles remains to be established.

In our initial investigation into the effect of hypokalemia on Na,K-ATPase expression we reported decreased abundance of α2, but not α1 or β1, in pooled hindlimb skeletal muscle, heart, and brain. The changes in skeletal muscle α2 were far the greatest: protein and mRNA levels decreased 80 and 40%, respectively, after 14 days of K+ deprivation (11). This study examined whether changes were restricted to a subset of muscle types found in the rat hindlimb. The results establish that β2 protein levels are also depressed more than 50% during 10 days of K+ deprivation in all muscles expressing this isoform (RG, EDL, and WG). The failure of our prior study to detect changes in β1 is likely a consequence of enhanced sensitivity of β1 subunit detection following deglycosylation of muscle samples prior to analysis. Overall, three distinct responses to K+ deprivation were observed in the panel of muscles: 1) in WG the response was the greatest, α2 and β2 decreased 94 and 70%, respectively; 2) in RG and EDL α2 and β2 decreased about 60%, and β1 decreased about 25%; and 3) in soleus and diaphragm α2 decreased 55 and 25%, respectively, with little if any accompanying change in β1 and no detection of β2. From this pattern we predicted that the WG would lose more intracellular K+ than soleus or diaphragm. However, intracellular K+ decreased equivalently in all five skeletal muscles after 10 days of K+ deprivation. In comparison, while we observed a 31% decrease in α2 abundance in K+-deprived cardiac left ventricular muscle, there was no significant decrease in cardiac intracellular K+, which can be attributed to the low percentage of α2 type pumps (25% or less) in the heart (6). Norgaard et al. (34) observed that after 4 weeks of K+-deficient diet there was only a 13% fall in left ventricle ICF K+ and a concomitant 43% decrease in [3H]ouabain binding (a measure of α2 abundance at the plasma membrane), the difference likely due to the longer deprivation period. Taken together, the results of this study demonstrate that the abundance of α2 and β2, and not the ubiquitous α1 isoform, is depressed in hypokalemia, and demonstrate small but significant decreases in β1 in a subset of muscles assayed, which is in contrast to our previous report of no change in β1 (11). These results, coupled with information on the relative ratios of α1 to α2 protein in various tissues, provide a molecular explanation for the significant and selective loss of K+ from skeletal muscle and only minor loss of K+ from cardiac muscle. We hypothesize that the presence and regulation of the α2 isoform in skeletal muscle provided an evolutionary advantage to complex organisms that needed to maintain transmembrane K+ gradients in the face of fluctuations in K+ availability (3).

We have hypothesized that expressing Na,K-ATPase subunits in a fiber type-specific pattern would provide an organism with the means to regulate fiber type involvement in the regulatory response to a K+ challenge (35). Specifically, one subset of muscles may be adapted to respond to hypokalemia by expressing isoforms that respond to insulin and rapidly move dietary K+ from ECF to ICF after a meal to avert cardiac complications from elevated extracellular K+, while another subset of muscles may be adapted to respond to hypokalemia by expressing isoforms that decrease in response to chronic K+ deprivation leading to the loss of K+ from the ICF to the ECF. Parallel specialization would allow an animal both to chronically lose K+ during deprivation and rapidly clear a ECF K+ load when a meal (and insulin surge) ends the K+ deprivation. Recent evidence from Lavoie et al. (31) supports this hypothesis by demonstrating that insulin increases Na,K-ATPase transport activity only in oxidative fiber type skeletal muscles (for example, soleus and most RG) by inducing translocation of α2 and β1 from intracellular to plasma membranes. Additional support comes from the study of Hsu and Guidotti (36), demonstrating that this insulin-stimulated K+ uptake response is still present in a hypokalemic animal with depressed sodium pump expression. Arguing against specialization during K+ deprivation comes from the study of Hsu and Guidotti (36), demonstrating that this insulin-stimulated K+ uptake response is still present in a hypokalemic animal with depressed sodium pump expression. Arguing against specialization during K+ deprivation comes from the study of Hsu and Guidotti (36), demonstrating that this insulin-stimulated K+ uptake response is still present in a hypokalemic animal with depressed sodium pump expression. Arguing against specialization during K+ deprivation comes from the study of Hsu and Guidotti (36), demonstrating that this insulin-stimulated K+ uptake response is still present in a hypokalemic animal with depressed sodium pump expression.
deprivation are the findings of this study. After 10 days of K⁺ deprivation, the oxidative fiber muscle types lose as much intracellular K⁺ as the fast glycolytic muscle even though there are much smaller decreases in α and β subunit pool sizes in oxidative fiber types. One hypothesis that reconciles these findings is that the molecular mechanisms mediating the responses may be muscle fiber type-specific. For example, sodium pump transport activity may be depressed in slow oxidative fiber muscle types by both decreasing abundance and translocation of the predominant αβ2 heterodimers from plasma membrane to internal stores (where they will be poised to respond to insulin), while sodium pump transport activity in fast glycolytic muscle fiber types, where αβ2 heterodimers predominate, may be depressed solely by decreasing pool sizes of this isoform. Such a scenario would allow skeletal muscle K⁺ stores to buffer the fall in extracellular K⁺ during K⁺ deprivation while retaining the ability to clear a potassium load from the ECF into the skeletal muscle during hyperkalemia.

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