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Role of Muscle in Regulating Extracellular [K^+]

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There is a positive association between diets rich in potassium, control of blood pressure, and prevention of stroke. Extracellular [K^+] is regulated closely to maintain normal membrane excitability by the concerted regulatory responses of muscle and kidney. Although kidney is responsible for ultimately matching K^+ output to K^+ intake each day, muscle contains more than 90% of the body's K^+ and can buffer changes in extracellular fluid [K^+] by either acutely taking up extracellular fluid K^+ or releasing intracellular fluid K^+ from muscle. It long has been assumed that the changes in muscle K^+ transport are a function of sodium pump (Na,K-adenosine triphosphatase [Na, K-ATPasell]) abundance, especially that of the α2 isoform, which predominates in skeletal muscle. To test the physiologic significance of changes in muscle Na,K-ATPase expression, we developed the K^+ clamp, which measures insulin-stimulated cellular K^+ uptake in vivo in the conscious rat. By using the K^+ clamp we discovered that significant insulin resistance to cell K^+ uptake occurs as follows: (1) early in K^+ deprivation before a decrease in muscle sodium pump pool size, and (2) during glucocorticoid treatment, which increases muscle Na,K-ATPase α2 levels greater than 50%. We also discovered that adaptation of renal and extrarenal K^+ handling to altered K^+ balance often occurs without changes in plasma [K^+], supporting a feedforward mechanism involving K^+ sensing in the splanchnic bed and adjustment of K^+ handling. These findings establish the advantage of combining molecular analyses of Na,K-ATPase expression and activity with systems analyses of cellular K^+ uptake and excretion in vivo to reveal regulatory mechanisms operating to control K^+ homeostasis.
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The recent Institute of Medicine project on "Dietary Reference Intakes for Electrolytes and Water"[1] reports that adults should consume at least 4.7 g of potassium per day to lower blood pressure, blunt the effects of sodium chloride, and reduce the risk for kidney stones and bone loss. The project also states that most American women consume no more than half of this recommended amount, and that men's intake is only moderately higher. In comparison, most Americans consume more than the tolerable upper limit for sodium consumption of 3.8 g.[1] There is a long-standing awareness of the positive association between diets rich in potassium and the control of blood pressure and prevention of stroke,[2-4] which is not surprising given the fact that potassium is the main intracellular cation and a key determinant of cell volume and nerve and muscle excitability. These properties are all dependent on the steep transmembrane K^+ gradient established by the ubiquitous sodium pump, Na,K-adenosine triphosphatase (ATPase). Sodium pump ATP hydrolysis fuels the coupled uphill transport of K^+ into the cell and Na^+ out of the cell. Extracellular [K^+] must be regulated closely (it usually is between 3.8-5 mmol/L) to maintain normal membrane excitability. This is accomplished by the concerted responses of kidneys, which matches K^+ excretion to K^+ intake by secreting or actively reabsorbing K^+[5-7] and muscle, which contains the major pool of K^+ and can regulate K^+ distribution between the intracellular fluid (ICF) and extracellular fluid (ECF) compartments (Fig. 1). This review focuses on recent in vivo studies examining the role of muscle Na,K-ATPase in potassium homeostasis. Because a recent comprehensive review of Na,K-ATPase regulation and skeletal muscle contractility is available,[8] this topic is not emphasized.

To show the importance of the interplay between kidney and muscle, consider the day-to-day challenges associated with potassium ingestion. Total ECF contains only about 70

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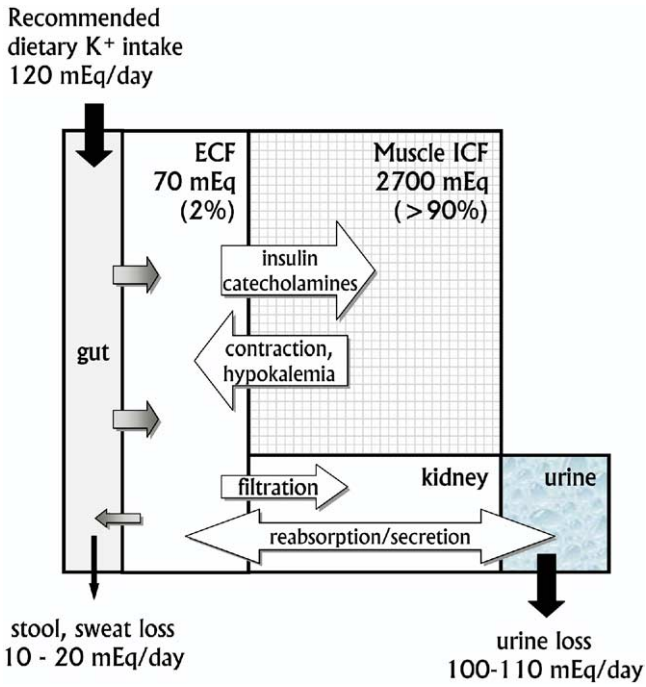

Figure 1 Compartmental diagram of potassium homeostasis in an average person. More than 90% of the body's K^+ is located in the muscle ICF and only about 2% is located in the ECF. Regulation of K^+ transport by both muscle and kidneys leads to fine control of extracellular [K^+]. After a meal, the gut absorbs K^+ and postprandial insulin acutely increases cellular K^+ uptake into muscle (and liver, not shown). Muscular contraction continuously releases K^+ into the ECF, which either will be pumped back into the muscle or filtered into the kidney and excreted to balance intake. When K^+ intake is high or low there are a variety of regulatory responses. Both the kidney and the colon can secrete or reabsorb K^+ in a regulated fashion depending on whether K^+ intake is high or low. During hypokalemia the muscle exhibits an altruistic specialization to donate cell [K^+] to balance the discrepancy between K^+ input and output over time. When significant exercise causes hyperkalemia, catecholamines drive an increase in muscle cellular K^+ uptake. (Color version of figure is available online.)

mEq K^+, whereas the recommended dietary potassium intake from the Institute of Medicine is nearly twice this at 120 mEq/d (4.7 g/d/0.039 g/mEq) (Fig. 1). A large meal may contain 70 mEq K^+, which would be added to the ECF within a short time of its ingestion. ECF [K^+] would double if there were not rapid adjustments to either transfer the K^+ to the ICF compartment or excrete it. In fact, after a meal there is little change in ECF K^+ levels because postprandial insulin stimulates muscle and liver to actively take up both glucose and the K^+ not excreted in the short term by the kidneys.[9] Between meals, muscle activity releases K^+ into the ECF and, mysteriously, kidneys excrete an amount equal to the daily K^+ intake. In patients with end-stage renal disease, muscle serves as a buffer to sequester some of the dietary K^+ between dialysis sessions. During fasting or when consuming a low K^+ diet, the kidney adapts to reabsorb more and secrete less K^+ to the point that renal output decreases to near zero.[5,10] This occurs primarily as a result of adjustments in the distal nephron: a decrease in surface expression and an abundance of apical K^+ channels that mediate K^+ secretion[11,12] and an increase in apical H,K-ATPases that can mediate K^+ reabsorption.[13,14] Because K^+ loss in the stools and sweat persists during fasting and low K^+ diets, potassium must be redistributed continuously from ICF muscle stores to the ECF to prevent a drastic decrease in ECF [K^+]. That is, muscle exhibits an altruistic specialization to donate cell [K^+] to balance the discrepancy between K^+ input and output over time.[15-17] An impressive illustration of this regulatory response was shown by Knochel et al[18] in a study of soldiers in summertime basic training. Soldiers lost more than 40 mEq K^+/d in sweat alone. Hypokalemia did not develop because high muscle activity shifted K^+ into the ECF. Nonetheless, after 11 days of training, a total body K^+ deficit of more than 400 mEq developed, perhaps exacerbated by aldosterone secreted in response to daily bouts of dehydration.[18]

Active transport of K^+ by muscle Na,K-ATPase plays a central role in these scenarios of acute and chronic challenges to potassium homeostasis. This review focuses on the molecular mechanisms in place in muscle that contribute to potassium homeostasis, in particular, muscle-specific regulation of sodium pump isoforms, and a method we developed to assess cellular K^+ uptake in vivo.

P-Type ATPases and K^+ Homeostasis

Potassium transport between the ECF and ICF is mediated by an array of transporters including P-type ATPases, cotransporters, and channels (shown in Fig. 2). Plasma membrane sodium pumps (Na,K-ATPase) actively transport K^+ from ECF into the cell and the renal hydrogen potassium pumps (H,K-ATPase) expressed during K^+-deficient states actively reabsorb K^+ from the renal tubular fluid back into the ECF.[14,19] These P-type ATPases are 1:1 heteromers of approximately 100-kd α-catalytic subunits and approximately 50-kd β-glycoprotein subunits that share 65% homology. Cells also express K^+ cotransporters including bumetanide-sensitive sodium potassium 2 chloride transporters[20] and potassium 2 chloride transporters,[21] which can drive cellular K^+ uptake, but their roles in regulating ECF K^+ homeostasis have not been investigated. Multiple isoforms of sodium pump α and β subunits exist and are expressed in a tissue-specific pattern.[13,14,19] Skeletal muscle expresses α1,α2, β1, and β2 isoforms. α2 expression is fairly uniform across muscles, whether oxidative or glycolytic, but α1 expression is twice as high in oxidative muscles such as soleus and diaphragm than in mixed or fast glycolytic muscles such as gastrocnemius and extensor digitorum longus.[22] Estimates place the percentage of α2 protein at 40% to 60%.[16,23] β1 is expressed without β2 in soleus and diaphragm, and β2 is expressed without β1 in white gastrocnemius, and both are expressed in mixed fiber muscles.[22,24]

Differential expression suggests differential function, regulation, or subcellular distribution, and there is evidence for all of these in muscle. Studies in mice lacking one copy of α1

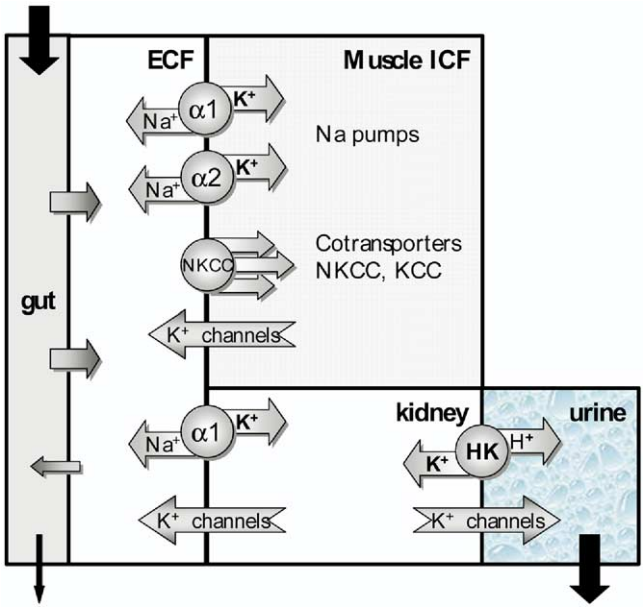

Figure 2 K^+ transporters between ECF, ICF, and external to the animal are shown. Muscle fibers express plasma membrane sodium pumps (Na,K-ATPase) composed of either α1 or α2 catalytic subunits along with β1 or β2 glycoprotein subunits. A number of K^+ channels mediate K^+ efflux from the skeletal muscle and K^+ cotransporters are found in muscle plasma membrane as well. ECF is filtered into the renal tubule fluid by glomerular filtration. All along the nephron basolateral Na,K-ATPase brings K^+ into the renal ICF, some is recycled back to the ECF via K^+ channels. In collecting duct principal cells, apical ROMK K^+ channels secrete K^+ into tubule fluid (urine). During K^+ deprivation or hypokalemia, collecting duct intercalated cells express the colonic isoform ($\alpha_c\beta$1) of H,K-ATPase in apical membranes to increase active K^+ reabsorption. (Color version of figure is available online.)

versus one copy of α2 indicate that these isoforms have distinct functions in regulating cardiac contractility: muscles from α1(+/−) mice show lower force compared with wild-type mice, whereas muscles from α2(+/−) mice show greater force.[25] Na,K-ATPase α2 (not α1) abundance is regulated specifically by K^+ restriction (decreased) and glucocorticoid treatment (increased).[16,22,26] Finally, there is evidence that $\alpha2\beta$1-type sodium pumps are located in internal endosomal vesicle membranes in red slow, but not in white fast, muscle,[27] and that insulin provokes a redistribution from internal membranes to the surface,[28,29] contributing to the insulin stimulation of active K^+ uptake. Taken together, these results suggest that α2 expression in skeletal muscle is a specialization to regulate K^+ cellular uptake from the ECF.

Passive K^+ fluxes from muscle are mediated by a number of K^+ channels including voltage-sensitive inward rectifiers, delayed rectifiers, Ca^{2+}-sensitive K^+ channels, and ATP-sensitive K^+ channels. After an action potential in muscle, the opening of K^+ channels rapidly restores membrane potential at the expense of a temporary increase in local ECF [K^+].[8] This K^+ either is pumped back into the cell by activation of sodium pumps or makes its way to the kidney for excretion. In summary, dietary potassium likely is processed as follows: (1) K^+ is absorbed into the ECF after a meal, (2) insulin stimulates K^+ uptake mediated by activation of muscle Na,K-ATPase, (3) muscle activity provokes the release of K^+ from muscle into the ECF, which either is pumped back into the muscle, or (4) ECF K^+ is filtered into the kidney and excreted to balance K^+ output to K^+ intake.

The fine control of K^+ excretion, effected in the collecting tubules and ducts, has been reviewed recently.[5,10,14] During K^+ restriction there is a shift in net K^+ handling from K^+ secretion to active K^+ reabsorption caused by decreased abundance of the renal outer medulla K secretory (ROM K) potassium channel,[11] and perhaps increased abundance and activity of nongastric, colonic isoform of the α subunit of H,K-ATPase in apical membranes.[30-33] Because colonic H,K-ATPase–deficient mice are able to reduce urinary K^+ excretion to nearly the same extent as mice with normal H,K-ATPase levels,[34] decreased ROM K surface expression and abundance is probably the most important adjustment during K^+ deprivation. The remainder of this review focuses on the role of muscle in potassium homeostasis.

Impact of Regulation of Muscle Na,K-ATPase on ECF/ICF K^+ Homeostasis

The K^+ Clamp to Measure Insulin-Stimulated Cellular K^+ Uptake In Vivo

To quantify insulin action on cellular K^+ uptake in vivo, we exploited the theory behind the glucose clamp technique,[35] which is used to measure insulin-stimulated glucose uptake in vivo, to develop a K^+ clamp. In this technique, conscious rats are infused with insulin to stimulate K^+ (and glucose) uptake and then, based on rapid and frequent assays of plasma [K^+], infused with appropriate K^+ to clamp plasma [K^+] at baseline (Fig. 3). The amount of K^+ infused (K^+_{inf}) over a defined time period is equivalent to the sum of insulin-stimulated portions of cellular K^+ uptake and K^+ excretion. Previous studies by Choi et al[35] and others[36] showed that insulin at physiologic concentrations does not increase renal K^+ excretion significantly. Therefore, K^+_{inf} appears to be a good measure of insulin-stimulated cellular K^+ uptake. The K^+ clamp is a useful tool to evaluate whether molecular changes in sodium pump expression or distribution impact insulin-sensitive cellular K^+ uptake.

Impairment of Insulin Action on K^+ Uptake During K^+ Deprivation

Long-term K^+ deprivation in rats has been shown to be associated with a greater than 50% decrease in the number of sodium pumps in various rat muscles (estimated with ^{3}H-ouabain binding), a similar decrease in enzymatic activity, and a decrease in K^+ influx into isolated soleus (by ^{86}Rb uptake).[7] In addition, we have established that K^+ deprivation provokes a specific decrease in the sodium pump α2- (not α1-) catalytic isoform abundance in skeletal muscle.[16,22]

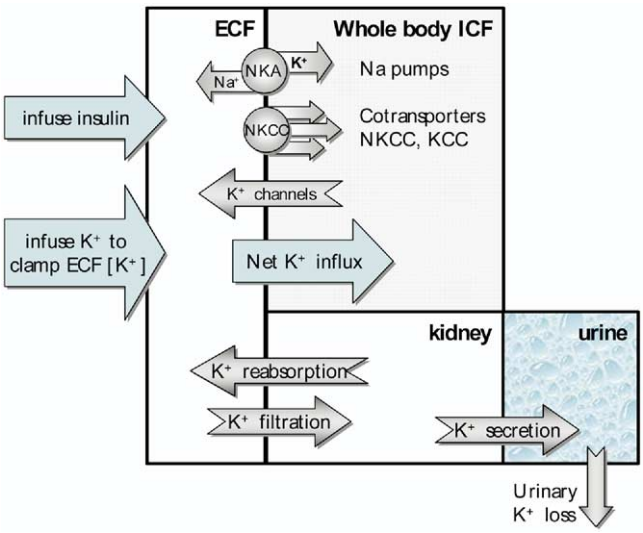

Figure 3 The K^+ clamp. Method described by Choi et al.[35] The insulin-stimulated clearance of K^+ from the ECF is quantitated by measuring the amount of K^+ infusion necessary to prevent a decrease in plasma [K^+] levels. Net K^+ influx into the ICF = infused K^+ (K^+inf) –renal K^+ excretion as a function of time. (Color version of figure is available online.)

We tested the hypothesis that insulin-stimulated cellular K^+ uptake decreases during K^+ deprivation as a function of muscle α2 abundance. After 10 days of K^+ deprivation, plasma [K^+] levels decreased to 2.9 mmol/L, Na,K-ATPase activity and α2-subunit levels decreased more than 50%, and insulin-mediated K^+ disappearance decreased to 6% of control, suggesting a relationship between the decrease in Na,K-ATPase α2 pool size and the decrease in insulin-stimulated K^+ uptake. However, when rats were deprived of K^+ for only 2 days, plasma [K^+] levels decreased slightly from 4.2 to 3.8 mmol/L, there was no significant decrease in muscle Na,K-ATPase activity or expression, but insulin-mediated cellular K^+ uptake decreased to 20% of control (Fig. 4). These data show that short-term K^+ deprivation leads to a significant insulin resistance of cellular K^+ uptake that precedes the decreases in muscle sodium pump expression and suggest that another mechanism must account for the early decrease in insulin-stimulated K^+ uptake (eg, inactivation or internalization). The decrease in α2 later in K^+ deprivation was associated with an even greater decrease in insulin-sensitive cellular K^+ uptake. Thus, the implementation of the K^+ clamp revealed a dissociation between changes in sodium pump expression and cellular K^+ uptake during early K^+ deprivation.

Restoring K^+ After Hypokalemia

Because hypokalemia is associated with decreases in muscle sodium pumps and cellular K^+ uptake, there was the concern that acute K^+ repletion after K^+ depletion would cause K^+ intoxication, that is, that it would not be cleared efficiently into the ICF. Recently, Bundgaard and Kjeldsen[37,38] investigated this possibility in rats fed a K^+-free diet for 2 weeks then acutely repleted by intravenous KCl infusion. After K^+ depletion, plasma [K^+] levels decreased to 2.0 mmol/L and muscle Na,K-ATPase α2 (not α1) and ouabain binding decreased 34% to 70%, depending on the muscle. During acute repletion, the rate of cellular K^+ uptake was higher in controls but the total K^+ clearance capacity before toxicity was 4-fold higher in the K^+-depleted group. During this K^+-depletion regimen, cardiac Na,K-ATPase α2 pool size was lower but the α1 pool actually was increased. During acute K^+ repletion there was no difference in myocardial K^+ uptake rate unless extracellular K^+ was clamped at 5.5 mmol/L, in

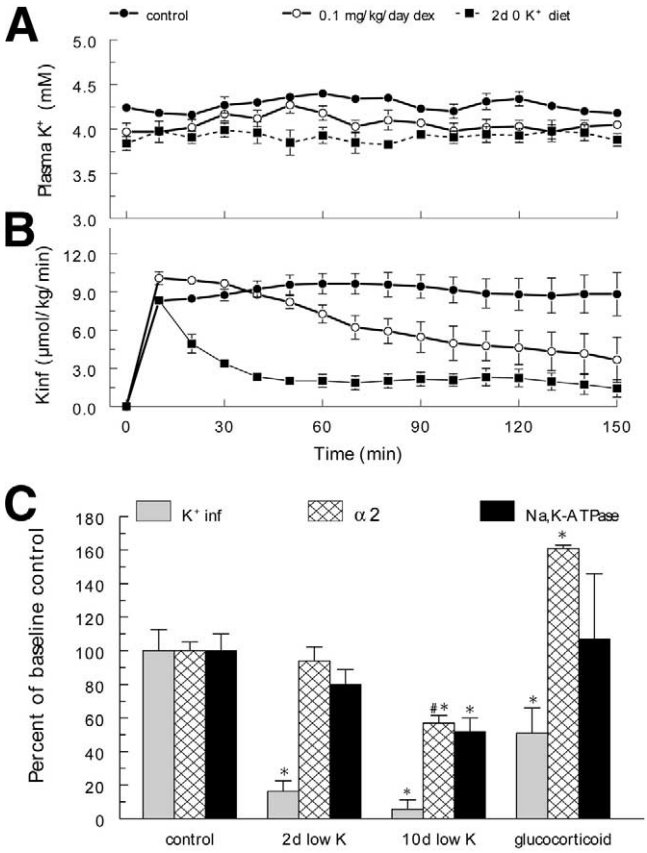

Figure 4 K^+ clamp results in conscious rats. (A) During the infusion of 5 mU insulin/kg/min via a tail-vein catheter, the plasma [K^+] level is measured every 10 minutes, and (B) K^+ infused (K^+inf) at a rate determined empirically to prevent a decrease in plasma [K^+] level below the initial baseline measurement. The K^+ clamp revealed that insulin-stimulated cellular uptake of K^+ is decreased in rats placed on 0 K^+ diet for 2 days or in rats treated with dexamethasone (0.1 mg/kg/d via osmotic minipumps) for 7 days. Renal K^+ excretion did not change with insulin infusion so K^+inf is a good estimate of insulin-stimulated cellular K^+ uptake. ●, Control; ○, 0.1 mg/kg/d dexamethasone; ■, 2-day 0 K^+ diet. (C) Total K^+inf during the last hour of the clamp, muscle Na,K-ATPase α2 abundance, and maximal muscle Na,K-ATPase activity all normalized to control levels defined as 100%. From this summary, it is apparent that insulin-stimulated cellular K^+ uptake (K^+inf) does not always change as a function of muscle Na,K-ATPase α2 levels or activity: K^+inf is suppressed significantly after 2-day 0 K^+ diet, before there is a change in Na,K-ATPase α2 levels (seen at 10 days) and after glucocorticoid treatment, which increased α2 levels. ▨, K^+ inf; ▨, α 2; ■, Na,K-ATPase.[35,48]

which case the K^+ uptake rate was twice as high in the K^+-depleted rat hearts. These findings indicate that risk for K^+ intoxification actually is lower, not higher as suspected, in K^+-depleted animals and that the heart is adapted (increased Na,K-ATPase α1 levels) to restore the lost K^+ quickly.

Independent Regulation of Insulin Action on Glucose Uptake Versus K^+ Uptake

Of the many homeostatic systems of the body, the K^+ and glucose homeostatic systems are unique in that they share acute regulation by insulin. This feature suggests the potential for interactions or cross-talk between the 2 systems. Insulin resistance with respect to glucose metabolism usually is associated with hyperinsulinemia because pancreatic β-cells increase insulin secretion to compensate for insulin resistance. It is conceivable that the resulting hyperinsulinemia would have a substantial impact on K^+ homeostasis (eg, provoke hypokalemia secondary to stimulating excessive cellular K^+ uptake) unless insulin's action on K^+ uptake was dampened similarly. Impaired insulin action on K^+ fluxes have been reported in obesity and diabetes,[39,40] both associated with insulin resistance with respect to glucose metabolism. On the other hand, insulin-mediated K^+ uptake is not altered in uremia whereas insulin-mediated glucose uptake is impaired markedly.[41] High-fat feeding is a well-established model of insulin resistance with respect to glucose metabolism.[42,43] By using the glucose and K-clamp techniques, we examined whether impaired insulin action on glucose uptake in high-fat fed rats is accompanied by impaired insulin action on cellular K^+ uptake. We discovered, inadvertently, that when rats were fed a typical high-fat diet, total dietary K^+ intake was reduced to one third of normal.[44] In addition to the expected decreases in insulin-stimulated glucose uptake, the low K^+ intake in high-fat fed rats led to decreases in both insulin-stimulated cellular K^+ uptake and urinary K^+ excretion. Interestingly, K^+ supplementation in high-fat fed rats restored insulin action on K^+ uptake to control levels, but did not correct insulin-stimulated glucose uptake. These data indicate that when K^+ intake was matched, insulin action on glucose uptake was impaired selectively (ie, without change in insulin action on K^+ uptake) by high-fat feeding. Thus, insulin's actions on glucose and K^+ uptake are regulated independently by dietary fat and K^+ content, respectively.

Dexamethasone Increases Muscle Sodium Pumps But Not Cellular K^+ Uptake

Chronic treatment of patients or experimental animals with glucocorticoids leads to an increase in pool size of skeletal muscle ouabain binding sites.[45,46] The McDonough laboratory determined that 14 days of treatment of rats with the synthetic glucocorticoid dexamethasone caused a greater than 50% increase in Na,K-ATPase α2 and β1 (not α1 or β2) isoform messenger RNA and protein levels (the major isoforms in muscle) in all muscle groups.[26] In a recent study we asked whether this increased abundance of Na,K-ATPase and ouabain binding was associated with increased muscle Na,K-ATPase activity in vivo. This question is important clinically because this large increase in functional sodium pumps in muscle (which contains the body's largest pool of sodium pumps) theoretically could provoke an increase in insulin-driven cellular K^+ uptake after a meal or increase catecholamine-driven cellular K^+ uptake during an asthma attack, resulting in an extra risk for hypokalemia and ensuing arrhythmias and cardiac arrest.[47]

Insulin-stimulated cellular K^+ uptake was measured by the K^+ clamp in rats chronically treated with dexamethasone sufficient to increase Na,K-ATPase α2 levels between 40% (soleus) and 65% (gastrocnemius). Surprisingly, insulin-stimulated cellular K^+ uptake was blunted significantly by 50%, rather than increased, in the dexamethasone-treated group.[48] It long has been established that chronic dexamethasone treatment causes insulin resistance of cellular glucose clearance[48] and these results provide evidence for insulin resistance of K^+ clearance as well: insulin-stimulated cellular K^+ uptake is depressed significantly despite increased muscle sodium pump pool size. This finding suggests the possibility that the increase in Na,K-ATPase synthesis and abundance is a compensation to counteract the dexamethasone-induced insulin resistance. In other words, insulin-stimulated cell K^+ uptake probably would be suppressed even more if Na,K-ATPase pool size was not increased.

Molecular Mechanisms

Sodium pump–mediated changes in active K^+ uptake could be mediated by changes in the number of sodium pumps in the plasma membrane and/or changes in the activity per pump mediated by allosteric or covalent modification. In skeletal muscle, there is strong support both for and against insulin-stimulated translocation of sodium pumps to the plasma membrane in skeletal muscle. The best evidence for translocation is a study by Marette et al,[49] using immunoelectron microscopy, which concluded that Na,K-ATPase α2 subunits could be detected in intratubular and vesicular structures and plasma membrane, and that insulin increased the density of gold particle–labeled sodium pumps per length of plasma membrane by either 3.7-fold or 1.5-fold (depending on protocol variables). However, identifying the location of intracellular stores of α2 was complicated because of α2-subunit labeling in tubular structures and in the triad region of transverse tubules. Whether some of the α2 identified as intracellular was actually in membranes contiguous with the plasma membrane is difficult to rule out. Also in support of translocation, Al-Khalili et al[29] studied epitrochlearis muscles incubated with or without insulin in vitro followed by cell surface biotinylation. The results indicated that insulin increased the cell surface pools of both α1 and α2 by more than 50%, and that this was prevented by coincident incubation with phosphoinositide 3-kinase or protein kinase C inhibitors. The caveat in this study is that it is possible that insulin treatment increased the overall efficacy of cell surface biotinylation. In the same study, analysis of translocation by subcellular fractionation indicated a 40% increase in α2 but not α1 in the plasma membrane–enriched fraction after insulin (which contained $<$10% of total plasma membranes), which

is at odds with the biotinylation results indicating that both α1 and α2 were translocated. These investigators conceded that subfractions of plasma membrane may not be recovered on the sucrose gradients, criticisms that may apply to the previous reports of translocation studied by subcellular fractionation.[27]

Studies refuting insulin-stimulated translocation of sodium pumps are based on ouabain binding to quantitate cell surface Na,K-ATPase α2 in muscle. Because ouabain binds to the extracellular face of the pump, has very high specificity for the rat α2 isoform, and binds only to active sodium pumps, significant translocation of Na,K-ATPase α2 to the plasma membrane should be detected as an increase in ^{3}H-ouabain binding to isolated muscles. Two different laboratories[6,50] have shown that, in isolated muscles, insulin doubles the initial rate of ouabain binding, a measure of activity, but does not increase steady-state binding. McKenna et al[51] recently readdressed the question of translocation of Na,K-ATPase in muscle and measured ^{86}Rb uptake (a measure of K^+ uptake) and ouabain binding in isolated soleus muscle in parallel: insulin increased ^{86}Rb uptake 23% and decreased cell [Na^+] 27%, both showed evidence of sodium pump activation but did not alter ^{3}H-ouabain binding. One caveat is that ouabain could cross the membrane and label all the Na,K-ATPase α2. Although there is no evidence that ouabain crosses through the plasma membrane by itself, there is evidence from cultured cell studies that ouabain bound to sodium pumps at the cell surface is internalized along with the sodium pumps[52-54]; if there is continuous recycling of the sodium pumps between cell membrane and intracellular pools, steady-state labeling may indicate equilibrium binding of ouabain to all the rat muscle cell's α2-type sodium pumps. Because the precise molecular mechanism of insulin stimulation of Na,K-ATPase activity remains an open question, it is not obvious to postulate how 2-day low K^+ treatment (with no change in total pool size of Na,K-ATPase) or dexamethasone treatment blunts insulin-stimulated cell K^+ uptake. The obvious future direction is to use cell-surface biotinylation (including assay of plasma membrane markers to assess overall biotinylation efficiency), or low-temperature ouabain binding assays to determine whether there is, in fact, detectable translocation of Na,K-ATPase and whether it is blunted in insulin-resistant states.

Feedback Versus Feedforward Control of K^+ Homeostasis

When dietary K^+ intake is increased or decreased, the kidneys respond by appropriately increasing or decreasing K^+ excretion.[55,56] This so-called *K^+ adaptation* is critical for chronic K^+ balance and has been well recognized for several decades. By using the K-clamp technique, we showed that insulin-stimulated cellular K^+ uptake also is suppressed profoundly during K^+ deprivation.[35,44] Thus, K^+ adaptation occurs both in the kidneys and extrarenal tissues in response to changes in K^+ intake. By using the high-fat diet model, we also showed that K^+ adaptation can occur without a decrease in plasma [K^+] level. According to the traditional view, extracellular K^+ is the major factor in the regulation of renal K^+ excretion.[55,56] Dietary K^+ intake increases extracellular [K^+] in the normal range, which stimulates renal K^+ excretion by a direct action on collecting duct K^+ secretion,[56] and, if plasma [K^+] increases, via aldosterone stimulation of this K^+ secretion, which helps to normalize extracellular [K^+] levels. Conversely, during K^+ restriction, decreased plasma [K^+] levels decreases renal K^+ excretion directly and indirectly (by decreasing aldosterone secretion), which normalizes extracellular [K^+] levels.[57] Thus, the maintenance of chronic K^+ balance has been understood traditionally based on the concept of negative feedback control (Fig. 5). Rabinowitz[58,59] challenged this traditional view and proposed a kaliuretic reflex arising from sensors in the splanchnic bed (ie, gut, portal circulation, and/or liver). According to this proposal, renal K^+ excretion can be increased, without (or before) increases in extracellular [K^+] levels, by a mechanism controlled by sensing of K^+ intake (ie, sensing of local increases in K^+ level in splanchnic areas during K^+ intake), a concept of feedforward control (Fig. 5).

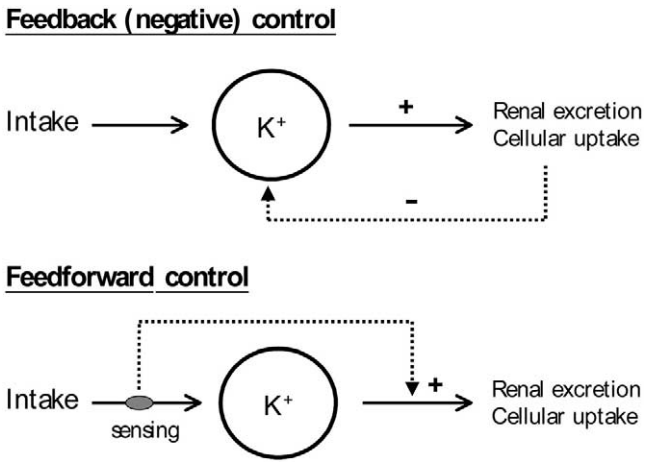

Figure 5 Models of feedback versus feedforward regulation of potassium homeostasis.

In studies by Choi et al[44] and Yamaji et al,[60] it was discovered that decreasing K^+ intake to one third of normal markedly decreased renal K^+ excretion and insulin-mediated cellular K^+ uptake. Interestingly, there was a strong correlation between changes in urinary K^+ excretion and insulin-stimulated K^+ uptake during reduced K^+ intake,[44] suggesting the possibility that the kidneys' function to excrete K^+ and insulin's action to promote cellular K^+ uptake are similarly (in concert) regulated in response to altered K^+ intake. Importantly, these changes were accompanied by no significant change in plasma K^+ or aldosterone levels. Thus, K^+ adaptation occurred in the absence of changes in extracellular K^+ (ie, the signal required for the feedback regulation). These data strongly support the operation of a feedforward control mechanism. A crucial component of feedforward control is sensing of K^+ intake. Morita et al[61] recently provided evidence that K^+ intake may be sensed by K^+ sensors in the

hepatoportal region. They found that hepatic afferent nerve activity increased in response to intraportal K^+ injection in a dose-dependent manner, and intraportal KCl infusion elicited an immediate kaliuresis with no significant change in the plasma K^+ concentration. Further studies are warranted to confirm and characterize K^+-sensing mechanisms in the hepatoportal region or other splanchnic regions.

Most homeostatic regulation in the body is under feedback control because it offers stable regulation. However, this type of control can be slow in adapting to an external disturbance because feedback mechanisms would not be activated until the system is disturbed significantly. In contrast, feedforward control may allow a speedy control of output function in anticipation of an increase of the signal, which may be critical in the acute regulation of K^+ homeostasis as discussed earlier. This type of control usually offers speed in control at the expense of stability or accuracy. The combination of both control mechanisms may provide both stability and speed of the regulation.

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