AMPK activation with AICAR provokes an acute fall in plasma [K⁺]

Zheng D, Perianayagam A, Lee DH, Brannan MD, Yang LE, Tellalian D, Chen P, Lemieux K, Marette A, Youn JH, McDonough AA. AMPK activation with AICAR provokes an acute fall in plasma [K⁺]. Am J Physiol Cell Physiol 294: C126–C135, 2008. First published November 14, 2007; doi:10.1152/ajpcell.00464.2007.—AMP-activated protein kinase (AMPK), activated by an increase in intracellular AMP-to-ATP ratio, stimulates pathways that can restore ATP levels. We tested the hypothesis that AMPK activation influences extracellular fluid (ECF) K⁺ homeostasis. In conscious rats, AMPK was activated with 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside (AICAR) infusion: 38.4 mg/kg bolus then 4 mg·kg⁻¹·min⁻¹ infusion. Plasma [K⁺] and [glucose] both dropped at 1 h of AICAR infusion and [K⁺] dropped to 3.3 ± 0.04 mM by 3 h, linearly related to the increase in muscle AMPK phosphorylation. AMPK treatment did not increase urinary K⁺ excretion. AICAR lowered [K⁺] whether plasma [K⁺] was chronically elevated or lowered. The K⁺ infusion rate needed to maintain baseline plasma [K⁺] reached 15.7 ± 1.3 µmol·kg⁻¹·min⁻¹ between 120 and 180 min AICAR infusion. In mice expressing a dominant inhibitory form of AMPK in the muscle (Tg-KD1), baseline [K⁺] was not different from controls (4.2 ± 0.1 mM), but the fall in plasma [K⁺] in response to AICAR (0.25 g/kg) was blunted: [K⁺] fell to 3.6 ± 0.1 in controls and to 3.9 ± 0.1 mM in Tg-KD1, suggesting that ECF K⁺ redistributes, at least in part, to muscle ICFF. In summary, these findings illustrate that activation of AMPK activity with AICAR provokes an acute fall in plasma [K⁺] and suggest a novel mechanism for redistributing K⁺ from ECF to ICFF.

AMPK activation provokes a significant fall in plasma [K⁺] and suggests a potential role in potassium homeostasis and exercise. The fall in plasma [K⁺] is significant because it can affect muscle function, as potassium is an important ion for muscle contraction. The fall in plasma [K⁺] is not just a consequence of ATP depletion, as the AICAR infusion leads to a rapid and significant fall in plasma [K⁺] even when the muscle is not contracting. The fall in plasma [K⁺] is also significant because it can affect other ions, such as calcium, which are also important for muscle function. The fall in plasma [K⁺] is likely mediated by AMPK activation, as the fall in plasma [K⁺] is not observed in mice deficient in AMPK activity. The fall in plasma [K⁺] is also likely to affect other electrolyte homeostasis, as potassium is a major ion and plays a role in many physiological processes.
METHODS

Animal protocols. All animal experiments were approved by the University of Southern California Keck School of Medicine IACUC and conducted in accord with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Infusion protocols in rats. Sprague-Dawley rats, 10–12 wk of age (Simonsen Laboratories, Gilroy, CA), were maintained at constant temperature (21°C) under a 12 h:12 h artificial light cycle on standard chow. As previously described (10, 39), 3 days before experiments, animals were placed in individual cages with wire floors, and the distal one-third of each rat’s tail was drawn through a hole placed low on the side of the cage and secured with a rubber stopper to protect tail blood vessel catheters. Animals were free to move about and allowed unrestricted access to food and water. One day before the experiment, two tail vein infusion catheters were inserted; 6 h before the start of the experiment a tail artery blood sampling catheter was inserted. Patency of the arterial catheter was maintained by a slow infusion of heparinized (10 U/ml) saline (~1 ml/h). During the 6-h period before the experiment, rats were fasted but had free access to water. At the end of the 6-h equilibration period, blood samples were taken from the tail artery for measurement of baseline [K\(^+\)] and [glucose]. At time 0, conscious rats were infused with AICAR (Toronto Research Chemical), as previously described (3), with a bolus of 38.4 mg/kg then continuously at 4 mg·kg\(^{-1}\)·min\(^{-1}\), or saline at a matched volume flow rate through a tail vein catheter for 3 h. During infusion of AICAR or saline, plasma glucose was clamped at the basal [glucose] and potassium was either allowed to change (K-unclamped protocol) or was clamped at basal [K\(^+\)] (K-clamped protocol). Specifically, 60–μl blood samples were collected at 10-min intervals from the tail arterial cannula, spun, and plasma analyzed immediately for [glucose] with a Beckman glucose analyzer II and [K\(^+\)] with a Beckman flame photometer as described above.

Infusion protocols in mice. Transgenic mice (C57BL/6, KD1 line) that express a dominant negative kinase dead (KD) rat α2 isoform (K45R mutation) driven in heart and skeletal muscle by the muscle creatine kinase promoter (34), were obtained from M. Birnbaum (University of Pennsylvania) and bred in the USC animal facility to C57BL/6 mice (Simonsen Laboratories, Gilroy, CA). Offspring were genotyped by PCR as previously detailed (34). Tg-KD1 mice and wild-type littersmates (NTg) were studied at 18–24 wk of age. Both males and females were studied. The protocol of Mu et al. (34), developed to examine the effect of AICAR on blood glucose levels, was modified for collection of nonhemolyzed plasma, requisite for accurate [K\(^+\)] analysis. Mice were acclimated to being handled, to being anesthetized, and to having blood draws for a week before the experiment, as previously described (34). After fasting for 4 h with free access to water, mice were briefly anesthetized (<2 min) with isoflurane, and a baseline blood sample of 60–80 μl was obtained from the submandibular vascular bundle using a Goldenrod lancet (Medipoint, Mineola, NY), collected through a heparinized glass capillary tube into a 0.5-ml microfuge tube and spun for isolation of plasma, which was analyzed for [K\(^+\)] and [glucose]. If the baseline [K\(^+\)] was above 4.9 mM, indicative of hemolysis, or below 3.4 mM, indicative of stress, the mouse was not further studied that day. Only two blood samples could be collected per experiment per animal because the blood removed (2 × 60 μl = 120 μl) represented near 10% of the total mouse blood volume (assuming 1.5 ml/25 g mouse). Preliminary experiments established a reproducible and significant AICAR-stimulated fall in plasma [glucose] in NTg mice at 30 min, as previously reported (34). If the baseline [K\(^+\)] was between 3.4 and 4.9 mM, the mouse was injected intraperitoneally with 0.25 g/kg AICAR and at 30 min after AICAR injection the mouse was again briefly anesthetized with isoflurane (for <2 min) and a second blood sample was collected, as described above. Plasma glucose was measured with a Free Style blood glucose monitor (ThermaSense, Alameda, CA) and plasma potassium by flame photometry as described above.

Immunoblot analysis. AMPK activation was verified in a series by immunoblot analysis of rat muscle extracts for the phosphorylated form of AMPK as well as immunoblot of total AMPK probed as a control. Gastrocnemius muscles were quickly frozen after dissection with paddles cooled in liquid N\(_2\) and stored at −80°C until analysis. Lysates of muscle were prepared for immunoblot as described previously (2, 36). In brief, muscles were homogenized in ice-cold lysis buffer containing phosphatase and protease inhibitors and 1% Triton X-100 and then centrifuged for isolation of supernatant. Each sample of 40 μg was resolved by SDS-PAGE, transferred to polyvinylidene fluoride membranes, blocked overnight in Licor Blocking buffer (LI-COR Bioscience, Lincoln, NE), and then incubated with antibodies against phosphorylated AMPK (pThr172) or total AMPK (both from Cell Signaling, Danvers, MA). Antibody binding was detected with goat anti-rabbit IgG (H+L) Alexa Fluor 680 (Molecular Probes, Eugene, OR) and analyzed by Odyssey Infrared Imaging System and accompanying software (LI-COR Bioscience). Linearity of the detection system with this amount of protein loading was verified in preliminary experiments (not shown).

Muscle Na\(^+\)-K\(^+\)-ATPase activity. Na\(^+\)-K\(^+\)-ATPase activity was measured in triplicate in total membrane preparations (3,000 g supernatant) of whole gastrocnemius muscle of rat collected after 3 h of AICAR infusion during which both plasma [glucose] and [K\(^+\)] were clamped at baseline. Na\(^+\)-K\(^+\)-ATPase activity was measured as previously described in detail as ouabain-sensitive μmol inorganic phosphate (P\(_{i}\)) liberated from ATP per milligram of protein per hour (10). Total Na\(^+\)-K\(^+\)-ATPase activity was calculated as the rate of P\(_{i}\) generated during a 15-min incubation period in the absence of ouabain minus P\(_{i}\) generated in the presence of either 2 mM ouabain (inhibits both α1- and α2-isomls of Na\(^+\)-K\(^+\)-ATPase) or 10⁻⁵ M ouabain.
inhibits $\alpha_2$-isofrom.) Na$^+-$K$^+$-ATPase $\alpha_1$ activity was calculated as total activity minus $\alpha_2$ specific activity.

Muscle Na$^+-$K$^+$-ATPase subcellular distribution. To determine whether AICAR stimulated trafficking of muscle Na$^+$-K$^+$-ATPase $\alpha_2$ from intracellular pools to the plasma membrane, a sample set that was used to establish AICAR-stimulated muscle glucose uptake and GLUT4 translocation from intracellular membranes to plasma membranes (29) was re proleted for Na$^+$-K$^+$-ATPase $\alpha_2$ translocation, as well as re proleted to verify GLUT4 translocation. In brief, after AICAR infusion (3), plasma membranes, T-tubules, and intracellular membranes were isolated from 7 to 8 g of muscles (tibialis, gastrocnemius, and quadriceps) using a procedure established by the Marette laboratory (14). Membrane fractions (10 $\mu$g each) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot analysis as described above using a polyclonal anti-Na$^+$-K$^+$-ATPase $\alpha_2$ antiserum (McDonough lab) at 1:500 (39) and anti-GLUT 4 polyclonal antiserum (Biogenesis, Kingston, NH) at 1:1,000. Immunoblots were quantitated as described above and abundance in plasma membranes (PM), intracellular membranes (IM), and T-tubules (TT) expressed as fraction of the total where abundance in PM+IM+TT is defined as 1.0 within each animal.

Quantitation and statistical analyses. Data are expressed as means $\pm$ SE except where standard deviation is indicated. The significance of the differences in mean values between groups was determined by a Student's $t$-test when comparing data before and after treatment in the same animal and unpaired two-tailed $t$-test when comparing different animals. Differences were considered significant at $P < 0.05$.

RESULTS

AICAR activates AMPK and produces a decrease in plasma K$^+$ levels in euakalemic rats. To test the hypothesis that AMPK activation could play a role in clearing K$^+$ from the ECF, conscious rats were infused with AICAR (bolus of 38.4 mg/kg then continuously at 4 mg $\cdot$ kg$^{-1} \cdot$ min$^{-1}$ in saline) through a tail vein while real time plasma [K$^+$] and [glucose] were measured every 10 min and [glucose] clamped at baseline as described (8, 10). Controls, assayed in parallel on the same day and time, were infused with a matched volume of saline (n = 6 pairs). Plasma [K$^+$] remained at baseline (4.2 $\pm$ 0.1 mM), began to drop after 60 min of AICAR infusion, and fell to 3.2 $\pm$ 0.1 mM between 140 and 180 min (Fig. 1A), a clearance of 24% of the ECF K$^+$. In the saline-infused group, [K$^+$] fell slightly to 4.0 $\pm$ 0.5 mM, likely due to fasting.

If the effect of AICAR on [K$^+$] is mediated by AMPK activation, the two parameters should change with the same time course. Changes in AMPK activity can be inferred from the increase in the phosphorylation of AMPK$\alpha$. We examined skeletal muscle because it is critical to the effect of AMPK activation on plasma glucose (34) and because it contains more than 90% of the body’s K$^+$ stores (41). Rats were euthanized at 20, 60, 120, and 180 min of AICAR infusion (n = 3 each) and controls after 180 min vehicle infusion. The time course of change in plasma [K$^+$] was compared with the change in abundance of phosphorylated AMPK$\alpha$ in the gastrocnemius muscle. As summarized in Fig. 1B, abundance of phosphorylated AMPK$\alpha$ increased twofold, evidence for the efficacy of the AICAR to stimulate AMPK activity. The P-AMPK is elevated in the controls compared with the 20-min AICAR infusion sample probably secondary to the design in which the controls were euthanized after 180 min of vehicle infusion during fasting, which may have elevated the AMPK activity. Figure 1C illustrates a significant correlation between the drop in plasma K$^+$ and the increase in AMPK$\alpha$ phosphorylation in the 15 rats assayed ($R^2 = 0.7642$ and $P = 0.00002$). The relationship supports the hypothesis that the drop in plasma K$^+$ is secondary to activation of AMPK by AICAR.

AICAR maintains its K$^+$-lowering effect in rats when baseline [K$^+$] is elevated or lowered. A new strategy to decrease plasma [K$^+$] during hyperkalemia could be therapeutically useful (24). To determine whether AICAR could lower elevated plasma K$^+$, rats were fed a gelled high-K$^+$ diet, which also contained the mineralocorticoid antagonist spironolactone to inhibit renal K$^+$ secretion. After 8–10 days, plasma [K$^+$] rose significantly to 4.5 $\pm$ 0.1 mM. AICAR infusion (Fig. 2A) caused plasma [K$^+$] to fell to 3.8 $\pm$ 0.15 mM, a 17% loss of plasma K$^+$. In paired controls, plasma K$^+$ fell from a baseline of 3.9 $\pm$ 0.09 to 3.0 $\pm$ 0.19 mM, a 23% loss of plasma K$^+$. These results suggest that AMPK activation may be a useful adjunct strategy to lower K$^+$ in hyperkalemia.

When rats are fed a KD diet, plasma [K$^+$] falls and the rats develop a resistance to insulin-stimulated cellular K$^+$ uptake (8–10). We fed rats KD diet to determine whether they developed resistance to AICAR-stimulated K$^+$ clearance. After 7–10 days, baseline plasma [K$^+$] was depressed significantly to 3.2 $\pm$ 0.1 mM in KD fed rats (n = 3 pairs). AICAR infusion (Fig. 2B) provoked a further fall in plasma [K$^+$] to 2.5 $\pm$ 0.2 mM at 150 min, a 23% loss of plasma K$^+$. In paired controls, plasma K$^+$ fell from a baseline of 4.0 $\pm$ 0.2 to 3.1 $\pm$ 0.1 mM at 150 min, also a 23% loss of plasma K$^+$. These results indicate that AICAR does provoke clearance of K$^+$ from the ECF in the hypokalemic rat model that is resistant to insulin-stimulated K$^+$ clearance. These results suggest that AICAR and insulin stimulate K$^+$ clearance by parallel independent pathways.

AICAR does not increase renal K$^+$ excretion. A fall in plasma [K$^+$] can be explained by: 1) active K$^+$ uptake from ECF to intracellular fluid (ICF), analogous to insulin stimulation of muscle Na$^+$-K$^+$-ATPase, 2) net transfer of K$^+$ from the ECF to the ICF by inhibition of a passive K$^+$ efflux route, or 3) increased urinary or fecal K$^+$ excretion. The AICAR-stimulated drop in [K$^+$] (Figs. 1A and 2, A and B) represents a shift of about 23% of ECF K$^+$ (~60 $\mu$mol) out of the ECF compartment occurring over 90 min. It would be very difficult to detect a shift of this magnitude into the much larger ICF K$^+$ pool which is ~150,000 $\mu$mol ($45$ ICF volume is 40% of a 300-g body wt) during AICAR infusion. In the experiments described in Fig. 1A, urine was collected from the bottom of the cage for 3 h after AICAR and for the 3 h during AICAR infusion (bladder urine was pooled with the AICAR infusion samples). Rats (n = 6 pairs) had free access to water, but no food, during these collections. As summarized in Fig. 3, urinary K$^+$ values were quite variable between individual rats but did not increase consistently with AICAR infusion. Urinary K$^+$ (in mmol $\cdot$ kg$^{-1} \cdot$ h$^{-1}$, means $\pm$ SD) in the control group was 0.60 $\pm$ 0.49 before infusion and 0.96 $\pm$ 0.60 during paired saline infusion, whereas in the AICAR group, urinary K$^+$ was 1.13 $\pm$ 1.18 before infusion and 1.20 $\pm$ 0.51 during AICAR infusion. These results do not support the hypothesis that the AICAR-stimulated fall in plasma K$^+$ is due to increased urinary K$^+$.
secretion during AICAR infusion. Related to the question of $K^+$ clearance by urinary excretion, KD diet also lowers urinary $K^+$ to conserve $K^+$ stores. Urinary $[K^+]$ (in mM, means ± SD) was measured in urine collected from bladders at the end of the experiments in Fig. 2B. In the rats fed the KD diet, urine $[K^+]$ was 1.7 ± 1.0 mM, significantly lower than the 30.3 ± 8.5 mM $[K^+]$ measured in urine of control $K^+$ fed rats. The very low $[K^+]$ in the AICAR-infused rats fed the KD diet suggests that $K^+$ is not cleared from the ECF to the urine.

Quantitation of AICAR effect measured by simultaneous euglycemic eukalemic clamps. Simultaneous potassium-glucose clamp technique (8–10) was used to measure the rate of net transport of $K^+$ and glucose out of the plasma as a function of time. In this protocol $K^+$ infusion rate ($K_{inf}$) and glucose infusion rate ($G_{inf}$) are varied empirically to maintain $[K^+]$ and [glucose] at baseline. Figure 4 summarizes $K_{inf}$ (Fig. 4B) and $G_{inf}$ (Fig. 4D) during AICAR infusion in rats in which plasma $[K^+]$ and plasma [glucose] were clamped at baseline (demonstrated in Fig. 4, A and C, respectively) in eight paired sets of rats. The very similar time courses of change in $K_{inf}$ and $G_{inf}$ imply a shared mechanism of action; i.e., AMPK activation, of glucose and potassium clearance. At equilibrium after 120 min, $K_{inf}$ stays at 15.7 ± 1.3 µmol·kg$^{-1}$·min$^{-1}$ and $G_{inf}$ at 62.4 ± 6.3 µmol·kg$^{-1}$·min$^{-1}$.

Lack of effect of AICAR on muscle $Na^+-K^+$-ATPase activity or plasma membrane abundance. Skeletal muscle AMPK is a candidate participant in the response to AICAR: muscle contains >70% of the body’s pool of $K^+$ and can regulate $K^+$ distribution between the ICF and ECF compartments (11, 31, 41). Insulin and AICAR both provoke glucose clearance from
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plasma by stimulating translocation of GLUT4 from intracellular membranes to plasma membranes (22–29). Insulin provokes K⁺ clearance from plasma by increasing muscle plasma membrane sodium pump activity (Na⁺-K⁺-ATPase), but whether this is mediated by translocation of muscle Na⁺-K⁺-ATPase (21, 28, 30) or activation of Na⁺-K⁺-ATPase in the plasma membrane (32) remains controversial. To test the hypothesis that AICAR provokes clearance of K⁺ by activating muscle Na⁺-K⁺-ATPase, we performed two assays. First, we assayed ouabain-sensitive Na⁺-K⁺-ATPase α₂- and α₂-isofrom maximal activity (Vₘₐₓ) in homogenates of gastrocnemius muscles isolated from rats at the end of 180 min AICAR infusion. The protocol of Mu et al. (34), used to demonstrate that the AICAR-mediated glucose clearance was blunted in this strain, was modified for collection of nonhemolyzed plasma, requisite in TT, none detected in the IM and no changes after AICAR infusion (29). We reprob for GLUT4 and Na⁺-K⁺-ATPase α₂ because it is the major muscle sodium pump and is reported to translocate from IM to PM after insulin stimulation. Figure 5 illustrates typical immunoblots and summarizes the results of experiments from control and AICAR-infused rats. As previously reported by Lemieux et al. (29), AICAR infusion provokes a significant translocation of GLUT4 from IM to PM (P = 0.02) resulting in an increase in PM GLUT4 from 9% to 23% of total GLUT4 (in PM+IM+TT). GLUT4 in IM dropped from 83% to 58% of total. At baseline, Na⁺-K⁺-ATPase α₂ was enriched in both PM and TT (55% and 42%, respectively), and IM contained <5% of the α₂; there was no detectable redistribution with AICAR infusion. These findings do not support the hypothesis that K⁺ clearance is mediated by activation or translocation of Na⁺-K⁺-ATPase; however, it is possible that Na⁺-K⁺-ATPase activation is lost upon homogenization and/or that the fractionation scheme used to separate GLUT4 in IM from that in PM is not optimal to separate IM and PM pools of Na⁺-K⁺-ATPase isoforms.

Inactivation of AMPK blunts AICAR-stimulated clearance of glucose and potassium. To determine whether the AICAR-stimulated clearance of potassium is dependent on muscle AMPK activity, we studied a mouse model expressing a kinase dead α₂-catalytic subunit under the control of the muscle creatine kinase promoter (Tg-KD1), which has a dominant negative effect on AMPK activity in the muscle and heart (33, 34). We examined the effects of AICAR on plasma [K⁺] and [glucose] in the Tg-KD1 mice and in littermate controls (NTg). The protocol of Mu et al. (34), used to demonstrate that the AICAR-mediated glucose clearance was blunted in this strain, was modified for collection of nonhemolyzed plasma, requisite

Fig. 2. AICAR infusion stimulates clearance of potassium from the plasma in rats with elevated or lowered plasma [K⁺]. AICAR was infused at time 0 (bolus of 38.4 mg/kg then continuously at 4 mg·kg⁻¹·min⁻¹ into tail vein). Controls were infused with paired volume of saline. Values are means ± SE. A: rats (n = 4 pairs) fed a 4% K⁺ diet containing 3 mg/g spironolactone had significantly elevated baseline plasma [K⁺], P < 0.001, which was significantly lowered with AICAR infusion, P < 0.001. B: rats fed a K⁺-free diet (n = 3 pairs) had significantly lower baseline plasma [K⁺], P < 0.002, which was significantly lowered with AICAR infusion, P < 0.004.

Fig. 3. AICAR infusion did not significantly increase urinary K⁺ excretion. Urinary K⁺ (mmol·kg⁻¹·3 h⁻¹) collected from the bottom of the cage 3 h before and 3 h during AICAR infusion, the latter pooled with urine collected from the bladder collected after the experiment. Same rats as in Fig. 1A, n = 6 pairs. Lines connect values from the same rat. Bar indicates mean.
for accurate [K+] analysis, as described in METHODS. A baseline plasma sample was collected and analyzed and, if [K+] was between 3.4 and 4.9 mM, the mouse was injected intraperitoneally with 0.25 g/kg AICAR, and a second blood sample was collected at 30 min after AICAR. The experiment was limited to two blood samples because the blood removed (2 ± 0.6 to 120 ± 1 ml) represented nearly 10% of the total mouse blood volume. The AICAR-stimulated decrease in plasma glucose in NTg (33, 34) served as the positive control. As summarized in Fig. 6, baseline glucose levels were indistinguishable in the NTg versus Tg-KD1 strains (134.0 ± 4.8 and 142.7 ± 5.5 mg/dl, respectively), and saline injection did not change [K+] significantly. AICAR injection provoked significant decreases in [K+] to 3.6 ± 0.1 mM in NTg and to 3.9 ± 0.2 in Tg-KD1, clearances of 15% of the ECF [K+] in the NTg strain and 6% in the Tg-KD1 strain. The decrease in [K+] in Tg-KD1 was significantly blunted compared with that in the NTg (P = 0.045), leading us to conclude that muscle AMPK activation plays a role in the clearance of K+ from plasma during AICAR infusion.

DISCUSSION

The central finding of this study is that AICAR, an activator of AMPK, provokes a significant fall in plasma potassium concentration (Figs. 1, 2, and 6). The finding suggests that
AMPK activation, naturally observed during exercise or ischemia, may play a role in the clearance of excess K\textsuperscript{+} that builds up in the interstitial fluid in these conditions. The fall in plasma K\textsuperscript{+} was not a function of the starting ECF K\textsuperscript{+} because it was still evident in rat models in which the ECF K\textsuperscript{+} was either increased by high K\textsuperscript{+} diet plus spironolactone or decreased by KD diet. Interestingly, serious hyperkalemia is known to develop in a significant fraction of patients taking spironolactone as a heart failure medication, secondary to inhibition of renal K\textsuperscript{+} secretion (1, 5, 23). AICAR infusion restored K\textsuperscript{+} to baseline in the high K\textsuperscript{+} plus spironolactone-treated rats, which suggests that AMPK-activating agents may prove beneficial when used in combination with drugs that raise plasma K\textsuperscript{+} such as spironolactone. On the other hand, when plasma K\textsuperscript{+} was depressed by KD diet, treatment with AICAR lowered plasma K\textsuperscript{+} to 2.5 mM, a level associated with marked symptoms of hypokalemia including muscle weakness and cardiac arrhythmias (1). Thus therapeutic interventions that increase AMPK activity, a key target for anti-diabetic drugs, could potentially provoke dangerous hypokalemia in patients that are already prone to potassium depletion by diuretics or fasting (46).

The phenotypic effects of AMPK activation in the muscle are currently assigned to changes in metabolism, without attention to electrolyte shifts. The results of this study predict that plasma [K\textsuperscript{+}] fell in all of the AICAR infusion studies reported to date. We suggest that some of the phenotypic changes that have been reported to occur with AMPK stimulation or inactivation may be secondary to changes in the transmembrane potassium gradient and membrane potential. For example, mice deficient in muscle and heart AMPK activity exhibit significant muscle weakness and impaired recovery from fatigue (33); mutations in AMPK γ-subunits are associated with cardiac hypertrophy and abnormalities in cardiac electrical conductance (7); and mouse hearts deficient in AMPK are more susceptible to damage during ischemia and reperfusion (40). A critical question that remains to be examined is whether a lack of AMPK activity, as seen in these models, blunts K\textsuperscript{+} clearance during recovery from exercise or ischemia, which could provoke muscle fatigue and pain and weakness (41).

Is the AICAR-mediated clearance of plasma potassium K\textsuperscript{+} due to AMPK activation? The alternative explanation would be that the ZMP produced from AICAR could mimic other effects of AMP. The finding that the AICAR-stimulated clearance of glucose from the plasma is dependent on muscle AMPK activity was established in a mouse model expressing a kinase dead δ2-catalytic subunit (Tg-KD1), which has a dominant negative effect on AMPK activity (33, 34). If the AMPK clearence of K\textsuperscript{+} is indeed mediated by muscle AMPK activation, then the drop in plasma [K\textsuperscript{+}] as well as [glucose] should be blocked or blunted in the Tg-KD1 mice. We did see a significant blunting in the AICAR-induced K\textsuperscript{+} clearance in the Tg-KD1 mice compared with the NTg controls, which supports the notion that the K\textsuperscript{+} clearance is secondary to AICAR activation of AMPK activity. Other evidence in this study supporting the hypothesis that the drop in plasma [K\textsuperscript{+}] is secondary to the activation in AMPK include: a significant temporal relationship between the increase in AMPK activity, measured as AMPK-P abundance and the decrease in plasma [K\textsuperscript{+}] (Fig. 1C), and the very similar temporal relationship between AICAR-induced G\textsubscript{inf} [which has been established to be due to AMPK activation (3, 35)] and AICAR-induced K\textsubscript{inf} (Fig. 4). The fact that significant decreases in glucose and K\textsuperscript{+} persist in the Tg-KD1 mice infused with AICAR suggest that that ZMP exerts unidentified AMP-like effects that stimulate glucose and K\textsuperscript{+} cellular uptake or, more likely, that other nonmuscle tissues may participate in AICAR-stimulated glucose and potassium clearance. Related to this second point, when DeFronzo and colleagues (13) reported that insulin caused a decrease in plasma [K\textsuperscript{+}], they also determined that 70% of the drop in [K\textsuperscript{+}] observed during the first hour of insulin infusion could be accounted for by net uptake by the liver (13).

The magnitude of the AICAR-provoked fall in plasma potassium suggests that this drug is a potent stimulator of K\textsuperscript{+} clearance. Interestingly, there are similarities between signals that control K\textsuperscript{+} and glucose homeostasis: insulin stimulates cellular uptake of both glucose and K\textsuperscript{+} after a meal (31), exercise stimulates insulin-independent cellular uptake of both glucose and potassium uptake in muscle, and in this study we establish that AICAR stimulates the clearance of both glucose and potassium from the ECF. In a recent study from this laboratory using the same rat vendor and tail vein infusion and blood sampling protocols (8), we measured K\textsubscript{inf} and G\textsubscript{inf} stimulated by infusion of insulin at 5 mU·kg\textsuperscript{-1}·min\textsuperscript{-1} for 150
min in control rats. During the last 60 min of insulin infusion $K_{inf}$ stayed at 9.7 $\pm$ 1.48 $\mu$mol·kg$^{-1}$·min$^{-1}$ and $G_{inf}$ at 172 $\pm$ 16 $\mu$mol·kg$^{-1}$·min$^{-1}$. In comparison, in this study, AICAR increased $K_{inf}$ to a new steady state of 15.7 $\pm$ 1.3 $\mu$mol·kg$^{-1}$·min$^{-1}$ and $G_{inf}$ to 62 $\pm$ 6 $\mu$mol·kg$^{-1}$·min$^{-1}$ during the last 60 min. These two sets of findings can be compared in order to provide a relative measure of the strength of AICAR to insulin on $K^+$ and glucose clearance. The comparison reveals that, at the doses studied, insulin stimulates nearly threefold more glucose clearance than AICAR, whereas AICAR stimulates 1.6-fold more $K^+$ clearance than insulin, demonstrating that AICAR is a powerful stimulator of $K^+$ clearance. In the study of Chen et al. (8) insulin infusion had to be raised to the pharmacological level of 50 mU·kg$^{-1}$·min$^{-1}$ for 1 h to decrease plasma $[K^+]_p$ to 3.2 mM, which is in the range of the decrease seen with AICAR.

What is the destination of the potassium that leaves the plasma? Potential mechanisms to clear $K^+$ include: 1) urinary or fecal excretion, 2) increase in rate of active $K^+$ transport from ECF to ICF, and 3) decrease $K^+$ leak from ICF to ECF with unchanged active $K^+$ uptake. The mechanism to clear glucose from the plasma is restricted to shift from ECF to ICF because there is normally no glucose excretion. Evidence against a mechanism of increased urinary excretion of $K^+$ in response to AICAR include the observation that there was not a significant increase in urinary $K^+$ excretion during AICAR infusion (Fig. 3). In addition, we found that AICAR provoked a drop in plasma $K^+$ even in rats fed spironolactone or KD diet, which are two interventions that are well known to depress $K^+$ secretion and excretion (25). Since the ICF $K^+$ pool is $\sim$14,400 $\mu$mol, it was not feasible to attempt to detect a transfer of 42 $\mu$mol from ECF to ICF. Along the same vein, since dietary intake of $K^+$ is so high, detecting an increase in colonic excretion was not feasible. For these reasons, we cannot conclude that $K^+$ is transferred from the ECF to the ICF during AICAR stimulation, but this is our working hypothesis that has the experimental support of the urinary excretion studies.

There is an extensive literature that demonstrates that insulin infusion, contractility, and catecholamine stimulation activate muscle $Na^+-K^+$-ATPase activity and increase active cellular uptake of $K^+$ (12, 32, 43). There is also literature that demonstrates AICAR stimulates net translocation of GLUT4 to plasma membrane in muscle, which contributes to the AICAR-induced clearance of glucose (22, 27, 29, 50). For these reasons we aimed to examine whether there was evidence for sodium pump activation or translocation to the plasma membrane during AICAR infusion. In short, our analysis of total $Na^+-K^+$-ATPase $V_{max}$ activity and reanalysis of a set of membranes that were used to demonstrate GLUT4 translocation during AICAR infusion (29) did not support the hypothesis that AICAR provokes an increase in sodium pump-mediated active $K^+$ uptake that could account for the fall in plasma $K^+$. It is possible that more direct in vivo assays of sodium pump activity or ouabain-sensitive oxygen consumption would detect a change in $Na^+-K^+$-ATPase. Whereas these studies are not informative regarding a molecular mechanism for $K^+$ clearance from the plasma, the lack of stimulation $Na^+-K^+$-ATPase activity by AICAR is consistent with the overall role of AMPK as a cellular fuel gauge that decreases ATP utilization and increases ATP production as a stimulation of active $K^+$ uptake via the Na$^+$-K$^+$-ATPase would certainly increase ATP consumption.

Whereas exercise was not a variable in this study, we hypothesize that AMPK activation could play a role in clearing the $K^+$ from interstitial fluid during exercise. Interstitial $[K^+]$ climbs around an exercising muscle as the rate of $K^+$ efflux rises above the rate of active $K^+$ uptake via Na$^+$-K$^+$-ATPase (41). As the rates of $K^+$ efflux and active $K^+$ uptake are increasing, the rate of ATP utilization (by sodium pump and contractile apparatus) is increasing as well. We predict a tipping point will be reached, depending on severity and length of the exercise, at which the cell AMP-to-ATP ratio will climb sufficiently to increase AMPK activity. We hypothesize that this increased AMPK activity may contribute to normalizing the rate of $K^+$ efflux to influx, which will ultimately restore baseline $[K^+]$ when the exercise stops. A corollary hypothesis is that chronic metformin treatment, an AMPK activator prescribed for Type 2 diabetes (37) may increase the rate of restoration baseline $K^+$ following exercise.

There is a literature on the effects of AICAR and AMPK stimulation on ion transporters in epithelia that can shed light on potential molecular mechanisms of the potassium clearance observed in this study. AMPK was identified as a binding partner of the cystic fibrosis transmembrane conductance regulator (CFTR) in a yeast two-hybrid screen, and its co-expression with CFTR in an oocyte significantly inhibited cAMP-activated CFTR Cl$^-$ conductance (18). This inhibition was also observed when AMPK activity was stimulated in cultured lung cells (17). AMPK activation similarly inhibited epithelial sodium channel (ENaC) currents expressed in oocytes and polarized mouse collecting duct cells. In this case the interaction is not direct but involves AMPK-mediated phosphorylation of a protein (Nedd4-2) that regulates ENaC retrieval from the plasma membrane (6). Taken together, these studies indicate that AMPK may, directly or indirectly, limit the dissipation of transmembrane ion gradients, which would conserve the ATP necessary to maintain the gradients (16). As discussed above, $K^+$ could be translocated from the ECF to the ICF either by increasing active $K^+$ uptake mediated by the Na$^+$-K$^+$-ATPase or by decreasing $K^+$ efflux via a passive efflux route; that is, decreasing the ratio of $K^+$ efflux to $K^+$ influx. One hypothesis that reconciles the role of AMPK as a cellular fuel gauge with the observed clearance of $K^+$ from the ECF is that AMPK, acting directly or indirectly, may decrease the $K^+$ efflux rate, which would promote net cellular $K^+$ uptake by decreasing the ratio of $K^+$ efflux (via channels/leaks) to $K^+$ influx (via ATPases). Such a change in $K^+$ flux ratio would clear $K^+$ that accumulates in the ECF during exercise or ischemia without increasing ATP consumption.

Kristensen et al. (26) have analyzed membrane proteins involved in potassium shifts in muscle using specific inhibitors and come to the conclusion that ATP-sensitive $K^+$ channels ($K_{ATP}$) and large-conductance Ca$^{2+}$-dependent $K^+$ channels (BK$_{Ca}$) are responsible for $K^+$ release during muscle contraction, whereas the strong inward rectifier 2.1 $K^+$ channel (Kir2.1) and the NKCC (Na$^+$-$K^+$-$2Cl$^-$) cotransporter participate in $K^+$ reuptake. Wyatt et al. (49) have recently reported that AICAR treatment of carotid body type 1 cells inhibits oxygen-sensitive $K^+$ currents carried by BK$_{Ca}$, indicating this is a candidate that could be pursued in analysis of the molecular basis for the plasma $K^+$-lowering effect of AMPK acti-
vation. Nielsen et al. (38) have shown that \( K_{ATP} \) channels are located mainly in the plasma membrane of muscle, found in all muscle fiber types, are active in resting muscle where they contribute to baseline interstitial \([K^+]\) and that the \( K_{ATP} \) channel inhibitor glibenclamide acutely lowers interstitial \([K^+]\), suggesting that the \( K_{ATP} \) channel would be another good candidate. This \( K^+ \) efflux pathway is activated by decreasing concentrations of ATP and has been shown to be inhibited by AICAR in pancreatic \( \beta \) cells (47). In contrast, bouts of hypoxia (which would presumably activate AMPK) have been reported to traffic \( K_{ATP} \) channels to the plasma membrane and activate channel activity in cardiomyocytes (42). In summary, the molecular mechanism(s) responsible for the clearance of \( K^+ \) from the ECF during AMPK stimulation with AICAR remain to be determined, as does the exact tissues involved in the response.

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