^{86}Rb is not a reliable tracer for potassium in skeletal muscle

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For technical reasons, ^{86}Rb is frequently preferred to ^{42}K as a tracer for K^+. Systematic comparisons of the two isotopes, however, are rarely done. In this paper we compare the transport of ^{42}K and ^{86}Rb in rat and mouse soleus muscle and in rat erythrocytes. Ouabain-suppressible K^+ uptake in rat soleus was the same whether measured with ^{42}K or ^{86}Rb, both when stimulated by insulin, salbutamol and calcitonin-gene-related peptide (CGRP), and when inhibited by graded concentrations of ouabain. Control experiments with rat erythrocytes, where Na^+–K^+–Cl^- co-transport has earlier been demonstrated, showed closely similar inhibitory effects of bumetanide on ^{42}K and ^{86}Rb uptake. In contrast, bumetanide produced no significant change in ^{42}K uptake of rat and mouse soleus muscle, but clearly inhibited ^{86}Rb uptake at concentrations down to 10^{-7} M ($P < 0.001$). Whereas the addition of 150 mM NaCl had no effect on ^{42}K uptake in rat soleus, ^{86}Rb uptake, and in particular the bumetanide-suppressible component, was markedly increased by this addition. The inhibitory effect of bumetanide on ^{86}Rb uptake gives rise to the false impression that skeletal muscle contains a $NaKCl_2$ co-transport system. Efflux studies showed that the fractional loss of ^{42}K from rat soleus muscle is 2.3 times larger than that of ^{86}Rb. Salbutamol and CGRP increased ^{86}Rb efflux, but inhibited ^{42}K efflux. This implies that for studies of K^+ efflux and bumetanide-sensitive K^+ transport, ^{86}Rb is not even an acceptable tracer for the detection of qualitative changes. Control experiments with ^{42}K are essential in any characterization of unknown K^+ transport processes.

INTRODUCTION

For the study of potassium transport, ^{86}Rb is frequently preferred to ^{42}K as a tracer for potassium (K^+), primarily because Rb^+ and K^+ have very similar physical and chemical properties (Ussing, 1960), but also because the half-life of ^{86}Rb is more convenient (18.7 days) than that of ^{42}K (12.4 h). When present in trace amounts, the two isotopes yield very similar values for the Na^+–K^+-pump-mediated fluxes of K^+ in smooth muscle cell preparations (Widdicombe, 1977; Smith et al., 1986) and skeletal muscle (Clausen et al., 1987). In contrast, a series of recent reports have pointed out that in measurements of K^+ efflux, the use of ^{86}Rb and ^{42}K gives rise to diverging results, in particular during exposure to channel openers (Henquin, 1980; Dawson et al., 1986; Smith et al., 1986, Videbæk et al., 1988; Bray and Weston, 1989; Newgreen et al., 1990). To our knowledge, however, no systematic comparison has so far been made in intact skeletal muscle. During a recent characterization of bumetanide-sensitive ion fluxes in isolated rat muscle (Dørup and Clausen, 1991), we observed that measurements performed using ^{86}Rb and ^{42}K give rise to markedly different results. This prompted a more comprehensive study from which it appeared that the fractional losses of the two isotopes differ by a factor of 2.3 and that following the addition of, for example, salbutamol or rat calcitonin gene related peptide (rCGRP) diametrically opposite changes are observed. This implies that for studies of channel-mediated K^+ efflux and bumetanide-sensitive K^+ transport, ^{86}Rb is not even an acceptable tracer for the detection of qualitative changes. On the other hand, dual isotope experiments demonstrated that, over a wide range of transport rates, ^{42}K and ^{86}Rb yield closely similar values for Na^+–K^+-pump-mediated K^+ uptake in rat soleus muscle.

MATERIALS AND METHODS

Animals and diets

Most experiments with soleus muscles were performed using 4-week-old male or female Wistar rats (60–70 g body weight). In some experiments 9-week-old male NMRI (Navy Medical Research Institute) mice (38–46 g body weight) were used. Erythrocytes were prepared from 12-week-old female Wistar rats. The animals had free access to food (Altromin, Lage, Germany) and water and were kept in an environment with constant temperature (21 °C) and day length (12 h).

Muscle preparations and incubations

Animals were killed by decapitation and the intact soleus muscle dissected out as previously described (Kohn and Clausen, 1971). The standard incubation medium was Krebs–Ringer bicarbonate buffer, pH 7.4, containing 120.2 mM NaCl, 25.1 mM $NaHCO_3$, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 1.3 mM $CaCl_2$ and 5 mM D-glucose. For wash-out following some of the incubations, a Na^+-free Tris/sucrose buffer (10 mM Tris/HCl, 263 mM sucrose, pH 7.4) was used. In order to allow adequate oxygenation (Maltin and Harris, 1985), the incubations in the standard medium took place at 30 °C under continuous gassing with humidified O_2/CO_2 (19:1) in a volume of 2–3 ml. Immediately after preparation, the muscles were equilibrated in the standard medium for 30–60 min and then taken for further incubations. This procedure has previously been shown to allow the maintenance of a constant membrane potential and a high intracellular $[K^+]/[Na^+]$ ratio for several hours *in vitro* (Kohn and Clausen, 1971; Clausen and Kohn, 1977; Clausen and Flatman, 1977).

Abbreviations used: CGRP, calcitonin-gene-related peptide; rCGRP, rat CGRP.

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^{42}K, ^{86}Rb, ^{134}Cs and ^{204}Tl uptake into skeletal muscle

Following equilibration, the muscles were preincubated for 15 min in Krebs–Ringer bicarbonate buffer in the absence or presence of ouabain and/or bumetanide. The muscles were then incubated for 10 or 20 min in Krebs–Ringer bicarbonate buffer containing ^{42}K (0.2 μCi/ml), ^{86}Rb (0.2 μCi/ml), ^{134}Cs (0.2 μCi/ml) or ^{204}Tl (0.01 μCi/ml) without or with the indicated additions. In some experiments the muscles were then washed for 4 × 15 min at 0 °C in non-radioactive Na^+-free Tris/sucrose buffer so as to remove isotope from the extracellular phase. Hereafter, the muscles were blotted, weighed, and homogenized in 2 ml of 0.3 M trichloroacetic acid. Following centrifugation, the ^{42}K, ^{86}Rb, ^{134}Cs or ^{204}Tl activity of the supernatant was determined by measurement of Cerenkow radiation (^{42}K, ^{86}Rb and ^{134}Cs) or by liquid scintillation counting (^{204}Tl). Assuming that the isotopes were representative for K^+ the specific radioactivity of the incubation medium was calculated as c.p.m./nmol of K^+. On the basis of tracer activity of the tissue extract, the uptake of isotope from the buffer was expressed as nmol of K^+/min per g wet wt.

In several experiments muscles were incubated with ^{42}K and ^{86}Rb simultaneously. In these experiments, the sum of the ^{42}K and ^{86}Rb activities of the supernatant was first determined by measurement of Čerenkov radiation. After the decay of ^{42}K, ^{86}Rb activity was determined and ^{42}K activity could be calculated by subtraction.

^{42}K and ^{86}Rb uptake into erythrocytes

Rats were decapitated and blood was collected in a heparinized tube. After centrifugation at 700 *g* for 5 min the white cell layer was removed, and the erythrocytes were washed three times by resuspension in Krebs–Ringer bicarbonate buffer. Following preincubation at 37 °C for 30 min as a 10 % (v/v) suspension in the same buffer, without or with ouabain and/or bumetanide, the cells were incubated for 60 min with ^{42}K (0.5 μCi/ml) and ^{86}Rb (0.5 μCi/ml) and the indicated additions. After incubation, 200 μl samples were transferred to microtubes containing dibutyl phthalate and centrifuged for 1 min at 9400 *g* in a Heraeus microfuge to separate incubation medium and cells. The sedimented cells were separated from the incubation medium using a scalpel blade and the two fractions were counted. ^{42}K and ^{86}Rb activities taken up by the cells was calculated and on the basis of the specific radioactivity of the buffer (c.p.m./nmol of K^+) expressed as nmol of K^+/h per ml of cells.

^{42}K and ^{86}Rb efflux

^{42}K and ^{86}Rb efflux was measured as described previously (Clausen and Kohn, 1977; Everts and Clausen, 1988). After equilibration, the muscles were incubated for 60 min in standard buffer containing ^{42}K or ^{86}Rb (1–2 μCi/ml). The wash-out of ^{42}K and ^{86}Rb was then followed by transferring the muscles through a series of tubes containing 2 ml of standard buffer without isotope. Following incubation, the muscles were blotted, weighed, homogenized in 2 ml of 0.3 M trichloroacetic acid, and taken for counting of ^{42}K and ^{86}Rb activity by Čerenkov radiation. After decay of ^{42}K, ^{86}Rb activity was determined and ^{42}K activity could be calculated by subtraction. By adding successively the activity in the wash-out tubes to that in the muscles at the end of the experiment, the isotope activity in the muscle at each transfer could be determined. On the basis of these values the fractional loss of ^{42}K or ^{86}Rb was calculated for each wash-out period.

Chemicals and isotopes

All chemicals were of analytical grade. ^{42}K (0.04 Ci/mmol), ^{86}Rb (0.3–0.8 Ci/mmol) and ^{134}Cs (0.03 Ci/mmol) were from the Danish Atomic Energy Commission Isotope Laboratory (Risø, Denmark). ^{204}Tl (0.25 Ci/mmol) was obtained from The Radiochemical Centre (Amersham, U.K.). Ouabain, salbutamol and rCGRP were products of Sigma Chemical Co. (St. Louis, MO, U.S.A.). Bumetanide was a gift from Leo Pharmaceuticals (Copenhagen, Denmark), and insulin was a gift from the Novo Nordisk A/S (Copenhagen, Denmark).

Statistics

All results are given as mean ± S.E.M. The significance of difference was assessed by the two-tailed Student's *t* test for groups of unpaired observations. Linear correlation analysis of unweighted values was performed by the method of least squares.

RESULTS

Na^+–K^+-pump-mediated K^+ uptake

Earlier studies have shown that when ^{86}Rb is used as a tracer for K^+ instead of ^{42}K, similar results for Na^+–K^+-pump-mediated K^+ uptake are obtained (Clausen et al., 1987). This observation was re-evaluated in rat soleus muscle using ^{86}Rb at three widely different specific activities. From Figure 1 it can be seen that ^{86}Rb in all instances gave lower values for total and ouabain-resistant K^+ uptake. Ouabain-sensitive ^{42}K uptake (196 ± 42 nmol/min per g wet wt.) was roughly the same as the ouabain-sensitive ^{86}Rb uptake measured using the isotope at low specific activity (205 ± 11 nmol/min per g wet wt.) and in buffer where all K^+ had been replaced by Rb^+ (196 ± 23 nmol/min per g wet wt.). It should be noted, however, that using ^{86}Rb at a high specific radioactivity (791 Ci/mol), ouabain-suppressible K^+ uptake was 252 ± 20 nmol/min per g wet wt., which is 28 % higher than the value obtained with ^{42}K as a tracer for K^+.

The results of this experiment suggested a difference between ouabain-suppressible ^{42}K and ^{86}Rb influx. In order to evaluate

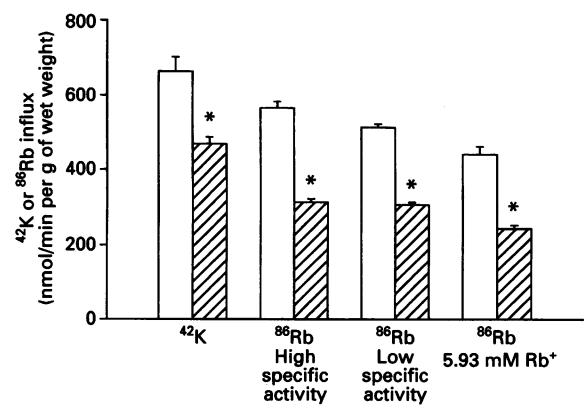

Figure 1 Effect of ouabain and specific radioactivity on ^{42}K or ^{86}Rb uptake in rat soleus muscle

After preincubation for 15 min without or with ouabain (1 mM), some muscles were incubated for 20 min with ^{42}K (0.2 μCi/ml) without or with ouabain. Two other groups of muscles were incubated for the same period with ^{86}Rb (0.2 μCi/ml) with high (791 Ci/mol) or low (18 Ci/mol) specific radioactivity. The last groups of muscles were incubated in buffer where all K^+ had been replaced by Rb^+ (5.93 mM Rb^+, corresponding to a specific radioactivity of 0.034 mCi/mol). After incubation, the muscles were blotted, weighed and homogenized in 2 ml of 0.3 M trichloroacetic acid for counting of ^{42}K or ^{86}Rb. Each bar represents the mean ± S.E.M. of measurements on eight muscles. The difference between muscles treated without (open bar) or with (hatched bar) ouabain was in all instances significant ($^*P < 0.001$).

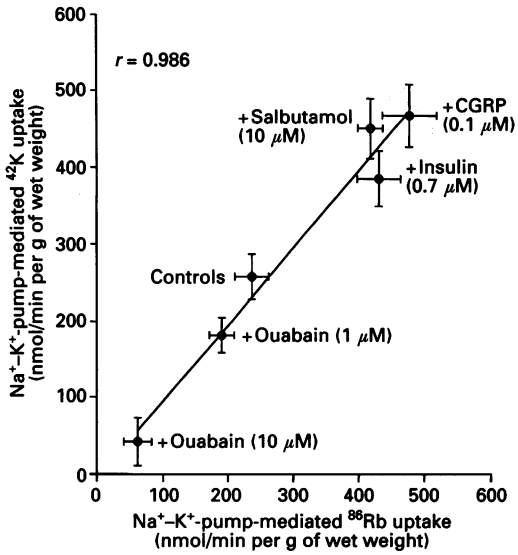

Figure 2 Correlation between Na^+–K^+-pump-mediated ^{86}Rb and ^{42}K uptake in rat soleus muscle

After equilibration for 30–60 min, soleus muscles were preincubated for 15 min in Krebs–Ringer bicarbonate buffer without or with ouabain (10^{-3} M). Then they were incubated in buffer containing ^{42}K (0.2 μCi/ml) and ^{86}Rb (0.2 μCi/ml) without or with ouabain and the indicated additions for 10 min. Two groups of muscles were pre-exposed for 15 min to ouabain at concentrations (10^{-6}–10^{-5} M) producing partial inhibition of the Na^+–K^+ pump. After the 10 min incubation with the isotopes, the muscles were blotted, weighed and homogenized in 2 ml of 0.3 M trichloroacetic acid for counting. The ^{42}K and ^{86}Rb activity of the supernatant obtained by centrifugation of the homogenate was first determined by measurement of Čerenkov radiation. After decay of ^{42}K, ^{86}Rb activity was determined and ^{42}K activity could be calculated by subtraction. Na^+–K^+-pump-mediated ^{86}Rb uptake was calculated as the difference between uptake measured in the absence and the presence of 10^{-3} M ouabain. Each point represents the difference ± S.E.M. between means of measurements performed on four to eight muscles incubated without or with 10^{-3} M ouabain.

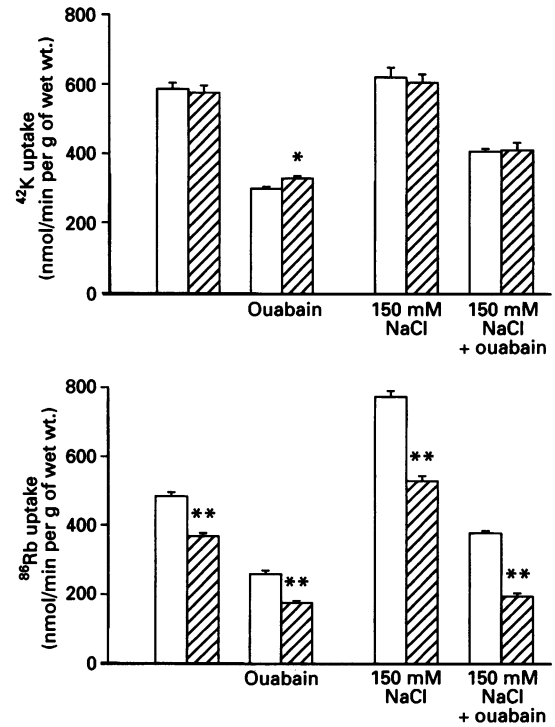

Figure 3 Effect of bumetanide on ^{42}K and ^{86}Rb uptake in rat soleus muscle

After preincubation for 15 min in Krebs–Ringer bicarbonate buffer without (open bar) or with (hatched bar) bumetanide (1 mM) and without or with ouabain (1 mM), the muscles were incubated for 10 min in the same buffer containing ^{42}K (0.2 μCi/ml) and ^{86}Rb (0.2 μCi/ml) without or with the addition of 150 mM NaCl. After washout for 4 × 15 min in ice-cold Na^+-free Tris/sucrose buffer, muscles were blotted, weighed and homogenized in 2 ml of 0.3 M trichloroacetic acid. The ^{42}K and ^{86}Rb activities of the supernatant was determined as described in the legend to Figure 2. Each result represents the mean ± S.E.M. of observations on 8–31 muscles. The significance of differences between muscles treated with or without bumetanide is indicated thus: $^*P < 0.01$, $^{**}P < 0.001$.

this in more detail, eight consecutive experiments determining the effect of ouabain (10^{-3} M) on ^{42}K and ^{86}Rb uptake (10 min incubations with each isotope separately) in soleus muscle were compared. The ouabain-suppressible ^{42}K uptake determined in eight experiments, each comprising comparison of four muscles incubated without ouabain with four muscles incubated with ouabain (i.e. 4 versus 4), gave a mean value of 242 ± 11 nmol/min per g wet wt.. Ouabain-suppressible ^{86}Rb uptake determined in eight other experiments (specific radioactivity of ^{86}Rb 200 Ci/mol) of the same format amounted to 248 ± 12 nmol/min per g wet wt. (not significant, $P > 0.5$).

The ouabain-suppressible components of ^{42}K and ^{86}Rb uptake were compared also when the Na^+–K^+ pump was partially blocked by 10^{-6}–10^{-5} M ouabain or stimulated by salbutamol, rCGRP or insulin (Clausen and Kohn, 1977; Clausen and Flatman, 1977; Andersen and Clausen, 1993). As shown in Figure 2, the Na^+–K^+-pump-mediated ^{86}Rb and ^{42}K uptakes were closely correlated for the six conditions tested ($r = 0.986$, $P < 0.001$). Taken together, these results confirm that it is justified to use ^{86}Rb as a tracer for the measurement of Na^+–K^+-pump-mediated K^+ uptake.

Effects of bumetanide on ^{42}K and ^{86}Rb uptake in soleus muscle

Figure 3 compares the effects of a supramaximal concentration of bumetanide (10^{-3} M) on the uptake of ^{42}K and ^{86}Rb in rat soleus muscle. Under basal conditions, bumetanide-suppressible ^{86}Rb uptake (Figure 3, lower panel) amounted to 114 ± 15 nmol/min per g wet wt. ($P < 0.001$), whereas in muscles preincubated with ouabain (1 mM) the value was 82 ± 12 nmol/min per g wet wt. ($P < 0.001$). The addition of 150 mM NaCl increased ^{86}Rb uptake by 60%, and bumetanide-sensitive ^{86}Rb uptake was stimulated (amounting to 244 ± 23 nmol/min per g wet wt., $P < 0.001$). Again, in the presence of ouabain a slightly lower bumetanide-suppressible component was obtained (182 nmol/min per g wet wt., $P < 0.001$).

These effects are all in striking contrast with the effects of bumetanide on ^{42}K uptake in the same muscles. Under none of the four conditions tested (basal, 10^{-3} M ouabain, and hyperosmotic buffer without or with ouabain) was any effect of bumetanide on ^{42}K uptake observed (Figure 3). Furthermore, whereas the hyperosmotic buffer produced a marked stimulation of ^{86}Rb uptake, it caused no change in ^{42}K uptake. In several control experiments performed using ^{42}K as the only tracer for K^+, bumetanide also failed to induce any detectable changes in ^{42}K uptake. Thus, the discrepancies between the uptake of ^{42}K and ^{86}Rb were not due to counting problems related to the simultaneous use of the two isotopes.

Table 1 shows the effect of increasing concentrations of bumetanide on ^{42}K and ^{86}Rb uptake in rat soleus muscle. The maximum inhibition of ^{86}Rb uptake amounted to 135 nmol/min

Table 1 Dose–response relationship for the effect of bumetanide on ^{42}K and ^{86}Rb uptake in rat soleus muscle

In experiment 1, muscles were preincubated for 15 min in Krebs–Ringer bicarbonate buffer without or with the indicated concentrations of bumetanide, and then incubated for 10 min in the same buffer plus ^{86}Rb (0.2 μCi/ml) or ^{42}K (0.2 μCi/ml). The muscles were then washed for 4 × 15 min in ice-cold Na^+-free Tris/sucrose buffer, blotted, weighed and homogenized in 2 ml of 0.3 M trichloroacetic acid. In Expts 2 and 3, the same procedure was used except that muscles were only incubated with ^{42}K. Values are means ± S.E.M., with numbers of muscles in parentheses. The significance of differences between muscles treated without or with bumetanide is indicated by *P*. N.S., not significant.

[Bumetanide] (M)	^{42}K uptake (nmol/min per g wet wt.)	*P*	^{86}Rb uptake (nmol/min per g wet wt.)	*P*
Expt. 1				
0	487 ± 30 (8)		456 ± 11 (32)	
10^{-8}	491 ± 7 (4)	N.S.	433 ± 14 (16)	N.S.
10^{-7}	476 ± 12 (4)	N.S.	390 ± 5 (19)	< 0.001
10^{-6}	497 ± 14 (4)	N.S.	361 ± 9 (16)	< 0.001
10^{-5}	470 ± 4 (4)	N.S.	336 ± 7 (16)	< 0.001
10^{-4}	442 ± 16 (3)	N.S.	319 ± 9 (16)	< 0.001
10^{-3}	427 ± 21 (4)	N.S.	309 ± 12 (8)	< 0.001
Expt. 2				
0	548 ± 11 (16)			
10^{-4}	553 ± 17 (16)	N.S.		
Expt. 3				
0	607 ± 20 (4)			
10^{-3}	595 ± 43 (4)	N.S.		

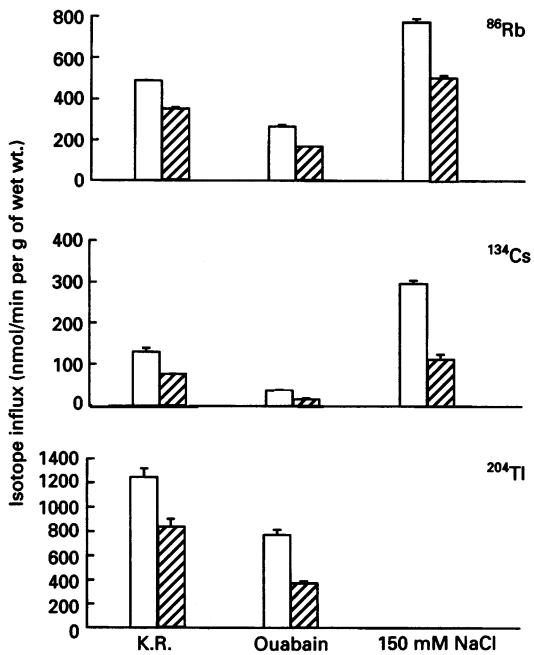

Figure 4 Effect of bumetanide (1 mM) on ^{86}Rb, ^{134}Cs and ^{204}Tl uptake in rat soleus muscle

After preincubation for 15 min in Krebs–Ringer bicarbonate buffer (K.R.) without (open bar) or with (hatched bar) bumetanide (1 mM) and without or with ouabain (1 mM), the muscles were incubated for 10 min in the same buffer containing ^{86}Rb (0.2 μCi/ml), ^{134}Cs (0.2 μCi/ml) or ^{204}Tl (0.01 μCi/ml) without or with the addition of 150 mM NaCl. After washout for 4 × 15 min in ice-cold Na^+-free Tris/sucrose buffer, muscles were blotted, weighed and homogenized in 2 ml of 0.3 M trichloroacetic acid for counting of ^{86}Rb, ^{134}Cs or ^{204}Tl. Each result represents the mean ± S.E. of measurements on 4–12 muscles.

per g wet wt., corresponding to 29% of total ^{86}Rb uptake. The concentration producing half-maximum inhibition ($K_{0.5}$) was calculated to be 10^{-7} M. In contrast, there was no significant effect of bumetanide (10^{-8}–10^{-3} M) on ^{42}K uptake. Parallel experiments showed that furosemide inhibited ^{86}Rb uptake in a dose-dependent way over the concentration range from 3×10^{-6} ($P < 0.001$) to 10^{-3} M ($P < 0.001$) ($K_{0.5} = 5 \times 10^{-6}$ M) (results not shown). These experiments included 16 control muscles and 40 muscles treated with furosemide.

Effects of bumetanide and ouabain on ^{86}Rb, ^{134}Cs and ^{204}Tl uptake

In order to assess whether bumetanide and ouabain influenced the transport of other K^+ congeners, the uptake of ^{134}Cs and ^{204}Tl in rat soleus was measured. Figure 4 compares the rates of K^+ uptake obtained using ^{86}Rb, ^{134}Cs and ^{204}Tl. Although the absolute values were not the same, the relative inhibitions of tracer uptake by ouabain and bumetanide were comparable. Under basal conditions, the bumetanide-suppressible uptake of ^{86}Rb, ^{134}Cs and ^{204}Tl amounted to 28, 42 and 38% respectively. Ouabain (10^{-3} M) suppressed the uptake of ^{86}Rb, ^{134}Cs and ^{204}Tl by 45, 72 and 33% respectively. In comparison, bumetanide and ouabain suppressed ^{42}K uptake by 0 and 51% respectively (Figure 3). It seems that the larger tracers for K^+ detect a bumetanide-suppressible fraction of K^+ influx of similar relative magnitude, but all in discrepancy with the values obtained using ^{42}K. Absolute and relative values for Na^+–K^+-pump-mediated ^{86}Rb uptake were again of the same magnitude as the values obtained with ^{42}K. In contrast, the ouabain-suppressible components of ^{134}Cs and ^{204}Tl uptake were significantly smaller and larger respectively than those measured using ^{86}Rb. The addition of 150 mM NaCl produced a clear-cut increase in bumetanide-suppressible ^{134}Cs uptake reminiscent of that seen in the ^{86}Rb-uptake experiments (^{204}Tl uptake was not measured after the addition of 150 mM NaCl).

^{42}K and ^{86}Rb uptake in mouse soleus

In order to evaluate whether the discrepancies between bumetanide-suppressible ^{42}K and ^{86}Rb influx in skeletal muscle were species-dependent, analogous dual-isotope experiments were performed using mouse soleus muscles. Table 2 shows that although bumetanide suppresses ^{86}Rb uptake under all four experimental conditions tested, no effect of bumetanide on ^{42}K

Table 2 Comparison between bumetanide-sensitive ^{42}K uptake and ^{86}Rb uptake in mouse soleus muscle

Experimental conditions as described in the legend to Figure 3. Each value represents the mean ± S.E.M. of observations on four muscles obtained in one experiment. The significance of differences between muscles treated without or with bumetanide is indicated by *P*. N.S., not significant.

Addition(s)	^{42}K uptake (nmol/min per g wet wt.)	*P*	^{86}Rb uptake (nmol/min per g wet wt.)	*P*
Control	672 ± 13		604 ± 19	
Bumetanide (1 mM)	607 ± 40	N.S.	458 ± 20	< 0.01
Ouabain (1 mM)	321 ± 12		248 ± 5	
Ouabain (1 mM) + bumetanide (1 mM)	324 ± 12	N.S.	194 ± 3	< 0.01
NaCl (150 mM)	602 ± 70		754 ± 76	
NaCl (150 mM) + bumetanide (1 mM)	648 ± 93	N.S.	480 ± 51	< 0.01
NaCl (150 mM) + ouabain (1 mM)	235 ± 10		273 ± 15	
NaCl (150 mM) + ouabain (1 mM) + bumetanide (1 mM)	275 ± 22	N.S.	145 ± 10	< 0.01

Table 3 Comparison between bumetanide-sensitive ^{42}K uptake and ^{86}Rb uptake in rat erythrocytes

Blood was collected and erythrocytes prepared as described in the legend to Figure 5. Following preincubation of a 10% (v/v) suspension for 30 min without or with bumetanide (1 mM) and/or ouabain (1 mM), the cells were incubated for 60 min with ^{42}K (0.5 μCi/ml) and ^{86}Rb (0.5 μCi/ml) without or with the addition of 300 mM sucrose. Preparation and counting of the samples was as described in the legend to Figure 5. Values represent the means ± S.E. of measurements on separate preparations of erythrocytes from three to seven rats (as indicated in parentheses). The significance of differences between erythrocytes treated without or with bumetanide is indicated by *P*.

Additions	^{42}K uptake (nmol/min per ml of cells)	*P*	^{86}Rb uptake (nmol/min per ml of cells)	*P*
Control	77.9 ± 3.8 (7)		78.9 ± 3.5 (7)	
Bumetanide (1 mM)	61.6 ± 3.5 (7)	< 0.01	59.3 ± 4.4 (7)	< 0.005
Ouabain (1 mM)	33.9 ± 4.6 (7)		37.8 ± 5.2 (7)	
Ouabain (1 mM) + bumetanide (1 mM)	20.5 ± 0.8 (7)	< 0.03	21.3 ± 1.0 (7)	< 0.02
Sucrose (300 mM)	130.4 ± 9.3 (3)		145.3 ± 11.3 (3)	
Sucrose (300 mM) + bumetanide (1 mM)	74.3 ± 4.0 (3)	< 0.01	80.0 ± 2.7 (3)	< 0.005

uptake could be detected. As in rat soleus, hyperosmotic buffer (addition of 150 mM NaCl) stimulated ^{86}Rb, but not ^{42}K, uptake. Ouabain-suppressible ^{42}K and ^{86}Rb uptake amounted to 351 and 356 nmol/min per g wet wt., indicating that ^{86}Rb is a reliable tracer for Na^+–K^+-pump-mediated K^+ influx also in mouse skeletal muscle.

Taken together, under a wide range of conditions ^{86}Rb and ^{42}K give similar results for Na^+–K^+-pump-mediated K^+ influx in rat and mouse soleus muscle, whereas in both preparations ^{86}Rb gives values for bumetanide-suppressible K^+ influx that are inconsistent with the lack of effect on ^{42}K influx.

The effects of bumetanide on K^+ uptake in erythrocytes

It was somewhat surprising that a bumetanide-suppressible ^{42}K influx could not be detected in skeletal muscle. Since this could be due to technical flaws, we felt that it was important to characterize the effects of bumetanide on ^{42}K and ^{86}Rb uptake in a rat cell type where bumetanide-suppressible Na^+–K^+–Cl^- co-transport is well documented (Lauf et al., 1987). For this purpose, rat erythrocytes were incubated with both ^{42}K and ^{86}Rb. Table 3 shows that a supramaximal concentration of bumetanide (1 mM) inhibited ^{42}K and ^{86}Rb uptake by 21 and 25% respectively. Ouabain-suppressible ^{42}K and ^{86}Rb uptake amounted to 56 and 52% respectively. When exposed to hyperosmotic buffer (300 mM sucrose), the erythrocytes showed a considerable (67–84%) increase in both ^{42}K and ^{86}Rb uptake, which in both instances was suppressible by bumetanide.

Figure 5 shows the effects of increasing concentrations of bumetanide on ^{42}K and ^{86}Rb uptake in rat erythrocytes. Neither basal K^+ uptake nor the dose–response relationship for bumetanide revealed any marked difference between the two isotopes. In conclusion, ^{86}Rb and ^{42}K give similar results for ouabain- and bumetanide-suppressible K^+ uptake in rat erythrocytes.

Efflux of ^{42}K and ^{86}Rb

It is well established that basal K^+ efflux is underestimated using ^{86}Rb as a tracer for K^+, giving values for the fractional loss of K^+ that are only 45–80% of those obtained with ^{42}K (Smith et al., 1986; Videbæk et al., 1988; Everts and Clausen, 1988). The following experiments with known stimulators of the Na^+–K^+ pump show that, in addition, widely diverging results are obtained using the two isotopes.

Figure 6 shows the effects of the β_2-agonist salbutamol on the fractional loss of ^{42}K and ^{86}Rb from rat soleus muscle. As observed earlier, the basal fractional loss of ^{86}Rb is only 45% of

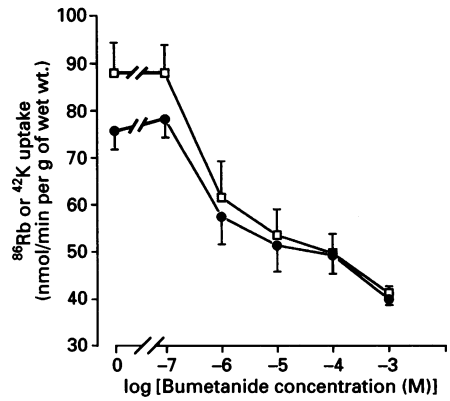

Figure 5 Dose–response relationship for the effect of bumetanide on ^{42}K and ^{86}Rb uptake in rat erythrocytes

Blood was collected from aorta in a heparinized tube. After centrifugation at 700 *g* for 5 min, the white cell layer was removed and the erythrocytes were washed three times, preincubated for 30 min in a 10% (v/v) suspension, without or with bumetanide at the indicated concentrations. The cells were incubated for 60 min with ^{42}K (0.5 μCi/ml) and ^{86}Rb (0.5 μCi/ml) without or with bumetanide. After incubation samples were taken to microtubes containing dibutyl phthalate, and centrifuged for 1 min in a bench microfuge to separate load and cells. The two fractions were counted for radioactivity, and ^{42}K (●) and ^{86}Rb (□) activities taken up by the cells calculated. Each result represents the mean ± S.E.M. of measurements on separate preparations of erythrocytes from three rats.

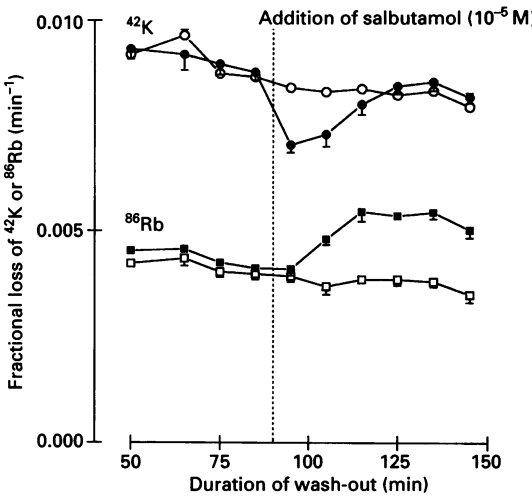

Figure 6 Effect of salbutamol on washout of ^{42}K and ^{86}Rb from rat soleus muscle

Muscles were loaded for 60 min at 30 °C in buffer containing either ^{42}K (2 μCi/ml) or ^{86}Rb (2 μCi/ml), and then washed for 15 × 10 min in 15 tubes containing unlabelled buffer without or with the indicated additions. The vertical dotted line indicates the application of salbutamol (10^{-5} M). By adding successively the ^{42}K or ^{86}Rb activities measured in wash-out tubes to that in the muscle at end of experiment, the activity in the muscle before each transfer was calculated. On the basis of these values, the fractional loss of ^{42}K or ^{86}Rb activity could be calculated for each wash-out period. Each result represents the mean ± S.E. of observations on six muscles.

that of ^{42}K. When salbutamol is added to the buffer, the fractional loss of ^{42}K is decreased for some 20 min, probably because a larger fraction of the ^{42}K lost from the cells is reaccumulated due to stimulation of the Na^+–K^+ pump. This interpretation is supported by the observation that in the presence of ouabain, catecholamines cause no decrease in ^{42}K efflux (for details, see Clausen and Flatman, 1977). In contrast, using ^{86}Rb as tracer for K^+, salbutamol elicits an increase in the fractional loss. Furthermore, this response is sustained, whereas the effect on ^{42}K efflux is transient. Also, when using another Na^+–K^+-pump activator, rCGRP (Andersen and Clausen, 1993), the effluxes of the two isotopes showed widely divergent responses. When exposed to rCGRP (10^{-7} M), the fractional loss of ^{42}K from rat soleus decreased from 0.00856 ± 0.00008 to 0.00721 ± 0.00012 ($P < 0.001$; 12 versus 12 observations in 6 versus 6 muscles) for 20 min, and then returned to the control level. As in the experiments with salbutamol, ouabain suppressed the transient decrease in fractional loss of ^{42}K induced by rCGRP. When using ^{86}Rb, the fractional loss increased from 0.00394 ± 0.00004 to 0.00487 ± 0.00015 ($P < 0.001$; 16 versus 16 observations in 4 versus 4 muscles).

DISCUSSION

The effects of bumetanide

The present study was initiated because the use of ^{86}Rb as a tracer for K^+ had given the misleading impression that bumetanide inhibits the uptake of K^+ in skeletal muscle, suggesting the existence of a $NaKCl_2$ cotransport system (Dørup and Clausen, 1991). It emerged that when the tracer used was ^{42}K, which is most likely to be representative for K^+, bumetanide had no detectable effect on K^+ influx in intact rat soleus muscle. In the same muscles, a clear-cut inhibitory effect of bumetanide on ^{86}Rb influx could repeatedly be demonstrated. This discrepancy is unlikely to reflect technical problems associated with the counting of the two isotopes in the same muscle extracts. Again, when comparing dose–response curves for the effect of bumetanide on ^{42}K and ^{86}Rb uptake, a marked divergence between the two isotopes was seen. The $K_{0.5}$ for the effect of bumetanide on ^{86}Rb uptake was 10^{-7} M, which corresponds well with the values for bumetanide-sensitive ^{86}Rb uptake obtained in human myoblasts (10^{-7} M) and in chick cardiac cells (6×10^{-7} M) (Frelin et al., 1986; Panet et al., 1987). Thus, when using ^{86}Rb as a tracer for K^+, our values were very comparable with those obtained earlier with other muscle cells and other species, whereas when using ^{42}K, no significant effect was obtained at any of the concentrations of bumetanide tested.

In rat erythrocytes, it could be demonstrated that bumetanide induces clear-cut and very similar inhibition of the uptake of both ^{42}K and ^{86}Rb (Figure 5). This observation confirms earlier reports describing Na^+–K^+–Cl^- co-transport in rat erythrocytes (Duhm and Göbel, 1984; Orlov et al., 1993), and suggests the existence of a similar system in skeletal muscle of the same species. The observation that bumetanide inhibits ^{86}Rb uptake in rat soleus muscle indicates that a bumetanide-sensitive transport system in the plasma membrane can recognize the K^+ congener and therefore has some similarities to the bumetanide-sensitive Na^+–K^+–Cl^- transport system in the erythrocyte membrane of the rat. One possible explanation for this phenomenon may be that in skeletal muscle, a Na^+–K^+–Cl^- transport system during evolution has lost its ability to carry K^+, but preserved its responsiveness to bumetanide and the capacity to carry ^{86}Rb or other K^+ congeners. The observation that the uptake of ^{134}Cs and ^{204}Tl in rat soleus were clearly suppressed by bumetanide supports this contention (Figure 4). Earlier reports have demonstrated bumetanide- and furosemide-sensitive uptake of ^{204}Tl in ascites tumour cells (Bakker-Grunwald, 1979; Sessler et al., 1983).

The Na^+–K^+-pump-mediated transport of ^{42}K, ^{86}Rb, ^{134}Cs and ^{204}Tl

The experiments with rat and mouse soleus as well as rat erythrocytes consistently show that when used as tracers for K^+, ^{42}K and ^{86}Rb gave virtually the same values for ouabain-suppressible K^+ uptake. This is in keeping with earlier studies on smooth muscle (Widdicombe, 1977; Smith et al., 1986) and skeletal muscle (Clausen et al., 1987). Furthermore, when the rate of ouabain-suppressible K^+ uptake in rat soleus was varied over one order of magnitude, the uptakes of ^{42}K and ^{86}Rb were closely correlated (Figure 2). This indicates that the Na^+–K^+ pump is capable of recognizing and transporting ^{86}Rb in almost the same manner as ^{42}K. Therefore, ^{86}Rb may be used as a tracer for the quantification of Na^+–K^+-pump-mediated K^+ influx in skeletal muscle. In view of the abovementioned discrepancies, however, it will be necessary to check for each condition whether the two isotopes are transported at the same rate by the Na^+–K^+ pump.

In contrast, when taking ^{134}Cs as a tracer for K^+, the ouabain-suppressible component of uptake was only 42 % of that obtained using ^{42}K or ^{86}Rb. Conversely, using ^{204}Tl, a value 85 % above that measured with ^{42}K was obtained (see Figure 4). This indicates that in skeletal muscle, ^{134}Cs and ^{204}Tl can only be used as tracers for ouabain-suppressible uptake of K^+ when relative changes are to be assessed.

Measurements of K^+ efflux

The most striking discrepancy observed in the present study is the diametrically opposite changes in the effluxes of ^{42}K and ^{86}Rb induced by the addition of either salbutamol (Figure 6) or rCGRP to rat soleus muscle. The transient inhibition of ^{42}K efflux induced by catecholamines or rCGRP has been observed before and is likely to reflect stimulation of Na^+–K^+-pump-mediated re-uptake of the isotope lost from the muscle cells into the interstitial water space (Clausen and Flatman, 1977; Andersen and Clausen, 1993). Due to the diffusional delay of ^{42}K throughout the interstitial water space, stimulation of the Na^+–K^+ pump will favour the intracellular re-accumulation of isotope that has not yet escaped into the surrounding buffer. This interpretation is supported by the observation that in the presence of ouabain, catecholamines or rCGRP cause no inhibition of ^{42}K efflux (Clausen and Flatman, 1977; Andersen and Clausen, 1993).

The stimulating effect of salbutamol and rCGRP on ^{86}Rb efflux indicates that both agents induce an activation of a transport system with some selectivity for ^{86}Rb compared with ^{42}K.

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Conclusions and practical implications

The results strongly indicate that ^{86}Rb can only be used as a tracer for K^+ under limited and very well defined conditions, e.g. the measurement of ouabain-suppressible K^+ uptake. It is likely that the demonstration of bumetanide-sensitive ^{86}Rb transport in a number of tissues cannot be taken as an indication of any effect of the diuretic on K^+ transport. Other misleading results may arise from uncritical use of ^{86}Rb as a tracer for K^+ efflux. In spite of the technical problems arising from the short half-life and limited commercial availability of ^{42}K, this isotope should be preferred for the characterization of K^+ transport processes.

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