Localization and function of ATP-sensitive potassium channels in human skeletal muscle

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Nielsen, Jens Jung, Michael Kristensen, Ylva Hellsten, Jens Bangsbo, and Carsten Juel. Localization and function of ATP-sensitive potassium channels in human skeletal muscle. Am J Physiol Regul Integr Comp Physiol 284: R558–R563, 2003. First published September 27, 2002; 10.1152/ajpregu.00303.2002.—The present study investigated the localization of ATP-sensitive K+ (KATP) channels in human skeletal muscle and the functional importance of these channels for human muscle K+ distribution at rest and during muscle activity. Membrane fractionation based on the giant vesicle technique or the sucrose-gradient technique in combination with Western blotting demonstrated that the KATP channels are mainly located in the sarcolemma. This localization was confirmed by immunohistochemical measurements. With the microdialysis technique, it was demonstrated that local application of the KATP channel inhibitor glibenclamide reduced P < 0.05) interstitial K+ at rest from ~4.5 to 4.0 mM, whereas the concentration in the control leg remained constant. Glibenclamide had no effect on the interstitial K+ accumulation during knee-extensor exercise at a power output of 60 W. In contrast to in vitro conditions, the present study demonstrated that under in vivo conditions the KATP channels are active at rest and contribute to the accumulation of interstitial K+.

There is a significant K+ release from active skeletal muscle. Thus, in a recent study using microdialysis, the interstitial K+ concentration increased progressively with increasing work intensity, reaching >10 mM (13). The extracellular K+ accumulation may impair muscle function by reducing membrane excitability (8). Part of the K+ release during contractions is due to the activity of the voltage-dependent K+ channels activated during the action potentials, but the large K+ release leads to the hypothesis that other channels may also be involved. Such channels could be the ATP-sensitive K+ (KATP) channels or the Ca2+-activated (BKCa2+) channels, are reported to be present in rat skeletal muscle T tubules (14). The function of these channels in muscle is unknown.

The aims of the present study were 1) to localize the KATP and BKCa2+ channels in human skeletal muscle and 2) to determine the functional importance of KATP channels in vivo for human muscle K+ distribution at rest and during muscle activity. To localize the channels in human skeletal muscle, membrane fractionation in combination with Western blotting, as well as immunohistochemistry, was used to localize the KATP and BKCa2+ channels at the subcellular level. Because

K+ current in metabolically stressed cells and their facilitation of recovery after such stress (2, 18).

The role of the KATP channels in skeletal muscle is less clear. KATP channels have been identified in skeletal muscle by electrophysiological methods (6, 21). It was demonstrated that ATP inhibits the KATP channels and that the inhibitory effect of ATP is reduced by lowering of pH (6). It was originally suggested that the channels are activated only in metabolically exhausted muscle fibers (4) and that the activity of the channels contributes to the decrease in force during fatigue in frog muscle (15). However, this hypothesis is not supported by later studies also using frog muscle (20). On the basis of recent studies in mice, it has been suggested that the channels can give some protection against ATP depletion and that the channels can influence force recovery after exercise (17). In addition, studies in KATP channel-deficient mice have led to the conclusion that the channels reduce the increase in resting (baseline) tension during fatigue and have no influence on the reduction in force, as originally suggested (9). The question is whether KATP channels are active at rest and influence the resting membrane potential. On the basis of Rh+ flux measurements in isolated mouse muscle, it has been concluded that KATP channels are inactive in unfatigued muscle (1, 17). However, flux measurements demonstrated a selective inhibition by the KATP channel inhibitor glibenclamide of the K+ efflux in resting rat hindlimbs perfused in situ (16). Another group of K+ channels, the large Ca2+-activated (BKCa2+) channels, are reported to present in rat skeletal muscle T tubules (14). The function of these channels in muscle is unknown.

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some of the fractionation techniques used for subcellular localization require a large muscle mass, the studies on human needle biopsy material were supplemented with studies on rat muscle. To determine the functional importance of $K_{ATP}$ channels in vivo, the $K_{ATP}$ channel inhibitor glibenclamide was used with the microdialysis technique for determination of the interstitial $K^+$ concentration at rest and during knee-extensor exercise.

METHODS

Microdialysis Experiments

Subjects. The subjects were six healthy men with age, weight, and height as follows: 25 ± 1 yr, 77.6 ± 6.7 kg, and 182.9 ± 0.9 cm. The subjects were informed of any risk associated with the experiments before giving their consent to participate. The work fully conforms to American Physiological Society guidelines for research involving animals and humans, and the study was approved by the local ethics committee of Frederiksberg and Copenhagen communities.

Microdialysis. Two types of microdialysis probes were used: CMA60 (30 mm long, 0.5 mm diameter, 20 kDa cutoff; CMA Microdialysis) and homemade (40 mm long, 0.2 mm ID, 6 kDa cutoff). Two CMA60 and two homemade probes were inserted into the vastus lateralis muscle of each leg using local anesthesia (lidocaine, 20 mg/ml) of the skin and subcutaneous tissue. The intention was to insert the probes parallel to the muscle fibers. The four probes in one leg (intervention leg) were used for drug infusion and $K^+$ measurements, and the four probes in the other leg (control leg) were used only for $K^+$ measurements.

The perfusate was Ringer acetate containing (in mM) 130 Na$^+$, 2 Ca$^{2+}$, 4 K$^+$, 1 Mg$^{2+}$, 30 acetate, 3 glucose, and 1 lactate. $^{201}$Tl (thallous chloride injection liquid, Amersham Life Science; 4.6 MBq/l) was added to the syringes used for dialysate infusion to determine fractional release. Glibenclamide (glyburide, Calbiochem) was dissolved in Ringer lactate infusion to determine fractional release. Glibenclamide in the probe, fractional release 0.2, pump rate 5 μl/min. It is impossible to calculate the concentration in the muscle close to the probe, because the dilution space and the volume of the outlet tubes was taken into account. The weight of the sample tubes was determined before and after sampling to validate the perfusion rate. Collection from probes having >10% deviation between expected and collected samples and probes having any sign of hemoglobin content in the dialysate was immediately stopped, and the samples were not analyzed. The $K^+$ concentrations of the collected samples were measured with a flame photometer (model FLM3, Radiometer) using lithium as an internal standard. Five microliters of each dialysate sample were counted in a Packard auto-gamma counter to determine thallium loss.

Calculations

The relative loss (RL) of $^{201}$Tl was calculated as follows

$$RL_{Tl} = \frac{\text{perfusate activity} - \text{dialysate activity/perfusate activity}}{\text{perfusate activity}}$$

The interstitial $K^+$ concentration was calculated from the dialysate samples with the assumption that fractional thallium loss was equal to fractional $K^+$ gain (13)

$$K_{perfusate} + \left( K_{dialysate} - K_{perfusate}/RL_{Tl} \right)$$

Channel Localization

Membrane separation. Giant sarcolemmal vesicles, used in a membrane separation procedure, were produced from rat and human muscle samples, as previously described (11). Marker enzyme analyses have shown that the vesicular membrane is mainly of sarcolemmal origin (12). Another membrane separation method based on ultracentrifugation and discontinuous sucrose gradients was used to isolate the T tubule fraction and the sarcolemmal (plasma membrane) fraction (7). The Na$^-\text{-}K^+$ pump α-subunit and the dihydropyridine (DHP) receptor α-subunit were used as marker enzymes for sarcolemma and T tubules, respectively, to characterize the membrane fractions.

Western blot. Rat and human muscle samples were homogenized in a sucrose buffer (in mM: 250 sucrose, 30 HEPES, 2 EGTA, 40 NaCl, 2 phenylmethylsulfonyl fluoride, pH 7.4) with a Polytron 2100 and centrifuged at 1,000 $^\circ$C, for 5 min. The supernatant was spun at 190,000 $^\circ$C, for 90 min at 4°C. The pellet was resuspended in Tris-SDS (10 mM Tris, 4% SDS, 1 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, pH 7.4), and protein content was determined with a BSA standard (DC protein assay, Bio-Rad). Samples were subjected to SDS-PAGE (Excell 8–18% gradient gel) and electrophobted to a Millipore Immobilon-P polyvinylidene difluoride membrane. The membrane was blocked by BSA, 0.1% Tween 20, and low-fat milk and incubated with the primary antibody diluted in a BSA-containing buffer. After treatment with the horseradish peroxidase-coupled secondary antibody and repeated washing, the membrane was incubated with enhanced chemiluminescence reagent (Amersham) and visual-
RESULTS

Interstitial $K^+$ at Rest

At the end of the 90-min restitution period after probe insertion, the interstitial $K^+$ concentration was $4.5 \pm 0.2$ mM in the control leg and $4.5 \pm 0.3$ mM in the intervention leg. A perfusate pH of 10 did not induce any difference in interstitial $K^+$ between the control and the intervention leg. Glibenclamide infusion for 30 min reduced ($P < 0.05$) interstitial $K^+$ in the intervention leg to $4.0 \pm 0.1$ mM (Fig. 1). After glibenclamide was washed out for 20 min, $K^+$ in the intervention leg was $4.2 \pm 0.3$ mM, which was not different from the concentration in the control leg ($4.4 \pm 0.1$ mM).

Interstitial $K^+$ During Exercise

Interstitial $K^+$ in the control and intervention leg was identical after the 40-W warm-up period. The first 60-W exercise period (without drugs) increased interstitial $K^+$ in both legs to 10–11 mM. Glibenclamide was then added to the perfusate in the intervention leg 15 min before the second 60-W bout. The peak interstitial $K^+$ during the second exercise bout ($~9$ mM) was lower ($P < 0.05$) than that during the first bout, but there was no difference between the control and the intervention leg (Fig. 2).

Localization of $K^+$ Channels in Rat Skeletal Muscle

Antibodies to the DHP receptor and the Na$^+$.K$^+$ pump $\alpha_2$-subunit were used to characterize the membrane fractions obtained from rat muscle. The T tubule marker DHP was absent in the sarcolemmal giant vesicles but could be detected in the sarcolemmal fraction obtained by sucrose-gradient ultracentrifugation. The highest density of the DHP receptor was found in the T tubule fraction. The Na$^+$.K$^+$ pump $\alpha_2$-subunit
was mainly found in vesicles and the sarcolemmal fraction, which were labeled 11–12 times more intensely than the muscle homogenate. This purification index is in agreement with a mainly sarcolemmal localization of the pump. The distribution of the $K_{ATP}$ channels demonstrated a great similarity to the distribution of the pump, suggesting that the $K_{ATP}$ channels are mainly located in the sarcolemma. In contrast, the BKCa$^{2+}$ channels were more abundant in the T tubule fraction, suggesting a higher density than in the sarcolemma (Fig. 3).

**Distribution of $K_{ATP}$ and BKCa$^{2+}$ Channels in Human Skeletal Muscle on the Basis of Western Blot Analysis**

Because of the limited amount of tissue available, only the giant vesicle method was used to purify sarcolemmal membranes from human muscle. The Kir 6.2 subunit of the $K_{ATP}$ channel was present in a higher density ($P < 0.05$) in vesicles than in homogenates ($n = 4$). In contrast, the BKCa$^{2+}$ protein content was clearly lower in vesicles than in homogenates (Fig. 4).

**Immunohistochemistry of Human Skeletal Muscle**

The immunohistochemical pictures demonstrated that the Kir 6.2 protein is present in the surface membrane of human skeletal muscle fibers. The staining was seen in all fibers, suggesting the presence of $K_{ATP}$ channels in all fiber types (Fig. 5A). The labeling of the surface membranes was not seen in negative control images (Fig. 5B), which were obtained with an identical procedure, but without primary antibodies. Fluorescence was also seen in some distinct areas near the surface. These spots might be in the area of the capillaries; this possibility is supported by the limited number at the surface of each fiber. Similar spots were also seen in the negative control situation (without primary and secondary antibodies) and are therefore considered to be autofluorescence from compounds near the capillaries.

**DISCUSSION**

The major findings in the present study are as follows. 1) $K_{ATP}$ channels are mainly located in the plasma membrane. 2) $K_{ATP}$ channels appear to be present in all fiber types. 3) $K_{ATP}$ channels are active in resting human muscle. In the present study, we used local drug application in combination with the microdialysis technique, instead of systemic ingestion. The advantage is that systemic effects, such as effects on pancreatic β-cells and increased insulin level (2), are avoided. Therefore, insulin-induced effects on K$^+$ bal-

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**Fig. 3. Distribution of the T tubule marker dihydropyridine (DHP) receptor (A), the Na$^+$-K$^+$ pump α2-subunit (B), ATP-sensitive K$^+$ ($K_{ATP}$, Kir 6.2) channel protein (C), and BKCa$^{2+}$ channel protein (D) in rat muscle homogenates, sarcolemmal giant vesicles, sarcolemmal fractions, and T tubule membrane fractions. Proteins were detected by specific antibodies. Values are means ± SE from 6–8 preparations of mixed hindlimb muscle. Arbitrary density units were obtained by densitometric scanning.**

**Fig. 4. Distribution of $K_{ATP}$ and BKCa$^{2+}$ channel proteins in human skeletal muscle on the basis of biopsy material and Western blotting analysis. Relative distributions of $K_{ATP}$ (Kir 6.2) and BKCa$^{2+}$ channel proteins in sarcolemmal giant vesicles were calculated relative to density in muscle homogenates. Values are means ± SE; $n = 4$ for Kir 6.2 and $n = 2$ for BKCa$^{2+}$.**

**Fig. 5. Immunohistochemistry of human skeletal muscle.**

- **A:** Kir 6.2 protein staining in the surface membrane of human skeletal muscle fibers.
- **B:** Negative control image with the same procedure but without primary antibodies.
Importance of KATP Channels

The interstitial K\(^+\) concentration can be described as a balance between K\(^+\) release mediated by various channels and reuptake mediated by the Na\(^+\)-K\(^+\) pump. The pronounced effect of the K\(_{ATP}\) channel inhibitor glibenclamide on interstitial K\(^+\) at rest suggests that KATP channels contribute significantly to the membrane permeability in resting human muscle.

The finding that KATP channels in human skeletal muscle are active at rest is surprising in light of the first hypothesis for the function of these channels, which were considered to be active only in exhausted cells (1, 4, 22). Voltage-clamp studies of frog muscle membranes have demonstrated that the channels are closed in patches from resting muscle (21), and studies in isolated mouse muscle have shown that K\(_{ATP}\) channel inhibition had no effect on membrane K\(^+\) permeability and the resting membrane potential in unstimulated isolated muscle (1, 17). In contrast, glibenclamide is reported to reduce K\(^+\) efflux in rat muscle perfused in situ (16). One explanation for these discrepancies could be species differences. Another explanation could be that the K\(_{ATP}\) channels are inactive under in vitro conditions but active under in vivo conditions: some factors that are responsible for the opening of K\(_{ATP}\) channels in resting muscle are lost during preparation of isolated frog and mouse muscle, and these factors are not present in the media used in patch-clamp studies. It has been reported that the K\(_{ATP}\) channels can be activated by lactate and pyruvate (10), protons (6, 22), adenosine and GTP (3), and nitric oxide (19, 23). The opening of K\(_{ATP}\) channels in resting human muscle could theoretically be due to a combined effect of several of these factors. However, inasmuch as the concentrations of these factors are low at rest, it is unlikely that they contribute to the opening of the KATP channels. Insulin, on the other hand, is likely to be involved in the opening of K\(_{ATP}\) channels at rest, inasmuch as insulin has been demonstrated to increase the K\(_{ATP}\) current in patch-clamped rat muscle fibers (24, 25).

During muscle activity, some K\(^+\) is released because of the activity of the voltage-dependent K\(^+\) channels associated with the action potentials. The present data suggest that glibenclamide has no effect on the interstitial K\(^+\) increase during exercise, which seems to indicate that K\(_{ATP}\) channels do not contribute to the increased K\(^+\) release. However, some reservations can be discussed. First, it could be argued that the increased blood flow during exercise removes glibenclamide, resulting in an insufficient inhibition. On the other hand, muscle movements have been found to nearly double the fractional release of compounds from the microdialysis probe. It is therefore likely that the release of glibenclamide from the probe is substantially increased during muscle exercise, which could compensate for an increased washout due to the increased blood flow. Second, it is not known whether glibenclamide inhibition results in a membrane depolarization in human muscle, but the apparent lack of effect of glibenclamide could be related to such changes in membrane potential, resulting in an increased driving force for K\(^+\), a mechanism that could blur the contribution of K\(_{ATP}\) channels during muscle activity. On the other hand, the lower interstitial K\(^+\) concentration in the glibenclamide experiments tends to hyperpolarize the fibers, which acts in the opposite direction. Although interference from the above-mentioned mechanisms cannot be completely ruled out, it is concluded that K\(_{ATP}\) channels are not major contributors to the increased K\(^+\) release during muscle exercise.

Subcellular Localization of K\(^+\) Channels

Membrane fractionation techniques and immunohistochemistry were used to localize the K\(^+\) channels in
muscle. Although the Western blotting technique does not allow measurements of absolute channel densities, some conclusions can be drawn from the relative densities. If the relative densities of the $K_{ATP}$ and BKCa$^{2+}$ channels in the different muscle fractions are compared with the density of the marker enzymes (Na$^{+}$-K$^{+}$ pump and DHP receptor), it can be concluded that the $K_{ATP}$ channels have a higher density in the sarclemma, whereas the BKCa$^{2+}$ channels have a higher density in the T tubule membranes. The sarcromemal localization of the $K_{ATP}$ channels and the effect of the specific inhibitor glibenclamide reported here are in agreement with a role for these channels in membrane permeability and regulation of the interstitial K$^{+}$ concentration at rest.

Summary

$K_{ATP}$ channels located in the sarclemma contribute to the permeability of resting human skeletal muscle and are therefore important for the interstitial K$^{+}$ balance. The finding that the inhibitor glibenclamide had no effect during exercise suggests that the $K_{ATP}$ channels are not important for the K$^{+}$ release during contractions in human muscle.

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REFERENCES


