The $K_{\text{ATP}}$ channel Kir6.2 subunit content is higher in glycolytic than oxidative skeletal muscle fibers

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Submitted 7 October 2010; accepted in final form 28 June 2011

Banas K, Clow C, Jasmin BJ, Renaud JM. The $K_{\text{ATP}}$ channel Kir6.2 subunit content is higher in glycolytic than oxidative skeletal muscle fibers. Am J Physiol Regul Integr Comp Physiol 301: R916–R925, 2011. First published June 29, 2011; doi:10.1152/ajpregu.00663.2010.—It has long been suggested that in skeletal muscle, the ATP-sensitive $K^+$ channel ($K_{\text{ATP}}$) channel is important in protecting energy levels and that abolishing its activity causes fiber damage and severely impairs function. The responses to a lack of $K_{\text{ATP}}$ channel activity vary between muscles and fibers, with the severity of the impairment being the highest in the most glycolytic muscle fibers. Furthermore, glycolytic muscle fibers are also expected to face metabolic stress more often than oxidative ones. The objective of this study was to determine whether the t-tubular $K_{\text{ATP}}$ channel content differs between muscles and fiber types. $K_{\text{ATP}}$ channel content was estimated using a semi-quantitative immunofluorescence approach by staining cross sections from soleus, extensor digitorum longus (EDL), and flexor digitorum brevis (FDB) muscles with anti-Kir6.2 antibody. Fiber types were determined using serial cross sections stained with specific antibodies: I; IIA, IIB, and IIX antibodies. Changes in Kir6.2 content were determined using serial cross sections stained with specific antimyosin antibodies. Fiber types were compared with changes in CaV1.1 content, as this Ca$^{2+}$ channel is responsible for triggering Ca$^{2+}$ release from sarcoplasmic reticulum. The Kir6.2 content was the lowest in the oxidative soleus and the highest in the glycolytic EDL and FDB. At the individual fiber level, the Kir6.2 content within a muscle was in the order of type IIB > IIX > IIA $\geq$ I. Interestingly, the Kir6.2 content for a given fiber type was significantly different between soleus, EDL, and FDB, and highest in FDB. Correlations of relative fluorescence intensities from the Kir6.2 and CaV1.1 antibodies were significant for all three muscles. However, the variability in content between the three muscles or individual fibers was much greater for Kir6.2 than for CaV1.1. It is suggested that the t-tubular $K_{\text{ATP}}$ channel content increases as the glycolytic capacity increases and as the oxidative capacity decreases and that the expression of $K_{\text{ATP}}$ channels may be linked to how often muscles/fibers face metabolic stress.

muscle fatigue; $K_{\text{ATP}}$ channels; fiber type

One mechanism by which the channel prevents fiber damage involves a reduction in action potential amplitude (16), which reduces Ca$^{2+}$ released by the sarcoplasmic reticulum and force produced by the sarcomere; i.e., the $K_{\text{ATP}}$ channel contributes to the decrease in force during fatigue (16, 25). It has been proposed that this effect reduces the activity of Ca$^{2+}$ ATPase pumps and myosin ATPase to preserve ATP (18, 25). A second mechanism is through avoidance of large and excessive resting membrane depolarization, which can be as large as 50 mV in the absence of $K_{\text{ATP}}$ channel activity, as opposed to 15–20 mV in control conditions (8, 18). Preventing excessive depolarization also prevents damaging increases in myoplasmic free Ca$^{2+}$, due to Ca$^{2+}$ influx through L-type Ca$^{2+}$ channels (8).

Skeletal muscles with different fiber-type composition and single fibers respond differently to modulation of $K_{\text{ATP}}$ channel activity. For example, the increases in resting force during fatigue in $K_{\text{ATP}}$ channel-deficient muscles are in the order of flexor digitorum brevis (FDB) >> extensor digitorum longus (EDL) > soleus, while the decreased capacity to recover force after fatigue is in the order of EDL >> FDB > soleus (9, 16, 17, 25). Soleus muscle is primarily composed of type I and IIA fibers, which are among the most oxidative and fatigue-resistant fibers in skeletal muscle (11). The major difference between the two types is that type I fibers have slower contraction and ATP utilization rates (19, 30). In comparison, EDL muscle is primarily composed of type IIB and IIX fibers, which have the lowest oxidative and highest glycolytic capacity combined with higher contraction and ATP utilization rates (24). FDB muscle consists mainly of type IIA and IIX fibers (31, 36).

As a consequence of these contractile and metabolic profiles, the fatigability and the extent of metabolic stress for a given muscle activity between muscles are in the order of EDL > FDB > soleus, while for the different fibers, it is type IIB > IIX > IIA ~ I (24). If fatigue is a mechanism that protects muscle against damaging ATP depletion (26), then for a similar muscular activity, fewer $K_{\text{ATP}}$ channels are expected to be activated in oxidative than in glycolytic muscles/fibers, and, as such, abolishing the channel activity has smaller effects. It can be further suggested that incidence of metabolic stress in situ will also be of the same order as that of the fatigability. The question then is whether muscles/fibers with low incidence of metabolic stress express less $K_{\text{ATP}}$ channel content than those with high incidence.

The objective of this study was to determine how the $K_{\text{ATP}}$ channel content varies between muscles and fiber types. We tested the hypothesis that the $K_{\text{ATP}}$ channel content in the cell membrane differs among muscles and fiber types and increases with the degree of glycolytic capacity (and decreases with the degree of oxidative capacity); i.e., in the order of EDL > FDB > soleus and of type IIB > IIX > IIA ~ I. To test this hypothesis, we determined the $K_{\text{ATP}}$ channel content in indi-
vidual fibers of soleus, EDL, and FDB. These muscles were chosen because 1) they have been extensively used to study the functional role of the $K_{ATP}$ channel; 2) together, it is possible to determine the channel content in all four fiber types; and 3) some fiber types are expressed in more than one muscle, allowing us to determine whether the channel content for a given fiber type is the same or different between muscles. Most studies have reported that the skeletal muscle $K_{ATP}$ channel is composed of the pore-forming subunit Kir6.2 and the regulatory subunit SUR2A (1). However, recent pharmacological studies have suggested that SUR2B and SUR1 may also be expressed in a small number of fibers (37). Therefore, we quantified the Kir6.2 content by using a semiquantitative approach in which muscle cross sections were stained with an anti-Kir6.2 antibody. Finally, while the $K_{ATP}$ channel is located in both the outer cell membrane and t-tubules (22), one can expect that the t-tubular $K_{ATP}$ channel is the most important channel for myoprotection. Thus, in this study, we measured the t-tubular Kir6.2 content.

MATERIALS AND METHODS

Animals

Two-to-four-month-old mice (Charles River Laboratories, Senneville, Quebec, Canada) were fed ad libitum and housed according to the guidelines of the Canadian Council for Animal Care. The Animal Care Committee of the University of Ottawa approved all experimental procedures used in this study. Prior to muscle excision, mice were anesthetized with a single intraperitoneal injection of 2.2 mg ketamine/0.4 mg xylazine/0.22 mg acepromazine per 10 g of animal body weight. The final volume of anesthetic injected was never greater than 0.06 ml.

Immunofluorescence Measurements

FDL, EDL, and soleus muscles were embedded in a small amount of Tissue-Tek optimum cutting temperature (Sakura Finetek, Torrance, CA) compound before being frozen in isopentane precooled in liquid nitrogen. Muscles were stored at $-80^\circ$C until analysis. Serial 10-μm-thick cross sections were cut from the midbelly of each muscle using a cryostat (Leica Microtome, HM 500M) cooled to $-18^\circ$C. Sections were then mounted on Superfrost Plus slides. Each slide contained a cross section of EDL, soleus, and FDB from the same animal.

Kir6.2 and CaV1.1 immunostaining. Cross sections were thawed for 15 min at $35^\circ$C on a slide dryer (Fisher Scientific, Gormley, Ontario, Canada) before being exposed 1 h at room temperature to 0.5% BSA in standard PBS. Sections were then exposed 2.5 h at room temperature to a primary antibody, either rabbit IgG anti-Kir6.2 (Alomone Laboratories, Jerusalem, Israel) or mouse IgG anti-CaV1.1 (Chemicon, Temecula, CA) antibody. Excess antibody was removed by three PBS washes (5 min per wash). Cross sections were incubated 45 min at $37^\circ$C with fluorescence-conjugated secondary antibodies: FITC-conjugated goat IgG anti-mouse IgG antibody (Sigma, Oakville, Ontario, Canada) or rhodamine-conjugated goat anti-rabbit IgG (Cedarlane Laboratories, Burlington, Ontario, Canada). After rinsing three times with PBS, sections were mounted using antifade reagent. Control cross sections were also stained concurrently to test for autofluorescence and nonspecific secondary antibody binding. The controls included cross sections incubated without any antibody or without primary antibody but with secondary antibody. These controls consistently did not exhibit autofluorescence or nonspecific secondary antibody binding (data not shown).

Fiber-type determination. Cross sections used for fiber typing were prepared as for Kir6.2 staining except that they were double labeled with rabbit IgG anti-laminin (Sigma) and a monoclonal antibody against either type I (mouse IgM anti-type I myosin A4.840), IIA
(mouse IgG anti-type IIA myosin SC71), IIX (mouse IgM anti-type IIX myosin 6A1), or IIB (mouse IgM anti-type IIB myosin BFF3) myosin isoforms (one fiber type per cross section). Antimyosin antibodies were obtained from the Developmental Studies Hybridoma Bank (Iowa City, IA). The secondary antibodies used were either FITC-conjugated goat IgG anti-mouse IgM (Chemicon), FITC-conjugated goat IgG anti-mouse IgG (Sigma), or rhodamine-conjugated goat IgG anti-rabbit IgG (Cedarlane Laboratories).

**Semiquantification of fluorescence intensity.** Images were obtained using a Sony digital camera (model DXC-950) attached to an Axioskop-2 fluorescence microscope (Zeiss, Toronto, Ontario, Canada). For all images captured of anti-Kir6.2- and CaV1.1-labeled cross sections, the acquisition settings (exposure time, gain, intensity, offset, and binning) were initially set to maximize the imaging of the antibody and then preserved to facilitate comparison of fluorescence intensity/quantification across experiments. Images of all muscles from a single animal were obtained during a single sitting.

Fluorescence intensity for the quantification of Kir6.2 and CaV1.1 was then determined using Northern Eclipse (EMPIX, Cheektowaga, NY). Each fiber was encircled within its inner perimeter to enclose as much of the fiber’s t-tubular network as possible, while excluding the cell membrane. The fluorescence intensity was then obtained and expressed per square micrometer.

**RT-PCR Measurement**

A semiquantitative RT-PCR approach was used to determine the relative abundance of the mRNA transcripts of myosin isoform I, IIA, IIB, and IIX in EDL, soleus, and FDB muscles that were freeze-clamped in liquid nitrogen. Extraction and RT-PCR procedures were as described by Chakkalakal et al. (6). Briefly, total mRNA was extracted by using TriPure (Boehringer Mannheim, Mannheim, Germany). The forward and reverse primers were respectively AGTTCCGCAAGGTTGCGAC and CCACCTAAAGGGATGTTGCAA for myosin I; CGGGTGAAGAGCCGGGAGGT and GAAGATGGTGCAAACGTGAC for myosin IIA; ACAGACTAAAGTGAAAGCTA and CACATTTTGTGATTTCTCCTGT for myosin IIB; CGGGTGAAGAGCCGGGAGTT and CTCTCCTGATGTACAAATGAT for myosin IIX. To correct for loading, the intensity of the myosin PCR products was standardized to the signal corresponding to the 28S ribosomal mRNA by dividing the intensity of each myosin band by that of the 28S ribosomal band. The forward and reverse primers for the 28S ribosome were TTGTTGCCATGGTAATCCTGCTCAG-

**Table 1. Myosin expression in soleus, EDL, and FDB muscles of CD-1 mice**

<table>
<thead>
<tr>
<th>Fiber Type</th>
<th>Soleus</th>
<th>EDL</th>
<th>FDB</th>
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<tbody>
<tr>
<td>I</td>
<td>26.9 ± 9.9</td>
<td>0.1 ± 0.1</td>
<td>2.3 ± 1.5</td>
</tr>
<tr>
<td>I-IIA</td>
<td>7.4 ± 1.4</td>
<td>0.7 ± 0.5</td>
<td>6.3 ± 2.2</td>
</tr>
<tr>
<td>I-IIX</td>
<td>32.9 ± 9.7</td>
<td>1.7 ± 1.0</td>
<td>7.5 ± 1.6</td>
</tr>
<tr>
<td>IIA</td>
<td>26.3 ± 2.7</td>
<td>1.0 ± 0.3</td>
<td>19.0 ± 3.6</td>
</tr>
<tr>
<td>IIA-IIX</td>
<td>3.9 ± 1.7</td>
<td>12.6 ± 3.0</td>
<td>32.1 ± 5.1</td>
</tr>
<tr>
<td>IIX</td>
<td>0.6 ± 0.6</td>
<td>23.6 ± 1.6</td>
<td>21.2 ± 3.7</td>
</tr>
<tr>
<td>IIX-IIB</td>
<td>14.5 ± 4.8</td>
<td>0.3 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>IIB</td>
<td>42.4 ± 4.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-IIX</td>
<td>1.9 ± 0.6</td>
<td>3.0 ± 1.4</td>
<td>8.8 ± 2.3</td>
</tr>
<tr>
<td>I-IIB-IIX</td>
<td>0.1 ± 0.1</td>
<td>0.4 ± 0.2</td>
<td>1.5 ± 1.1</td>
</tr>
<tr>
<td>I-IIA-IIX</td>
<td>0.8 ± 0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-IIB-IIX-IIX</td>
<td>0.2 ± 0.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are given as means ± SE of 5 mice. Fiber type was determined using specific antimyosin for type I, IIA, IIB, and IIX. The total number of fibers analyzed for EDL, soleus, and FDB was 1,447, 1,231, and 758, respectively (note these numbers are the same for the analysis of Kir6.2 and CaV1.1 data).
The significant differences (34). When the interaction between mice. So, fluorescence intensity values for a given fiber type in a muscle were averaged for each mouse. The averages obtained for each mouse were then used to 1) calculate the final mean (i.e., the number of samples was equivalent to the number of mice and not the number of fibers tested) and 2) to run the ANOVA used to determine significant differences. A split-plot ANOVA was used in which muscles were in the whole plot, and data from different fiber types were in the split plot. ANOVA calculations were made using the version 9.0 GLM (general linear model) procedures of the Statistical Analysis Software (SAS Institute, Cary, NC). When the interaction was significant, the least square difference (LSD) was used to locate the significant differences (34).

**RESULTS**

**Fiber Type Composition**

Many past studies in which fiber types were determined did not have access to an antibody against IIX myosin, so the number of type IIX fibers was often calculated by subtracting the number of type I, IIA, and IIB fibers from the total number of fibers (36). Here, using an antibody for each of the four myosin isoforms, we better ascertained the fiber type composition of CD-1 mouse soleus, EDL, and FDB muscles.

In soleus, most fibers stained for myosin I isoform and fewer for myosin IIA (Fig. 1, A and B). For both myosins, the fluorescence was intense and evenly distributed across most fibers. Although myosin IIB isoform was basically absent in soleus, many fibers also expressed myosin IIX isoform (Fig. 1, C and D), except that the staining was much less intense compared with that of myosin I and IIA. As previously reported in other studies (5, 33), several fibers expressed more than one myosin isoform, and in the soleus, many fibers coexpressed either myosin I and IIA or myosin I and IIX.

On an average basis, a total of 67% of soleus fibers expressed myosin I (Table 1). However, less than half of these fibers (27%) solely expressed myosin I. A large proportion of fibers coexpressed myosin I and IIX, herein referred to as type I-IIX fibers. A small portion of soleus fibers coexpressed myosin I and IIA (7%, type I-IIA fibers) or myosin I, IIA, and IIX (2%, type I-IIA-IIX fibers). A total of 40% of the soleus fibers expressed myosin IIA, but only two-thirds of these fibers just expressed that myosin isoform. Although a total of 39% of the soleus fibers expressed myosin IIX, less than 1% of all soleus fibers only expressed myosin IIX; i.e., the large majority of these fibers coexpressed another myosin isoform. For all soleus type I-IIIX and IIA-IIIX fibers, the fluorescence intensity for myosin IIX was much less than for myosin I and IIA; i.e., myosin I and IIA were the dominant myosin isoforms being expressed in those fibers. The same was not observed for type I-IIIA fibers. As shown in Fig. 1, A and B, some fibers stained strongly for myosin I and weakly for myosin IIA, while for other fibers, the reverse was observed. Thus, it appears that in the soleus muscle, the expression of myosin I and IIA gives rise to a spectrum of type I → I-IIIA → IIA-I → IIA fibers.

Most EDL fibers stained for type IIX and IIB (Fig. 2). Like the soleus, EDL also contained fibers expressing more than one myosin isoform. A total of 17% of EDL fibers expressed myosin IIA, but the majority of them actually coexpressed myosin IIA and IIX (Table 1). The number of EDL fibers expressing myosin IIB and IIX amounted to 57% and 55%, respectively, of all fibers. However, fibers expressing just myosin IIB and IIX were 42% and 24%, respectively. In EDL, there was a large proportion of type IIA-IIX and IIB-IIX fibers. As for the type I-IIIA fibers in soleus, type IIA-IIX and IIB-IIX in EDL form a spectrum of fiber types in which, for example, some fibers expressed more myosin IIB than IIX, while others expressed more IIX than IIB.

FDB expressed three myosin isoforms: I, IIA, and IIX. Type I, IIA, and IIX fibers constituted 2%, 19%, and 21% of all FDB fibers, respectively, for a total of 42%. Thus, the majority of FDB fibers contain more than one myosin isoform. The largest
group was the type IIA-IIX fiber. FDB was also the muscle with the highest proportion of type I-IIA-IIX, which represented close to 9% of all FDB fibers, compared with 2% in soleus and 3% in EDL.

While it is well established that both EDL and FDB express myosin IIX (31, 36), there is only one other study reporting expression of myosin IIX in mouse soleus (12). To further substantiate the fiber type composition of all muscles, a semi-quantitative RT-PCR was performed to determine the mRNA content of the different myosin isoforms. The fluorescence intensity and mRNA content for myosin I were both in the order of soleus > FDB > EDL (Fig. 3A). For myosin IIA, the order was FDB ~ soleus > EDL (Fig. 3B). EDL muscle was the only one expressing myosin IIB in very large quantities (Fig. 3C). However, while EDL had the greatest amount of myosin IIB mRNA, there was substantial expression of the IIB mRNA in both soleus and FDB. Finally, there was a gradual increase in myosin IIX from soleus to EDL to FDB and a weak similar trend for the mRNA. Overall, for each myosin isoform, the trend in differences for protein content across muscles was similar to the trend observed for mRNA content, except that on a relative scale, the differences were much greater for the protein than for the mRNA.

Kir6.2 Content

Striking differences in fluorescence intensity were observed between fibers and between soleus and EDL when cross sections were stained with anti-Kir6.2 (Fig. 4, A and B). In some soleus fibers, a clear staining was observed outlining the cell membrane with little fluorescence inside the fibers, where the t-tubules are located; these fibers were found to express myosin I. Other fibers were clearly stained inside the fibers with various fluorescence intensities; these fibers were found to express myosin IIA. In general, the fluorescence intensity was much greater in EDL muscles. Again, the inside of some EDL fibers had barely any fluorescence, but interestingly, these fibers were those expressing myosin IIA, while fibers with the

Fig. 4. EDL and FDB had greater Kir6.2 and CaV1.1 content than soleus. Cross sections of soleus (A, C) and EDL (B, D) were stained with anti-Kir6.2 (red) or anti-CaV1.1 (green), respectively. E: total t-tubular fluorescence intensity in arbitrary units (AU) per square micrometer in soleus, EDL and FDB muscles. Data are given as means ± SE of 5 mice from a total of 1,302 fibers for soleus, 1,309 for EDL and 718 for FDB. *Significantly different mean fluorescence intensity than that of soleus, ANOVA and least square difference (LSD) P < 0.05.
greatest fluorescence intensity were fibers expressing myosin IIB or IIX. On an average basis, the total fluorescence intensity was the largest in EDL and FDB, being respectively 8- and 9-fold greater than in soleus (Fig. 4E). Differences in fluorescence intensity for CaV1.1 were also observed between muscles and between fibers within a muscle (Fig. 4, C and D). As observed for Kir6.2, the fluorescence intensity for CaV1.1 was larger in EDL and FDB than in soleus (Fig. 4E). However, the relative differences in fluorescence intensities for CaV1.1 were much smaller than for Kir6.2, as both EDL and FDB only had 3-times greater fluorescence intensity than soleus.

**Differences in Kir6.2 content among fibers.** In soleus, the fluorescence intensity for the Kir6.2 immunostaining was the lowest in type I fibers (Fig. 5A). Type IIA fibers had five times more fluorescence intensity than type I fibers. Type I-IIX fibers had similar fluorescence intensity as type I fibers, while type I-IIA fibers had an intermediate level between type I and I-IIA. The greatest fluorescence intensity was in type IIA-IIX fibers. In EDL, type I and IIA fibers had similar fluorescence intensity. Fluorescence intensities in type IIX and IIB fibers were 60% and 190% greater, respectively, than in type IIA fibers (Fig. 6A). Fluorescence intensities in type I-IIX and IIA-IIX fibers were similar to those of type I and IIA, while type IIB-IIX fibers had similar fluorescence intensity to that of type IIB fibers. In FDB muscles, there were gradual increases in fluorescence intensities from type I to IIA to IIX, as well as from type I-IIX to I-IIA to IIA-IIX (Fig. 7A). So, in most cases, the fluorescence intensity for Kir6.2 increased in the order of I ≤ IIA < IIX < IIB in a given muscle.

While differences in Kir6.2 fluorescence intensities were observed between fiber types within a muscle, differences were also observed for a given fiber type between muscles (Fig. 8A).
The fluorescence in FDB type I fibers was 11-fold greater than in similar fibers in soleus. Soleus and EDL type IIA fibers had similar mean fluorescence intensity, which was twofold less than in FDB IIA fibers. A similar twofold difference was observed between EDL and FDB type IIX fibers. It is also worth noting that while the mean fluorescence intensity was greater in EDL type IIB than EDL IIX fibers, FDB IIX fibers had similar mean fluorescence intensity than EDL IIB fibers. Differences in fluorescence intensity between muscles for a given fiber type were also observed in fibers expressing more than one myosin isoform.

**CaV1.1. Content**

One factor that can affect t-tubular Kir6.2 content is the fiber t-tubular content itself. Although there are discrepancies as to whether t-tubule surface area per fiber volume is lower in soleus than in EDL, there is agreement regarding the relative length of t-tubules associated in junctional contacts with the sarcoplasmic reticulum and the frequency of triads being lower in soleus than in EDL (13, 15). Furthermore, it is also known that the Ca^{2+} current and L-type Ca^{2+} channel densities are much lower in soleus than EDL fibers (23), which is in agreement with lower triad density. It is, thus, possible that differences in Kir6.2 content between muscles and fiber types are also linked to differences within t-tubules. If this is the case, then the relative differences in Kir6.2 and CaV1.1 between muscles and fibers should be similar. This comparison is also important because both proteins are involved in controlling muscle contractility. CaV1.1 is crucial in triggering Ca^{2+} release and thus contraction when action potentials travel down the t-tubules, while Kir6.2 is responsible for reducing membrane excitability and contractility during metabolic stress.

The mean fluorescence intensity for the CaV1.1 immunostaining in soleus type IIA fibers was twice the intensity in type I fibers (Fig. 5B), a much smaller difference than the 5-fold difference for Kir6.2. For fibers expressing more than one myosin isoform, the fluorescence intensity increased from I-IIX to I-IIA to IIA-IIX, but again the differences between fibers were much less for CaV1.1 than for Kir6.2. In EDL muscles, the largest difference in fluorescence intensity for...
CaV1.1 was between type I and IIA fibers with smaller differences between type IIA, IIB, and IIX fibers (Fig. 6B). This is, again, in contrast with the fluorescence intensities observed for Kir6.2 where small differences existed between type I, IIA, and IIX and a large difference existed between type IIB and IIX fibers. A gradual increase in mean fluorescence intensities was observed from type I-IIX to I-IIA to IIA-IIX to IIB-IIX. In FDB fibers, the CaV1.1 fluorescence intensities were in the order of IIX > IIA > I and IIA-IIX > I-IIA > I-IIX (Fig. 7B). Contrary to the situation in soleus and EDL, the differences in fluorescence intensity for CaV1.1 between fiber types were almost a mirror image of the differences for Kir6.2. Finally, there were also differences in fluorescence intensities for CaV1.1 for a given fiber type between muscles, but the number of significant differences was much less compared with the number of significant differences for Kir6.2 (Fig. 8B).

**Correlation Between Kir6.2 and CaV1.1 Content**

Here, the mean fluorescence intensity (AU/μm²) for Kir6.2 and CaV1.1 was first determined for type IIA fibers of each animal. The fluorescence intensities of all fibers were then expressed as a ratio of that mean. After averaging the ratios for each fiber type, the mean fluorescence ratios for Kir6.2 were plotted against those for CaV1.1. All regressions were significant (P < 0.05) with R values of 0.885, 0.808, and 0.981 for soleus, EDL and FDB, respectively (Fig. 9). The slopes were also significantly (P < 0.05) greater than 1 for soleus and EDL, being respectively 2.44 and 1.65, but not significant for FDB.

**DISCUSSION**

The major findings of this study are 1) within a muscle, the t-tubular Kir6.2 content was generally in the order of type IIB > IIX > IIA ≥ I fibers; 2) for a given fiber type, t-tubular Kir6.2 content varied among different muscles; and 3) the variation in t-tubular Kir6.2 content between fibers was greater than the variation in CaV1.1 content for soleus and EDL, but not for FDB.

In this study, the content of the different myosin isoforms, t-tubular KATP channel and CaV1.1 was estimated using a semiquantitative immunohistochemistry measurement. This approach was used over Western blot analysis because measurements were done in several fibers and to specifically measure t-tubular Kir6.2 and CaV1.1 content without any contamination from the outer cell membrane. While antibody penetration is a problem with immunohistochemistry, we reduced the extent of this problem and others by using a large number of fibers, thus reducing measurement variability through increased sample size. Furthermore, cross sections from different muscles from the same mouse were simultaneously stained on the same slide, and pictures were taken under the same conditions to permit comparisons between muscles. Finally, at least for CaV1.1, the mean fluorescence intensity was significantly greater in EDL than soleus, suggesting a greater channel content in EDL, which is in agreement with greater L-type Ca²⁺ currents in EDL (23).

**Fiber-Type Composition**

As previously reported (2, 7, 12, 27, 32, 36), in this study, myosin I and IIA were the two main myosin isoforms expressed in soleus muscle fibers. However, we now show that myosin IIX was also expressed in 40% of soleus fibers, and the presence of its mRNA transcript further confirms that this isoform is expressed in soleus. However, pure type IIX fibers were rarely expressed in soleus; i.e., myosin IIX was most of the time coexpressed with myosin I. So, the major fiber-type composition of soleus muscle should now be considered as type I, I-IIX, and IIA. In EDL, the proportions of fibers expressing myosin I, IIA, and IIB were also similar to previous reports. However, contrary to previous studies, most fibers expressing myosin IIA actually coexpressed myosin IIX, while one-third of the fibers expressing myosin IIB coexpressed myosin IIX. As previously reported (28, 32, 36), 24% of EDL fibers expressed just myosin IIX, but another 32% coexpressed myosin IIX with either myosin IIA or IIB. Thus, in EDL, the major fiber types are IIA-IIX, IIX, IIX-IIB, and IIB. For FDB, they are type IIA, IIA-IIX, and IIX fibers.

So, our study further confirms that skeletal muscle fiber-type composition forms a dynamic continuum rather than four clearly delineated fiber types (33, 35). Future functional contractility studies are now needed to determine how myosin coexpression affects the contractile profile and what role they play in the mechanism of muscle plasticity.

![Graphs showing variations of fluorescence intensities for Kir6.2 and CaV1.1 for fibers expressing one myosin isoform](http://ajpregu.physiology.org/).
**Kir6.2 Content**

The fast-twitch glycolytic EDL and FDB had higher t-tubular Kir6.2 content than the slow-twitch oxidative soleus. Within a muscle, the t-tubular content was in the order of type IIB > IIX > IIA ≥ I. The situation was more complex for fibers expressing more than one myosin isoform, but in most cases, the Kir6.2 content was either close to or between the content of the fibers expressing either myosin isoform. For example, in the soleus muscle, the Kir6.2 content of type I-IX fibers was close to that of type I, while for type I-IIA fibers, the Kir6.2 content was between that of type I and IIA fibers. As mentioned in the results, differences in t-tubular content, in the relative length of t-tubules associated in junctional contacts with the sarcoplasmic reticulum and/or in the frequency of triads may be the reason for the differences in Kir6.2 content between muscles and fiber types. Indeed, correlations of Kir6.2 relative fluorescence intensities vs. that of CaV1.1 were significant for all three muscles. However, the Kir6.2 vs. CaV1.1 correlations gave slopes that were significantly greater than one for soleus and EDL. Furthermore, FDB fibers had up to twice the amount of Kir6.2 compared with the same fiber types in EDL and soleus, whereas for CaV1.1, the differences were much less and, in many cases, nonsignificant. Finally, the difference between the fiber type with the lowest (soleus type I fiber) and highest (EDL type IIB fiber) fluorescence intensity was 47-fold for Kir6.2 and just 7-fold for CaV1.1. Thus, the relative differences in fluorescence intensities between muscles/fibers were much greater for Kir6.2 than CaV1.1. So, while the t-tubular membrane content may, in part, determine the expression of $K_{\text{ATP}}$ channel in t-tubules, it cannot be the only factor.

Several studies have now reported differences in the physiological responses to a modulation of $K_{\text{ATP}}$ channel activity during exercise and fatigue among different muscles. Muscle fatigue studies have demonstrated that abolishing the channel activity causes increases in resting force above that of control muscles in the order of FDB >> EDL > soleus and greater depression of force recovery following fatigue in the order of EDL ~ FDB > soleus (8, 9, 16, 17, 25). One reason for the effect being the smallest in the oxidative soleus is undoubtedly because of a smaller metabolic stress, resulting in less $K_{\text{ATP}}$ channel activation; so, abolishing the channel activity has the smallest effect. However, soleus muscle also had the smallest t-tubularKir6.2 content, especially in fibers expressing myosin I, which constitute the majority of soleus fibers.

Different responses to a lack of $K_{\text{ATP}}$ channel activity have also been reported at the fiber level. When mice were elicited to run on a treadmill for 4 – 5 wk, between 10 and 25% of fibers in EDL, plantaris, and tibialis had been damaged and eventually regenerated, an effect not observed in muscles of wild-type mice (36). More importantly, 98% of the regenerated fibers were type IIB fibers, and the remaining 2% type IIA or IIX. Thus, the most glycolytic fibers appear to be the most dependent on $K_{\text{ATP}}$ channel for myoprotection, at least during treadmill running, and this correlates with the fact that type IIB fibers are among those with the highest $K_{\text{ATP}}$ channel content, at least in EDL. Finally, during fatigue in vitro, a large number of single $K_{\text{ATP}}$ channel-deficient FDB fibers had excessive resting membrane depolarization and increases in resting myoplasmic [Ca$^{2+}$] ([Ca$^{2+}$]i), while other fibers had membrane depolarizations and increases in [Ca$^{2+}$] that were similar to those observed in FDB fibers from wild-type mice. Very few FDB fibers expressed myosin IIB, but the fibers expressing myosin IIX have as much $K_{\text{ATP}}$ channel as the EDL type IIB fibers. It is, therefore, most likely that the most affected $K_{\text{ATP}}$ channel-deficient FDB fibers are those expressing myosin IIX, the most glycolytic fibers, while the least affected ones are probably fibers expressing myosin I and/or IIA, the most oxidative fibers. Together, these results suggest that the regulation of $K_{\text{ATP}}$ channel expression in muscles/fibers depends on either the metabolic profile and/or the incidence of metabolic stress, resulting in greater channel content as the glycolytic capacity increases and oxidative capacity decreases.

We had hypothesized that $K_{\text{ATP}}$ channel content between muscles would be greater in EDL than in FDB because the EDL contains many type IIB fibers, being the most glycolytic fibers, while FDB does not. There was however, no difference in channel content between the two muscles. Moreover, FDB type IIX fibers contain more $K_{\text{ATP}}$ channels than EDL type IIX and as much as EDL type IIB fibers. Finally, EDL type IIA express more channels than soleus IIA. It is known that the daily stimulation pattern is very different between EDL and soleus (20). It is, thus, possible that the expression of $K_{\text{ATP}}$ channel is also influenced by the daily pattern of muscular activity.

In summary, we have shown based on Kir6.2 content that the t-tubular $K_{\text{ATP}}$ channel content varies significantly among different muscles and fiber types. We provided evidence that $K_{\text{ATP}}$ channel content cannot be fully related to differences in t-tubular membrane content. In fact, the content (this study) and physiological effects (previous studies) of $K_{\text{ATP}}$ channels are the highest in the most glycolytic muscles (i.e., EDL and FDB) and fibers (i.e., type IIB and IIX) and the lowest in the most oxidative muscle (i.e., soleus) and fibers (i.e., type I and IIA). Three possible factors have been proposed to affect $K_{\text{ATP}}$ channel expression: the metabolic profile, incidence of metabolic stress and pattern of muscle activity. It will be of interest in future studies to determine how these three factors influence $K_{\text{ATP}}$ channel expression.

**ACKNOWLEDGMENTS**

This study was supported by a grant from the National Science and Engineering Research Council to Jean-Marc Renaud.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**REFERENCES**


KATP CHANNEL CONTENT IN SKELETAL MUSCLE FIBERS


