Deficiency of α-sarcoglycan differently affects fast- and slow-twitch skeletal muscles

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Danieli-Betto, Daniela, Alessandra Esposito, Elena Germinario, Dorianna Sandonà, Tiziana Martinello, Anna Jakubiec-Puka, Donatella Biral, and Romeo Betto. Deficiency of α-sarcoglycan differently affects fast- and slow-twitch skeletal muscles. Am J Physiol Regul Integr Comp Physiol 289: R1328–R1337, 2005. First published July 7, 2005; doi:10.1152/ajpregu.00673.2004. —α-Sarcoglycan (Sgca) is a transmembrane glycoprotein of the dystrophin complex located at skeletal and cardiac muscle sarcolemma. Defects in the α-sarcoglycan gene (Sgca) cause the severe human-type 2D limb girdle muscular dystrophy. Because Sgca-null mice develop progressive muscular dystrophy similar to human disorder they are a valuable animal model for investigating the physiopathology of the disorder. In this study, biochemical and functional properties of fast-twitch extensor digitorum longus (EDL) and slow-twitch soleus muscles of the Sgca-null mice were analyzed. EDL muscle of Sgca-null mice showed twitch and tetanic kinetics comparable with those of wild-type controls. In contrast, soleus muscle showed reduction of twitch half-relaxation time, prolongation of tetanic half-relaxation time, and increase of maximal rate of rise of tetanus. EDL muscle of Sgca-null mice demonstrated a marked reduction of specific twitch and tetanic tensions and a higher resistance to fatigue compared with controls, changes that were not evident in dystrophic soleus. Contrary to EDL fibers, soleus muscle fibers of Sgca-null mice distinctively showed right shift of the pCa-tension (pCa is the negative log of Ca2+ concentration) relationships and reduced sensitivity to caffeine of sarcoplasmic reticulum. Both EDL and soleus muscles showed striking changes in myosin heavy-chain (MHC) isoform composition, whereas EDL showed a larger number of hybrid fibers than soleus. In contrast to the EDL, soleus muscle of Sgca-null mice contained a higher number of regenerating fibers and thus higher levels of embryonic MHC. In conclusion, this study revealed profound distinctive biochemical and physiological modifications in fast- and slow-twitch muscles resulting from α-sarcoglycan deficiency.

limb girdle muscular dystrophy 2D; skeletal muscle fiber types; skeletal muscle contractile properties; caffeine sensitivity of sarcoplasmic reticulum; calcium sensitivity of myofibrillar proteins

THE SARCOCGLYCAN COMPLEX IS a group of four single-pass transmembrane proteins (α, β, δ-, and γ-sarcoglycan) associated with the major dystrophin-based membrane cytoskeletal complex (4, 23, 29). Although the exact function of the sarcoglycan complex is not well defined, it is well established that mutations in any of the sarcoglycan genes result in distinct forms of limb-girdle muscular dystrophy, collectively named sarcoglycanopathies (5, 29, 36, 37, 45). A clear sign that the four sarcoglycans act as a complex is the fact that mutations in any one of the sarcoglycan genes cause the loss of or defects in cognate proteins and typically produces deficiencies of other sarcoglycan proteins (23, 50).

Besides the sarcoglycans, the multimeric complex formed by dystrophin includes also dystroglycan (α and β), syntrophins, dystrobrevin, and sarcospan (4). Dystrophin and dystrophin-associated proteins (DAP) provide muscle cell membrane for a mechanical connection between myofibrils and the extracellular matrix. Particularly, dystrophin links in the intracellular side actin cytoskeleton with the transmembrane anchor point of β-dystroglycan, whereas, on the extracellular side, α-dystroglycan connects to laminin of the extracellular matrix (4, 32). The DAP complex is regularly distributed all over muscle cell membrane and forms a supportive cytoskeletal structure devoted to protect muscle membrane from lateral solicitation due to contractile activity (38, 41). As a consequence, the lack of dystrophin as it occurs in Duchenne muscular dystrophy (DMD), in causing full dismantling of the entire structure makes the muscle fiber more prone to membrane damage (4, 50). The sarcoglycans, with sarcospan, form a distinct subcomplex that stabilize the association of dystroglycans to dystrophin and thus reinforce the overall structure (9). Therefore, deficiencies in the sarcoglycans also determine destabilization of dystrophin complex leading to membrane instability and higher sensitivity to membrane injury (14, 24, 25, 51).

It is worth reporting that additional sarcoglycans functions have been described that could help revealing additional loss-of-function mechanisms in the pathogenesis of the dystrophy (23). It has been shown that sarcoglycans, within the dystrophin complex, represent an additional anchor point for neuronal nitric oxide (NO) synthase (nNOS) via α-syntrophin and α-dystrobrevin (62). Consistently, the loss of sarcoglycans determines the loss of nNOS both in sarcoglycan-null mice and in patients of sarcoglycanopathies (8). Moreover, through γ- and δ-sarcoglycan, the sarcoglycan complex associates filamin-2, a protein normally involved in transduction signaling pathways (55). Also, because all sarcoglycan can be phosphorylated (61) and β-, γ- and δ-sarcoglycan possess an extracellular epidermal growth factor-like domain, the complex has been proposed as an independent signaling module (23). Finally, we have demonstrated that α-sarcoglycan is an ecto-ATPase, indicating that α-sarcoglycan might control the ATP

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level and the signaling action of the nucleotide at the surface of muscle cells (3, 49). Thus the combined loss of mechanical and signaling functions associated with sarcoglycan deficiency might contribute to the pathological phenotype of dystrophic muscle.

Identification of animal models of muscular dystrophy has been helpful in research of the pathogenesis, physiopathology, and treatment of these disorders (15, 57). The most widely used model of muscle dystrophy is the mdx mouse, the dystrophin-null animal model for DMD. In general, it is reported (17, 31, 48) that the absence of dystrophin does not alter twitch kinetics of muscles from mdx mice, whereas specific twitch and tetanic forces are significantly reduced. Because it has been demonstrated that fast-twitch fibers are preferentially affected in DMD patients (58), studies were undertaken to reveal any possible difference in the contractile characteristics of fast- and slow-twitch muscles. Although not all studies of hind-limb muscles from mdx mice were performed at the same age, it emerged that specific tensions of dystrophic extensor digitorum longus (EDL) and soleus are equally reduced compared with wild-type controls (1, 17, 31, 39). On the other hand, studies at the single-fiber level revealed that EDL and soleus muscles of mdx mice are affected differently by the absence of dystrophin (13, 29). The different dysfunction of the mdx dystrophic muscles has been correlated with changes of myosin heavy-chain (MHC) composition and enzymatic kinetic of myofibrillar proteins Ca2+ -sensitivity as well as of excitation-contraction coupling (1, 12, 13, 28, 35, 41, 43).

Murine models of the diverse sarcoglycanopathies have also been generated and, with the naturally occurring δ-sarcoglycan-null Bio 14.6 hamster, have been used to investigate the functional consequence of sarcoglycan-deficiency. All sarcoglycan-null animals show progressive muscular dystrophy of variable severity (2, 14, 16, 24, 30).

Functionally, fast-twitch muscles from Bio 14.6 dystrophic hamster showed either no changes (34) or the significant reduction (53, 60) of absolute and specific twitch and tetanic forces compared with wild-type control. Soleus muscle of dystrophic hamster equally showed the dramatic decrease of specific tensions (7). In contrast to the Bio 14.6 dystrophic hamster, mice lacking δ-sarcoglycan showed no changes in the absolute tetanic force produced by EDL (23).

Mice lacking γ-sarcoglycan develop progressive muscular dystrophy similar to human muscular dystrophy (24). However, fast EDL muscle from γ-sarcoglycan-null and wild-type mice showed no significant differences in the absolute and specific twitch and tetanic tensions (P0) and, surprisingly, in the resistance to mechanical strain (22). Unfortunately, it is not possible to compare force production of fast- and slow-twitch muscles from Sgcd- and Sgcg-null mice, because, so far, contractile properties of slow muscles have not been analyzed.

From the physiological point of view, Sgca-null mice show the significant reduction of specific force of hind-limb and diaphragm muscles, compared with wild-type controls (14, 40, 48). However, it is evident that fast EDL muscle reacts differently than slow-twitch soleus muscle to the absence of α-sarcoglycan, particularly in the ability to generate absolute force (14, 48). An additional difference between fast and slow muscles is that EDL muscle of Sgca-null mice has a very high resistance to passive stretch compared with control muscle, whereas soleus is not different from control (14). In contrast, both fast and slow muscles of Sgca-null mice are susceptible to contraction-induced injury after exercise, a sensitivity evident also in fibers from Sgcb- and Sgcd-null mice but not in fibers from γ-sarcoglycan-deficient muscles (22).

In summary, functional studies have revealed that consequences of primary gene defects are more complex and variable than predicted (57). Defects in individual sarcoglycans seem to cause either similar or opposing responses to fast- and slow-twitch muscles. In particular, Sgca-null muscles show a dramatic difference in the adaptive response of fast- and slow-twitch muscles, an evidence that evoked the need for additional investigation (14). In the present study, contractile properties and fatigue characteristics of fast-twitch EDL and slow-twitch soleus muscles from Sgca-null mice were investigated. These data were complemented with analyses at the single-fiber level of Ca2+ -sensitivity of myofibrillar proteins, of sarcoplasmic reticulum calcium sensitivity, and of MHC isoforms expression. Results make evident distinct functional and contractile differences between EDL and soleus muscles from Sgca-null mice.

MATERIALS AND METHODS

Animals. This study was approved by the Ethics Committee of the Medical Faculty of the University of Padova. The protocol utilized in the study has been authorized by the Public Veterinary Health Department of the Italian Ministry of Health. The experiments were carried out on the Sgca-null mice previously characterized (30) by using the congenic C57BL/6j strain (Charles River) as control. All experiments were performed on male mice of the same age (12–13 wk). The animals were first weighed and then killed by cervical dislocation. Soleus and EDL muscles were excised, weighed, and used for the experimental analyses.

Histological and immunofluorescence analysis. Muscles were frozen in liquid nitrogen in a slightly stretched position. Serial cross sections (8-μm thick) were cut in a cryostat microtome set at −24 ± 2°C (Slee Pearson). For the histochemical analysis, hematoxylin and eosin staining was performed on muscle sections to examine the general morphology and to determine the cross-sectional area (CSA) of individual fibers. Muscle cryostat sections were also stained for the SDH activity. Digital photographs were taken of each muscle section and analyzed by the ImageJ NIH imaging software.

MHC expression in muscle fibers was detected by immunofluorescence using specific antibodies. Serial sections from control and Sgca-null soleus and EDL muscles were probed with the following antibodies: BA-D5 (1:400) for type 1 MHC, A4–74 (1:50) for type 2A, BF-F3 (1:400) for type 2B, RT-D9 (1:400) for type 2B/2X, and F1.652 (1:5) for the embryonic isoform. A4–74 and F1.652 were from Developmental Studies Hybridoma Bank, (University of Iowa, Iowa City, Iowa). BA-D5, BF-F3, and RT-D9 antibodies were from S. Schiaffino (University of Padova, Padova, Italy). All primary antibodies were diluted in PBS and incubated at room temperature for 45 min. Tetramethylrhodamine isothiocyanate-conjugated rabbit antimouse immunoglobulins (Dako, Denmark, 1:100), incubated at room temperature for 30 min, were used as secondary antibodies.

Whole muscle mechanical properties. Contractile properties were investigated in vitro at 30 ± 1°C. Muscle bathing, stimulation conditions, and tension recordings were performed as previously described (33). Twitches were obtained by applying single supramaximal stimuli (0.5 ms duration), whereas tetani were obtained by applying trains of stimuli at frequency of 100 and 140 Hz for soleus and EDL, respectively. The following parameters were measured: contraction time, half-relaxation time of the twitch, and twitch (P0) and P0. The specific force (i.e., force per CSA) produced by each muscle was calculated from the absolute force, muscle weight, and...
muscle length, assuming a density of 1.056 g/ml (25). The maximum rate of rise of tetanus was also measured (expressed both as Nm/s and N·ms⁻¹·mm⁻²). Muscle responses were recorded via an AT-MIO 16 AD card and data were analyzed by the LabView computer program (National Instruments). Force-frequency curves were determined by stimulating soleus muscle at 20, 40, 60, 80, and 100 Hz and EDL muscle at 30, 60, 100, 140, and 160 Hz. Fatigue was produced, as previously reported (11, 19), by submitting muscles to a 10-min protocol consisting of a train of short tetani at low frequency (40 Hz for 300 ms, 0.5/s, in soleus and 60 Hz for 300 ms, 0.3/s in EDL). Force recorded during the fatigue protocol was normalized to the initial tension. At the end of each experiment, the muscles were weighed.

Preparation of single fibers. Muscle fibers were chemically skinned as previously described (10, 20, 33). Briefly, muscles were tied to a wooden stick and quickly immersed into an ice-cold skimming solution containing (in mM): 170 K-propionate, 2.5 Mg-propionate, 2.5 Na₂K₂ATP, 5 K₂EGTA, and 10 imidazole buffer, pH 7.0. Skinned fibers were stored at ~20°C for no more than 4 wk in a skimming solution supplemented with 50% (vol/vol) glycerol.

Single fiber functional studies-Ca²⁺ sensitivity of tension development. pCa-tension curves (pCa is the negative log of Ca²⁺ concentration) were obtained by exposing the fiber sequentially to solutions with different free Ca²⁺ concentration (10, 20). Single-fiber segments (~1.5-mm length) were inserted between two clamps, one fixed and the other connected to a tension transducer (model 801; AK Sensonor, Horten, Norway). Fibers were immersed in a relaxing solution at room temperature (22–24°C) with the following composition (in mM): 170 K-propionate, 2.5 Mg-propionate, 5 Na₂K₂ATP, 5 K₂EGTA, and 10 imidazole buffer, pH 7.0. Fibers were stretched manually to a sarcomere length of 3 μm as measured by light diffraction with a helium neon laser lamp (33). The tension generated in each pCa solution was continuously recorded, and the baseline tension was established as the steady-state voltage output recorded with the fiber in the relaxing solution. Specific tension for each single fiber was calculated by normalizing the maximum tension measured at pCa 5 to the fiber CSA, as calculated by three different diameter determinations along the fiber length considering the fiber immersed in solution as a cylinder (11).

Functional studies of single fiber-sarcoplasmic reticulum caffeine response. The threshold for sarcoplasmic reticulum Ca²⁺-release in response to caffeine was determined as previously described (11, 20, 33). Fibers were first incubated for 30 s in a Ca²⁺-loading solution (pCa 7.0 solution) and then in a relaxing solution deprived of EGTA. The fibers were stepwise challenged with increasing concentrations of caffeine until tension was recorded (11, 20, 33). To completely deplete sarcoplasmic reticulum cisterns of Ca²⁺, 20 mM caffeine was used. The tensions developed at caffeine threshold (Pₐ) and by 20 mM caffeine (Pₐ₀), were measured. Caffeine threshold was defined as the lowest concentration (usually at least 3–5% Pₐ₀) of caffeine that was able to induce an appreciable tension (11, 47).

**RESULTS**

**Histological analysis of Sgca-null muscles.** Mice lacking α-sarcoglycan develop progressive muscular dystrophy similar to human muscular dystrophy (14, 27, 48). At 3 mo of age, EDL and soleus muscles from Sgca-null mice showed a significantly larger mass compared with wild-type controls (Table 1). Moreover, both muscles showed an ample variability of

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<th>Table 1. Contractile properties of Sgca-null muscles</th>
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Values are means ± SE. The number of muscles utilized for the analysis of contractile properties was 13 wild-type and 14 Sgca-null extensor digitorum longus (EDL) and 9 wild-type and 5 Sgca-null soleus. P₀, maximal twitch tension; CT, contraction time; HRT, half-relaxation time; P₀/P₀, twitch tension-to-tetanic tension ratio; fiber CSA, muscle fiber cross-sectional area, as determined in fibers from Sgca-null and wild-type EDL (667 and 673, respectively) and soleus (844 and 887, respectively). The muscle-to-body mass ratio was calculated taking into account that the body mass of control and Sgca-null mice were 23.9 ± 0.6 (n = 6) and 25.9 ± 0.3 (n = 6) g, respectively, with the difference being significant (P < 0.005). Table data significant differences: *P < 0.05; †P < 0.005.
muscle fiber size, with the presence of very small fibers with very large ones. EDL muscle fibers from Sgca-null mice showed a mean CSA 43% larger compared with wild-type control fibers (Table 1), whereas a minor, still significant, CSA increase (24%) was shown in soleus muscle fibers (Table 1). A significant number of the large fibers of EDL muscles from Sgca-null mice showed partial or complete splitting, a rare occurrence in soleus fibers (Fig. 1). Moreover, EDL and soleus muscle fibers from Sgca-null mice showed a high number of internally located nuclei (62.6 ± 4.7%, n = 1,243, and 65.4 ± 2.3%, n = 975, respectively), with some large fibers containing two or three internal nuclei, particularly in EDL muscles (Fig. 1). In contrast, the number of fibers with internally-located nuclei was very low (>0.2%) in both EDL and soleus wild-type controls. Figure 1 also shows that soleus muscles from Sgca-null mice contain a larger number of small regenerating fibers (~18%) compared with dystrophic EDL muscles (~2%).

EDL muscle fibers from Sgca-null mice showed an overall more intense succinate dehydrogenase (SDH) staining intensity, an estimate of mitochondria number, compared with wild-type controls (Fig. 1). In contrast, soleus muscle fibers from Sgca-null mice showed no significant difference in SDH reaction compared with controls (not shown).

Contractile properties of Sgca-null muscles. Figure 2 shows typical examples of twitches and tetani produced in EDL and soleus muscles from Sgca-null and wild-type mice. EDL muscles from Sgca-null and wild-type mice showed no significant differences in the average absolute twitch tension (Table 1). In contrast, soleus muscles from Sgca-null mice produced a higher absolute twitch tension than wild-type controls (Fig. 2 and Table 1). EDL muscles from Sgca-null and wild-type mice showed no significant differences in the contraction and relaxation times, whereas the latter was shorter in Sgca-null soleus compared with wild-type controls (Fig. 2C and Table 1).

In EDL muscles from Sgca-null mice, the absolute P0 was significantly lower than in wild-type controls (Table 1). In contrast, in soleus muscles from Sgca-null mice, the absolute P0 was slightly higher (Table 1). When normalized to the CSA, on the other hand, the average specific twitch tension was significantly lower in Sgca-null EDL compared with controls, whereas that of soleus muscles was not different (Table 1). The muscle twitch-to-tetanus ratio was significantly higher in Sgca-null EDL mice compared with controls, whereas that of soleus was not different in Sgca-null and wild-type mice (Table 1).

EDL muscles from Sgca-null mice showed no significant differences in the absolute maximum rate of rise of tetanus, whereas in soleus muscles from Sgca-null mice the rate was significantly higher than in wild-type controls (Table 1). Similarly, the half-relaxation P0 time was higher only in soleus muscles from Sgca-null mice compared with controls (Table 1).

Force-frequency relationship of Sgca-null muscles. Figure 3 shows the force-frequency relationship in EDL and soleus muscles from Sgca-null and wild-type mice. EDL muscles from Sgca-null mice produced a higher relative force at all frequencies up to 100 Hz compared with controls, thus showing a clear leftward shift of the force-frequency relationship. In fact, the frequency giving 50% force was ~45 Hz in Sgca-null EDL muscles and 60 Hz in controls. Soleus muscles from Sgca-null mice produced a higher relative force at frequencies above 40 Hz compared with controls (Fig. 3B). Soleus muscles from Sgca-null and wild-type mice showed no significant differences in the frequency giving 50% force (~22 Hz).

Fatigue of Sgca-null muscles. Force production during fatigue produced by low-frequency tetanic stimulation of muscles is shown in Fig. 3, C and D. EDL muscles from Sgca-null mice were more fatigue resistant than controls (Fig. 3C). In fact, EDL muscles from Sgca-null mice produced significantly higher mean relative forces during the whole fatiguing protocol, which includes an initial transient potentiation phase. In EDL muscles from Sgca-null mice, relative force at the end of the 10-min stimulation was 39.0 ± 1.5 and 20.1 ± 2.9% in controls. In contrast, there was no significant difference in the fatigue resistance in soleus muscles compared with wild-type controls (Fig. 3D).

MHC composition of Sgca-null muscles. Figure 4 shows MHC isoform composition of EDL and soleus muscles from Sgca-null mice as determined by SDS-PAGE analysis (Fig. 4A) performed with the method described by Talmadge and Roy (54). EDL muscles from Sgca-null and wild-type mice

Fig. 1. Histological analysis of Sgca-null muscles. Hematoxylin and eosin (A and B) and succinate dehydrogenase (C and D) staining of cryostat sections of extensor digitorum longus (EDL) and soleus muscles from Sgca-null mice. Hematoxylin and eosin staining performed in EDL (A) and soleus (B) muscles from Sgca-null mice reveals very large fibers with small regenerating ones whose number is higher in soleus than in EDL muscle. Some large fibers of EDL muscle from Sgca-null mice show longitudinal splitting (arrows). Numerous fibers of both EDL and soleus muscles from Sgca-null mice show internally-located nuclei, with some fibers containing 2 or 3 nuclei. Succinate staining performed in EDL muscle sections from wild-type (C) and Sgca-null mice (D) show an overall higher staining in the dystrophic muscle.
showed no significant difference in the overall MHC isoforms composition, both muscles showing a comparable net prevalence of type 2B MHC isoform (Fig. 4B). On the other hand, in soleus muscles from *Sgca*-null mice, expression level of type 1 MHC was significantly lower, and that of type 2X MHC was significantly higher compared with wild-type controls. Moreover, the protein band comprising both type 2A and embryonic MHC isoforms (the electrophoretic method does not permit the
separation of these two bands) was significantly reduced in soleus muscles from Sgca-null mice compared with controls (Fig. 4B).

pCa-tension relationship of Sgca-null muscle fibers. Ca$^{2+}$ sensitivity of myofibrillar proteins was measured in chemically skinned fibers isolated from wild-type and Sgca-null muscles. EDL muscle fibers isolated from Sgca-null and wild-type mice showed no difference in the pCa/tension relationships (Fig. 5 and Table 2). In contrast, soleus muscle fibers isolated from Sgca-null mice showed a significant right shift of the pCa-tension curve compared with controls (Fig. 5). In fact, the pCa threshold for tension development and the pCa$_{50}$ were significantly lower in soleus fibers of Sgca-null mice compared with controls (Table 2). The Hill coefficient, an estimate of cooperative interactions between myofibrillar elements, was not different between soleus muscle fibers of Sgca-null and control mice (Table 2).

Sarcoplasmic reticulum caffeine sensitivity of Sgca-null muscle fibers. Figure 6 shows caffeine sensitivity of Ca$^{2+}$ release from sarcoplasmic reticulum of muscle fibers isolated from wild-type and Sgca-null muscles. In wild-type mice, the mean threshold caffeine concentration able to stimulate the release of enough Ca$^{2+}$ to cause a detectable fiber contraction (11, 20) was significantly lower in soleus fibers compared with EDL fibers (Table 3 and Fig. 6A). In soleus muscle fibers from Sgca-null mice, the mean caffeine threshold was significantly higher compared with control fibers, but it was not different compared with wild-type EDL fibers. Moreover, sarcoplasmic reticulum of soleus fibers isolated from Sgca-null mice released less calcium than control fibers (Table 3). In fact, the ratio between tension developed at threshold caffeine concentration (P$_{th}$/P$_{20}$) and that developed at 20 mM caffeine (P$_{20}$, a concentration able to completely empty sarcoplasmic reticulum) was significantly reduced (Table 3). Contracture amplitude produced by 20 mM caffeine, corresponding to the amount of Ca$^{2+}$ accumulated by sarcoplasmic reticulum during 30 s of incubation, was also significantly lower in soleus fibers isolated from Sgca-null mice compared with wild-type controls (Table 3).

MHC composition of single fibers from Sgca-null muscles. Figure 7 shows SDS-PAGE analysis of MHC isoforms expressed by the chemically skinned fibers used for the physiological analysis described in Figs. 5 and 6.

Seventy-four percent of fibers isolated from EDL muscles of wild-type mice (n = 34) contained type 2B MHC isoform, whereas the remaining fibers (26%) contained type 2A MHC. In contrast, 67% of fibers isolated from EDL muscles of Sgca-null mice (n = 42) were hybrid fibers coexpressing 2B and 2X MHC isoforms, whereas the remaining fibers contained the type 2B MHC only.

Single fibers isolated from soleus muscles (n = 38) presented a more variable composition of MHC isoforms with a large number of hybrid fibers (Fig. 7). Approximately 37% of the single soleus fibers isolated from wild-type muscles ex-
between control and Sgca-null EDL, 15 in wild-type, and Sgca-null soleus.

**DISCUSSION**

The present study shows that the absence of α-sarcoglycan in the Sgca-null mice produces profound modifications of contractile properties that are largely muscle-type specific, in that changes noted in the fast-twitch EDL muscle were often either not present in dystrophic soleus muscles or changed in the opposite direction.

Limb-girdle muscular dystrophy 2D is an autosomal recessive disorder caused by mutations in the Sgca gene (45). The Sgca-null mice was developed to determine how the sarcoglycan-sarcospan complex, destabilizes the dystrophin complex by greatly reducing the amount of bound α-dystroglycan (9, 14). This results in the structural instability of sarcolemma, as indicated by the uptake of Evans blue dye into muscle fibers and the elevated serum levels of cytoplasmic enzymes (14).

Functionally, Sgca-null muscles show a dramatic difference in the adaptive response of fast- and slow-twitch muscles, an evidence that evoked additional investigation (14). Likewise in mdx mice (17, 39), EDL and soleus muscles from 3-mo-old Sgca-null mice display a larger mass (27 and 36%, respectively) and mean CSA than age-matched controls. Hypertrophy of EDL and soleus muscles in younger (8-wk-old) Sgca-null mice was much larger, 40 and 62%, respectively (14), than that reported here in older animals, suggesting that the phenomenon could be progressively attenuated with age.

**Table 2. pCa-tension relationships of single fibers**

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<td>control</td>
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<tr>
<td>pCα0</td>
<td>6.24±0.06</td>
<td>6.28±0.05</td>
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<td>5.83±0.07</td>
<td>5.84±0.04</td>
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<td>N</td>
<td>2.66±0.46</td>
<td>2.10±0.15</td>
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<td>1.38±0.07</td>
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Values are means ± SE; n is number fibers, pCα0 is the lowest log Ca2+ concentration giving a detectable tension; pCα50 is the pCa value corresponding to 50% of maximum tension; N, the Hill coefficient. Significant differences between control and Sgca-null mice are indicated. *P < 0.01; †P < 0.04.

**Table 3. Sarcoplasmic reticulum caffeine sensitivity of single fibers**

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<td>pCα50</td>
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<td>70.5±8.2</td>
<td>46.2±4.9</td>
</tr>
</tbody>
</table>

Values are means ± SE; n is number fibers, Pth/P20, ratio between the tension developed at caffeine threshold level and 20 mM caffeine. Significant differences between control and Sgca-null mice are indicated. *P < 0.05; †P < 0.01.
A first important functional difference between fast- and slow-twitch 
Sgca-null hindlimb muscles is that, despite a similar level of hypertrophy, 
absolute tetanic force of 3-mo-old EDL was reduced by 39%, whereas 
that of soleus was increased by 38%, compared with age-matched controls. As a 
consequence, specific P_0 of Sgca-null EDL was about half that of control 
(−46%), whereas that of dystrophic soleus was not different. In contrast, it was reported that the absolute tetanic 
force of 8-wk-old Sgca-null EDL was not different from that of controls, whereas absolute force of the 8-wk-old dystrophic 
soleus increased in the same way as in the 3-mo-old animals 
analyzed in the present study (14). Thus from these data, it results that also specific P_0 of 8-wk-old Sgca-null EDL is reduced by −31%. Interestingly, another fast-twitch muscle,
regulative proteins (troponin and tropomyosin) and/or changes in the phosphorylation state and processes of regulative myosin light chains. Moreover, the higher content of fibers expressing type 2X MHC could also explain the higher maximum rate of rise of tetanus and capacity to generate force of dystrophic soleus.

Because the number of hybrid 2X/2B fibers was much higher in Sgca-null EDL (83%) than in the control muscle (61%), the lack of any difference in the pCa sensitivity of myofibrillar proteins of the two muscles could be explained by the possibility that pCa sensitivity of type 2B fibers is similar to that of hybrid-type 2X/2B fibers.

Sgca-null EDL muscle showed a higher resistance to fatigue than control, whereas soleus muscle was not different than control. Several factors may be responsible for the lower fatigability of dystrophic EDL. It has been reported that hind-limb muscles of mdx mice are less fatigable than those of control animals (44, 46), a property that was attributed to the lower relative force produced by the dystrophic muscles and thus to the lower energy demand. The same explanation could be applied to the Sgca-null EDL, which has a relative maximal tension half that of normal EDL and thus uses less energy and develops a slower rate of fatigue. An additional explanation could be found in the observed disproportional of MHC isoform composition where the reduction of glycolytic fibers expressing the fast-fatiguing 2B MHC is compensated by the large increase of hybrid fibers, resulting in a more oxidative phenotype of dystrophic EDL. Consistently, the overall SDH staining of Sgca-null EDL, an estimate of mitochondria number, was higher than that of control muscle.

An additional difference associated with the absence of α-sarcoglycan was found in the excitation-contraction coupling mechanism of dystrophic muscle fibers. Caffeine sensitivity of the sarcoplasmic reticulum (an indication of how easily the opening of the Ca²⁺ release channel) of soleus fibers was significantly decreased with respect to control fibers, whereas that of EDL fibers was unaffected. This alteration of sarcoplasmic reticulum properties of the slow-twitch Sgca-null soleus fibers would make them similar to fast fibers. It is in fact known in several species that sarcoplasmic reticulum of slow muscle fibers is more sensitive to caffeine than that of fast fibers (18, 47). Whereas in mouse, the different caffeine sensitivity of sarcoplasmic reticulum from fast and slow muscles was demonstrated at the muscle level (52), our data represent the first confirmation at the fiber level. Mdx muscles also display profound modifications of Ca²⁺ handling to the extent that this characteristic is considered a critical pathogenic feature of muscle dystrophy (21). A recent study, performed in chemically skinned fibers isolated from soleus and EDL mdx muscles (13), showed that sarcoplasmic reticulum properties of these muscles are differently affected by the downstream effects associated with the absence of dystrophin. In the present study, we show that also the absence of α-sarcoglycan produces distinctive effects on fast and slow muscles, and we hypothesize that caffeine sensitivity changes of Sgca-null soleus could be attributed to the shift of the muscle toward the type 2X phenotype.

In conclusion, the present work demonstrated that substantial modifications occur to muscles as a consequence of α-sarcoglycan deficiency characterized by changes that are muscle fiber-type dependent. In DMD patients, the absence of dystrophin seems to affect more fast- than slow-twitch fibers (58). In Sgca-null mice, the absence of α-sarcoglycan also perturbs more fast-twitch than slow-twitch fibers. Therefore, we speculate that within the dystrophin complex, probably in the sarcoglycan-sarcospan subcomplex, reside structural/signaling properties that distinctively affect the design of muscle fiber phenotype, perhaps also under physiological conditions.

REFERENCES

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