β₂-Agonist administration increases sarcoplasmic reticulum Ca²⁺-ATPase activity in aged rat skeletal muscle

Jonathan D. Schertzer, David R. Plant, James G. Ryall, Felice Beitzel, Nicole Stupka, and Gordon S. Lynch

Department of Physiology, The University of Melbourne, Victoria, Australia

Submitted 26 August 2004; accepted in final form 6 October 2004

β₂-Agonist administration increases sarcoplasmic reticulum Ca²⁺-ATPase activity in aged rat skeletal muscle. Am J Physiol Endocrinol Metab 288:E526–E533, 2005. First published October 12, 2004; doi:10.1152/ajpendo.00399.2004.—Aging is associated with a slowing of skeletal muscle contractile properties, including a decreased rate of relaxation. In rats, the age-related decrease in the maximal rate of relaxation is reversed after 4-wk administration with the β₂-adrenoceptor agonist (β₂-agonist) fenoterol. Given the critical role of the sarcoplasmic reticulum (SR) in regulating intracellular Ca²⁺ transients and ultimately the time course of muscle contraction and relaxation, we tested the hypothesis that the mechanisms of action of fenoterol are mediated by alterations in SR proteins. Sarcoendoplasmic reticulum Ca²⁺-ATPase (SERCA) kinetic properties were assessed in muscle homogenates and enriched SR membranes isolated from the red (RG) and white (WG) portions of the gastrocnemius muscle in adult (16 mo) and aged (28 mo) F344 rats that had been administered fenoterol for 4 wk (1.4 mg/kg/day ip, in saline) or vehicle only. Aging was associated with a 29% decrease in the maximal activity (Vₘₐₓ) of SERCA in the RG but not in the WG muscles. Fenoterol treatment increased the Vₘₐₓ of SERCA and SERCA1 protein levels in RG and WG. In the RG, fenoterol administration reversed an age-related selective nitration of the SERCA2a isoform. Our findings demonstrate that the mechanisms underlying age-related changes in contractile properties are fiber type dependent, whereas the effects of fenoterol administration are independent of age and fiber type.

aging; calcium; β₂-adrenoceptor; contractility

Sarcopenia, the progressive loss of skeletal muscle mass with advancing age, is associated with a decline in muscle strength that leads to a loss of functional independence and a reduced quality of life (17). Aging is also associated with a slowing of the time course of skeletal muscle contraction and relaxation (15). These changes can reduce the accuracy and precision of movements, impair the ability to perform simple tasks, and increase the risk of sudden falls and related injuries. Ideally, therapies to prevent or reverse the age-related changes in skeletal muscle should also target the slowing of contraction and relaxation, in addition to preserving or increasing muscle mass and strength.

Neurogenic factors, such as motor unit remodeling and denervation, have been implicated in the age-related changes to skeletal muscle (11). However, myogenic factors are also involved, since the slowing of contraction and relaxation occurs before the onset of muscle wasting (18, 20, 21). Intrinsic changes to skeletal muscle fibers appear to be involved in the deterioration of function with age. Specifically, age-related changes in the proteins regulating Ca²⁺ handling during excitation-contraction coupling and relaxation have been described (15).

Relaxation of skeletal muscle is thought to involve at least three processes: dissociation of Ca²⁺ from troponin, detachment of cross bridges, and uptake of Ca²⁺ by the sarcoplasmic reticulum and myoplasmic Ca²⁺ buffers (33). The factors regulating intracellular [Ca²⁺] during skeletal muscle relaxation are fiber type specific. In fast-twitch muscle, it has been shown that parvalbumin contributes at least 50% to the final rate constant of decay of [Ca²⁺] after a single action potential (2). In slow-twitch muscle (where parvalbumin levels are very low), the sarcoplasmic reticulum Ca²⁺-ATPase (SERCA) makes the major contribution to the decay of [Ca²⁺] (2). Aging has been associated with a decrease in the amount of Ca²⁺ available for triggering contraction (20) due to dihydropyrindine receptor (DHPR) uncoupling from the sarcoplasmic reticulum (SR) Ca²⁺ release channel (CRC) (5). However, the effects of aging on the kinetic properties of the SERCA are not clear. The reductions in SR Ca²⁺ resequestration from chemically skinned fast-twitch muscle fibers from aged rats were hypothesized to be caused by an inactivation of SERCA (13). However, predominantly fast-twitch muscles show no age-related alteration in SERCA protein levels, isoform composition, or kinetic properties (4, 7).

To date, the accumulated data have only excluded an effect of aging on the intrinsic properties of SERCA from predominantly fast-twitch muscle. However, SR vesicles isolated from rat slow-twitch muscles (soleus, adductor longus, and vastus intermedius) do show an age-dependent reduction in both SERCA activity and Ca²⁺ resequestration (32). Furthermore, the age-related inactivation of SERCA activity was associated with a selective nitration of the SERCA2a isoform predominantly at Tyr²⁹⁴-Tyr²⁹⁵ (32), which is close to the Ca²⁺ translocation sites, suggesting that an age-related decrease in SERCA activity could be related to a decreased binding affinity for Ca²⁺. It has also been shown that the conformation of the nucleotide-binding domain of SERCA is altered in aged skeletal muscle (3). Therefore, current evidence suggests that an age-related reduction in SERCA activity in slow-twitch muscle may be associated with changes in the environment of nucleotide and/or Ca²⁺-binding domains.

We have demonstrated previously that 4 wk of daily administration of the β₂-adrenoceptor agonist (β₂-agonist) fenoterol can attenuate the age-related decrease in rat skeletal muscle mass and strength and decrease the time course of the isometric relaxation.
twitch (22). However, the mechanisms responsible for this change in contractility have not been elucidated. It has been demonstrated that a 6-wk administration of the β2-agonist salbutamol can reduce contraction time in canine skeletal muscle (36). Salbutamol treatment also increased the rate of SR Ca\textsuperscript{2+} reserequestration, which suggested that changes in skeletal muscle contractile parameters were mediated by alterations in SR proteins, namely a β2-agonist-mediated induction of SERCA1 protein levels (36). In addition, salbutamol treatment attenuated the fast twitch-to-slow twitch transformation of myosin heavy-chain (MHC) isoforms, SR protein characteristics, and muscle mechanics that accompany chronic low-frequency stimulation (CLFS) (10). Therefore, it is possible that β2-agonist administration could attenuate an age-related decrease in skeletal muscle relaxation rate by reversing age-related modifications of SR proteins.

We tested the hypothesis that the age-related decrease and the β2-agonist-induced increase in the rate of skeletal muscle relaxation are mediated through alterations in the properties of the SR. We also hypothesized that fenoterol treatment in rats would attenuate age-related changes in the maximal activity (V\text{max}) of SERCA and determined the underlying mechanisms that could account for changes in SERCA activity, including assessments of SERCA isoform protein levels, SERCA2a nitration levels, and indirect measures of the Ca\textsuperscript{2+} and ATP-binding affinities of SERCA.

MATERIALS AND METHODS

Adult (16 mo old) and aged (28 mo old) male Fischer 344 rats (460–500 g) obtained from Harlan Sprague Dawley (Indianapolis, IN) were allocated at random to a control or fenoterol-treated group (n = 8/group). Fenoterol-treated rats received 1.4 mg/kg fenoterol (Sigma-Aldrich, Castle Hill, NSW, Australia) administered via intraperitoneal injections in 0.5 ml of isotonic saline every day for 4 wk, and control rats received a daily injection of 0.5 ml of saline vehicle, as described previously (22). All experiments were approved by the Animal Experimentation Ethics Committee of The University of Melbourne and adhered to the code of practice for the care and use of animals for scientific purposes as described by the National Health and Medical Research Council of Australia.

Contractile properties: maximal rate of twitch and tetanus relaxation. At the completion of the treatment period, rats were anesthetized with pentobarbital sodium (Nembutal; Rhone Merieux, Pinkenba, QLD, Australia; 60 mg/kg ip). The extensor digitorum longus (EDL) and soleus muscles were surgically excised, and isometric contractile properties were measured in vitro as reported previously (22). In that study, we reported that although absolute one-half relaxation time of the twitch was not different after fenoterol treatment, twitch force was significantly greater. Hence, compared with untreated control rats, the actual rate of relaxation of the twitch response was significantly faster in treated rats (22). Although twitch force of either EDL or soleus muscles was not different between adult and aged rats (22), the ability of fenoterol treatment to increase twitch force warranted closer investigation. Therefore, to further examine the maximal rate of relaxation of the twitch and tetanic responses, we studied the maximum negative derivative of a linear-fit curve calculated over 5 ms for the EDL and 25 ms for soleus muscle by use of custom-written software applications (DR Stom Software Solutions, Ann Arbor, MI) and LabView software (National Instruments, Austin, TX). The maximal rate of relaxation was determined during supra-maximal twitches and fused tetani (150 Hz EDL, 120 Hz soleus). Although the EDL and soleus muscles from both hindlimbs were used previously for examination of the effects of fenoterol on contractile properties and β-adrenoceptor densities (22), the gastrocnemius muscles were also excised and stored for later biochemical analysis, and these muscles served as the basis for the present study investigating fenoterol’s effects on SERCA kinetic properties.

Sample preparation: SR characteristics. While rats were anesthetized, the gastrocnemius muscles were excised from both hindlimbs. The deep red portion of the gastrocnemius muscle (RG) was separated from the predominantly fast-twitch superficial white portion (WG). The rats were killed by the opening of the thoracic cavity and immediate cardiac excision. Crude muscle homogenates were prepared, as described previously (24, 25). SR vesicles were prepared, utilizing a combination of two SR isolation protocols (6, 9), as described previously (24). During the entire homogenization and SR vesicle isolation procedure, samples were immersed in ice. It is critical to keep the samples on ice to avoid temperature-dependent reductions in SERCA activity (27). SR isolation was carried out on the same day as muscle homogenization and was accomplished by sucrose gradient differential centrifugation, using a Beckman ultracentrifuge with a 70Ti fixed-angle rotor. Protein determination of homogenates and SR vesicles was made by the method of Bradford and analyzed in triplicate.

SERCA activity. Ca\textsuperscript{2+}-induced SERCA activity in enriched SR membranes was analyzed according to the methods of Leberer et al. (14), described previously (24). Ca\textsuperscript{2+}-induced SERCA activity in crude muscle homogenates was analyzed with the use of modifications according to Simonides and van Hardeveld (29), as described previously (25). The reaction buffer for crude muscle homogenates (with SR vesicles in parentheses) contained, in mM, 200 (100) KCl, 20 HEPES, 15 (10) MgCl\textsubscript{2}, 10 Na\textsubscript{2}HPO\textsubscript{4}/Na\textsubscript{2}H\textsubscript{2}PO\textsubscript{4} (PEP), 5 ATP, and 1 EGTA. The pH of the reaction buffer was adjusted to 7.0 at 37°C. Immediately before the reaction was started, 18 μM lactate dehydrogenase (LDH), 18 U/ml pyruvate kinase (PK), 0.3 mM NADH, 1 μM Ca\textsuperscript{2+} ionophore A-23187 (Sigma-Aldrich), and 15–30 μl of muscle homogenate or 1–5 μl of enriched SR membrane were added to 1 ml of reaction buffer. The markers of SERCA enzyme kinetics in muscle homogenates included V\text{max}, cytosolic free [Ca\textsuperscript{2+}], ([Ca\textsuperscript{2+}]\textsubscript{i}) required for half-maximal activity (pCa\textsubscript{50}), and the Hill coefficient (nH), which is a measure of the cooperative Ca\textsuperscript{2+}-binding affinity of the SERCA enzyme. In SR vesicles, only the V\text{max} was assessed. To assess changes in SR membrane permeability to Ca\textsuperscript{2+}, the assay was also performed without the Ca\textsuperscript{2+} ionophore A-23187 in a subset of samples for each condition (muscle homogenates only; n = 3). Assays were performed at 37°C and 340 nm (Multiscan Spectrum; Thermo Electron, Waltham, MA). In all cases, the V\text{max} of SERCA activity was established by progressively raising the [Ca\textsuperscript{2+}]\textsubscript{i} until a plateau and subsequent decline in SERCA activity occurred. Basal (or Mg\textsuperscript{2+}-ATPase) activity was determined by adding cyclopiazonic acid (CPA), a specific inhibitor of the SERCA enzyme, to a final concentration of 40 μM. The [Ca\textsuperscript{2+}]\textsubscript{i} for a given day of analysis was measured in triplicate on separate aliquots of reaction buffer with a calcium electrode (model 97-20; Orion, Beverly, MA). SERCA activity was then plotted against the negative logarithm of [Ca\textsuperscript{2+}]\textsubscript{i} (pCa), as described previously (31). Nonlinear regression analysis was performed with GraphPad Prism v. 4.00 for Windows (GraphPad Software, San Diego, CA) using the following sigmoidal dose-response (variable slope) relationship

\[ Y = \frac{Y_{\text{top}} - Y_{\text{bot}}}{1 + 10^{log \text{Ca}_{50} \times X}} \cdot Y_{	ext{bot}} \]

where Y\text{bot} is the value at the bottom of the plateau, Y\text{top} is the value at the top of the plateau, and X and Y are the dose-response variables. The Hill coefficient was calculated with values ranging between 10 and 90% of the V\text{max}.

Fluorescence measurements. The fluorescent probes fluorescein 5-isothiocyanate (FITC; Sigma-Aldrich) and N-cyclohexyl-N’-(di-methylamino-α-naphthyl)carbodiimide (NCD-4; Molecular Probes, Eugene, OR) were used to indirectly assess the nucleotide- and Ca\textsuperscript{2+}-binding affinities of SERCA, respectively, as previously described (24, 31). An identical amount of enriched SR vesicle protein...
Table 1. Maximum rate of relaxation of twitch and tetanus

<table>
<thead>
<tr>
<th></th>
<th>Adult (16 mo)</th>
<th>Aged (28 mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Fenoterol</td>
</tr>
<tr>
<td>Soleus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(dF_{\text{twitch}}/dt)</td>
<td>1.7±0.1</td>
<td>2.2±0.2*</td>
</tr>
<tr>
<td>(dF_{\text{tetanus}}/dt)</td>
<td>9.5±0.3</td>
<td>13.1±1.3*</td>
</tr>
<tr>
<td>EDL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(dF_{\text{twitch}}/dt)</td>
<td>33.7±1.7</td>
<td>50.1±1.6*</td>
</tr>
<tr>
<td>(dF_{\text{tetanus}}/dt)</td>
<td>101.1±4.1</td>
<td>125.2±3.8*</td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n = 8\)/group. Maximum rate of relaxation of twitch and tetanus in soleus and extensor digitorum longus (EDL) muscles from adult and aged rats after treatment with fenoterol (1.4 mg·kg\(^{-1}\)·day\(^{-1}\)·ip) or saline for 4 wk. Maximum rate of relaxation (\(dF/dt\)) is in mN/ms. *\(P < 0.05\), adult control vs. adult treated; †\(P < 0.05\), adult control vs. aged control; ‡\(P < 0.05\), adult control vs. aged treated.

Table 2. SERCA enzyme activity kinetic parameters

<table>
<thead>
<tr>
<th></th>
<th>Adult (16 mo)</th>
<th>Aged (28 mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Fenoterol</td>
</tr>
<tr>
<td>RG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal-ATPase</td>
<td>38.3±3.7</td>
<td>32.9±4.7</td>
</tr>
<tr>
<td>(V_{max})</td>
<td>335±15</td>
<td>438±13*</td>
</tr>
<tr>
<td>(n_H)</td>
<td>1.91±0.17</td>
<td>1.79±0.08</td>
</tr>
<tr>
<td>pCa(_{50})</td>
<td>6.02±0.07</td>
<td>6.01±0.10</td>
</tr>
<tr>
<td>Fold increase due to A-23187</td>
<td>1.52±0.14</td>
<td>1.58±0.24</td>
</tr>
<tr>
<td>WG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal-ATPase</td>
<td>72.8±8.0</td>
<td>66.2±17</td>
</tr>
<tr>
<td>(V_{max})</td>
<td>742±81</td>
<td>1044±55*</td>
</tr>
<tr>
<td>(n_H)</td>
<td>1.69±0.07</td>
<td>1.76±0.17</td>
</tr>
<tr>
<td>pCa(_{50})</td>
<td>5.80±0.08</td>
<td>5.75±0.05</td>
</tr>
<tr>
<td>Fold increase due to A-23187</td>
<td>2.51±0.79</td>
<td>2.29±0.13</td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n = 8\)/group, except for fold increase due to A-23187 (\(n = 3\)). Sarcoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) enzyme activity parameters of crude homogenates from red (RG) and white (WG) gastrocnemius muscles from adult and aged rats after treatment with fenoterol (1.4 mg·kg\(^{-1}\)·day\(^{-1}\)·ip) or saline for 4 wk. Basal ATPase and maximum activity (\(V_{max}\)) are in nmol·mg protein\(^{-1}\)·min\(^{-1}\). Ca\(^{2+}\)-ATPase activity-pCa curves allowed for determination of kinetic parameters. Hill coefficient (\(n_H\)) was determined on the basis of Ca\(^{2+}\)-ATPase activity between 10 and 90% of maximum value. pCa\(_{50}\), the cytosolic free Ca\(^{2+}\) concentration required to elicit 50% maximal Ca\(^{2+}\)-ATPase activity; A-23187, a Ca\(^{2+}\) ionophore. *\(P < 0.05\), adult control vs. adult treated; †\(P < 0.05\), adult control vs. aged control; ‡\(P < 0.05\), aged control vs. aged treated; §\(P < 0.05\), aged vs. aged (main effect).

Compared with RG, WG generally demonstrated a higher \(V_{max}\), basal ATPase activity, and fold stimulation by A-23187 and a lower pCa\(_{50}\) (main effect, \(P < 0.05\)).
were determined by scanning densitometry. Values (n ≥ 5 for each condition) were expressed as a percentage of total MHC protein.

Statistical analyses. Individual variables were compared between groups with a one-way or two-way ANOVA as appropriate. Bonferroni’s post hoc multiple comparison procedure was used to detect differences between specific means. Significance was set at P ≤ 0.05.

All values are expressed as means ± SE unless specified otherwise.

RESULTS

Contractile properties: maximal rate of twitch and tetanus relaxation. In soleus muscles, the maximal rate of relaxation of the twitch and tetanus was decreased by 27 and 28%, respectively, in untreated aged rats compared with untreated adult rats (P ≤ 0.05; Table 1 and Fig. 1A). Fenoterol treatment increased the maximal rate of relaxation of the tetanus in EDL muscles of adult and aged rats by 24 and 33%, respectively, and a similar effect was observed for the twitch response (P < 0.05; Table 1 and Fig. 1B).

were determined by scanning densitometry. Values (n ≥ 5 for each condition) were expressed as a percentage of total MHC protein.

Statistical analyses. Individual variables were compared between groups with a one-way or two-way ANOVA as appropriate. Bonferroni’s post hoc multiple comparison procedure was used to detect differences between specific means. Significance was set at P < 0.05. All values are expressed as means ± SE unless specified otherwise.

RESULTS

Contractile properties: maximal rate of twitch and tetanus relaxation. In soleus muscles, the maximal rate of relaxation of the twitch and tetanus was decreased by 27 and 28%, respectively, in untreated aged rats compared with untreated adult rats (P < 0.05; Table 1 and Fig. 1A). Fenoterol treatment increased the maximal rate of relaxation of the tetanus in soleus muscles of adult and aged rats by 38 and 51%, respectively, and a similar effect was observed for the twitch response (P < 0.05; Table 1 and Fig. 1A). In EDL muscles, the maximal rate of relaxation of the twitch and tetanus was decreased by 21 and 24% in untreated aged rats compared with untreated adult rats, respectively (P < 0.05; Table 1 and Fig. 1B). Fenoterol treatment increased the maximal rate of relaxation of the tetanus in EDL muscles of adult and aged rats by 24 and 33%, respectively, and a similar effect was observed for the twitch response (P < 0.05; Table 1 and Fig. 1B).

were determined by scanning densitometry. Values (n ≥ 5 for each condition) were expressed as a percentage of total MHC protein.

Statistical analyses. Individual variables were compared between groups with a one-way or two-way ANOVA as appropriate. Bonferroni’s post hoc multiple comparison procedure was used to detect differences between specific means. Significance was set at P < 0.05. All values are expressed as means ± SE unless specified otherwise.

RESULTS

Contractile properties: maximal rate of twitch and tetanus relaxation. In soleus muscles, the maximal rate of relaxation of the twitch and tetanus was decreased by 27 and 28%, respectively, in untreated aged rats compared with untreated adult rats (P < 0.05; Table 1 and Fig. 1A). Fenoterol treatment increased the maximal rate of relaxation of the tetanus in soleus muscles of adult and aged rats by 38 and 51%, respectively, and a similar effect was observed for the twitch response (P < 0.05; Table 1 and Fig. 1A). In EDL muscles, the maximal rate of relaxation of the twitch and tetanus was decreased by 21 and 24% in untreated aged rats compared with untreated adult rats, respectively (P < 0.05; Table 1 and Fig. 1B). Fenoterol treatment increased the maximal rate of relaxation of the tetanus in EDL muscles of adult and aged rats by 24 and 33%, respectively, and a similar effect was observed for the twitch response (P < 0.05; Table 1 and Fig. 1B).

were determined by scanning densitometry. Values (n ≥ 5 for each condition) were expressed as a percentage of total MHC protein.

Statistical analyses. Individual variables were compared between groups with a one-way or two-way ANOVA as appropriate. Bonferroni’s post hoc multiple comparison procedure was used to detect differences between specific means. Significance was set at P ≤ 0.05. All values are expressed as means ± SE unless specified otherwise.

RESULTS

Contractile properties: maximal rate of twitch and tetanus relaxation. In soleus muscles, the maximal rate of relaxation of the twitch and tetanus was decreased by 27 and 28%, respectively, in untreated aged rats compared with untreated adult rats (P ≤ 0.05; Table 1 and Fig. 1A). Fenoterol treatment increased the maximal rate of relaxation of the tetanus in soleus muscles of adult and aged rats by 38 and 51%, respectively, and a similar effect was observed for the twitch response (P ≤ 0.05; Table 1 and Fig. 1A). In EDL muscles, the maximal rate of relaxation of the twitch and tetanus was decreased by 21 and 24% in untreated aged rats compared with untreated adult rats, respectively (P ≤ 0.05; Table 1 and Fig. 1B). Fenoterol treatment increased the maximal rate of relaxation of the tetanus in EDL muscles of adult and aged rats by 24 and 33%, respectively, and a similar effect was observed for the twitch response (P ≤ 0.05; Table 1 and Fig. 1B).
SERCA activity. The $V_{\text{max}}$ of SERCA activity measured in RG muscle homogenates was 29% lower ($P < 0.05$) in untreated aged rats compared with untreated adult rats (Table 2 and Fig. 2A). Treatment with fenoterol increased the $V_{\text{max}}$ in RG muscle homogenates from adult and aged rats by 24 and 34%, respectively ($P < 0.05$; Table 2 and Fig. 2A). In WG muscle homogenates, no age-related alteration in the $V_{\text{max}}$ was detected. However, fenoterol treatment increased the $V_{\text{max}}$ in WG muscle homogenates from adult and aged rats by 29 and 35%, respectively ($P < 0.05$; Table 2 and Fig. 2B).

The $V_{\text{max}}$ in SR vesicles enriched from RG muscles was 26% lower in untreated aged rats compared with untreated adult rats ($P < 0.05$; Fig. 3C). In fenoterol-treated adult and aged rats, the $V_{\text{max}}$ in SR vesicles enriched from RG muscle was 19 and 27% higher than in control rats, respectively ($P < 0.05$; Fig. 3C). The $V_{\text{max}}$ of SR vesicles enriched from WG muscles of fenoterol-treated adult and aged rats was 25% higher than from rats administered vehicle alone, but no age-dependent alteration was detected ($P < 0.05$ data not shown).

In muscle homogenates, the sensitivity of the SERCA enzyme to Ca$^{2+}$ was reduced in RG and WG muscles from aged rats, as shown by lower pCa$_{50}$ values and the right shift of the activity-pCa relationship ($P < 0.05$, main effect; Table 2 and Fig. 2). Fenoterol treatment did not alter pCa$_{50}$ values in either age group of rats (Table 2 and Fig. 2). The $n_H$ of the SERCA activity-pCa relationship was not altered by age or treatment. In crude homogenates, fenoterol treatment or aging did not alter stimulation due to A-23187, indicating that SR membrane permeability to Ca$^{2+}$ was not altered. However, WG demonstrated a generally higher stimulation by the Ca$^{2+}$ ionophore A-23187 compared with RG (main effect, $P < 0.05$).

Fluorescence measurements. The maximum fluorescence intensity of SR vesicles labeled with FITC was 12, 13, and 19% lower in samples from RG of untreated aged rats compared with those from treated aged rats, untreated adult rats, and treated adult rats, respectively ($P < 0.05$; Fig. 3, A and C). FITC fluorescence was not altered in SR vesicles isolated from WG muscle (Fig. 3A). The maximum emission intensity was unchanged in all conditions for NCD-4 (Fig. 3B).

SDS-PAGE and Western blotting. SERCA1 protein levels in SR vesicles isolated from RG and WG muscle were higher in fenoterol-treated rats. Compared with values for untreated adult rats, RG SERCA1 protein levels were 46 and 42% higher in treated adult and aged rats, respectively ($P < 0.05$; Fig. 4A). No age-dependent changes in SERCA1 protein levels were

![Fig. 4](http://ajpendo.physiology.org/)
apparent in RG or WG. SERCA2a protein levels were not different in WG or RG (Fig. 4B). The SERCA1-to-SERCA2a ratio in RG and WG muscles was greater in fenoterol-treated rats than in untreated rats ($P < 0.05$, data not shown).

In both RG and WG muscle, Western blotting detected a nitrated protein migrating at an apparent molecular mass of 95 kDa, which corresponded to the molecular mass of SERCA2a (Fig. 4C). Several nitrated proteins were also evident at ~70 kDa (Fig. 4C) that align with the lower-molecular-mass bands detected in SERCA2a immunoblotting (Fig. 4B). There was no evidence of age-related SERCA1 (110 kDa) tyrosine nitrination. In SR vesicles isolated from RG muscle, an age-dependent increase in nitration of SERCA2a was evident, with nitrotyrosine levels in RG from untreated aged rats being 74% higher than values for untreated adult rats. Importantly, RG muscles from treated aged rats had SERCA2a nitrotyrosine levels equivalent to those from untreated adult rats. Unlike RG, no alterations in nitrotyrosine levels were observed in WG (Fig. 4C).

Determination of the relative MHC composition confirmed that RG muscle contained a different MHC isoform profile compared with WG muscle. Specifically, RG muscle contained 8–28% of MHC type IIB and 20–30% of MHC type I. In contrast, WG muscle contained 45–60% MHC type IIB. No MHC type I was detected in WG muscle. In general, aging was associated with a shift toward a higher proportion of slow MHC isoforms (IIB → IIA/IIX → I), whereas fenoterol treatment was associated with a shift toward fast MHC isoforms (I → IIA/IIX → IIB; Fig. 5).

**Data correlation.** The $V_{\text{max}}$ of SERCA activity in enriched SR vesicles was statistically correlated ($P < 0.05$) to measurements in crude muscle homogenates for both RG ($r^2 = 0.68$) and WG muscles ($r^2 = 0.36$), indicating no selective yield of SR vesicles during the isolation protocol ($P < 0.05$). In RG, maximum FITC fluorescence was statistically correlated ($r^2 = 0.62$) with the $V_{\text{max}}$ of SERCA activity in enriched SR vesicles ($P < 0.05$; Fig. 3C).

**DISCUSSION**

Aging is associated with deleterious changes in skeletal muscle contractility, including factors that contribute to an overall slowing of movement. The findings of this study indicate that treatment of aged rats with the $\beta_2$-agonist fenoterol can reverse the slowing of relaxation in slow- and fast-twitch skeletal muscle due to increased SERCA activity and SERCA protein levels. We also provide evidence that there is an age-related alteration in the environment of the nucleotide-binding domain and/or a selective nitration of the SERCA2a isoform that is associated with the depression in SERCA activity. Treatment with fenoterol ameliorated the age-related decrease in nucleotide-binding affinity and reversed the age-related accumulation of nitrotyrosine residues on the SERCA2a isoform. These changes, in combination with increases in SERCA1 protein levels, appear to be the underlying mechanisms of fenoterol treatment in reversing age-related decreases in the $V_{\text{max}}$ of SERCA. Our results demonstrate that the underlying mechanisms resulting in age-related changes in contractile properties are fiber type specific. An age-related decrease in SERCA activity occurred in muscles with a slow or mixed fiber type (RG) but not in predominantly fast-twitch muscles (WG). In slow-twitch muscle (where parvalbumin levels are low or negligible), the age-related slowing of relaxation was associated with a decrease in SERCA $V_{\text{max}}$. Conversely, in fast-twitch muscle (where parvalbumin levels are high), SERCA $V_{\text{max}}$ was unchanged despite the age-related slowing of relaxation. The effects of fenoterol are clearly age independent, since its administration increased SERCA activity and SERCA1 protein levels and increased the maximal rate of relaxation in muscles from both adult and aged rats. These results demonstrate that muscles from aged rats retain their capacity to adapt to different stimuli, including anabolic agents.

On the basis of the literature to date, the effects of aging on SERCA activity are controversial. It has been argued that aging has no effect on the intrinsic properties of SERCA (15). However, the studies that failed to demonstrate age-related changes in SERCA activity were performed on muscles comprised of fibers with predominantly fast-twitch characteristics (4, 7), with one exception. Narayanan et al. (18) showed that SERCA activity in rat soleus muscle was unchanged despite an age-related depression in Ca$^{2+}$ resequestration. However, because they assessed SERCA activity at only a single $[\text{Ca}^{2+}]_i$ (8.21 μM), it cannot be said definitively that the activity measured was the $V_{\text{max}}$. Differences in SERCA activity could be masked by possible age-related changes in pCa$_{50}$ and/or the Hill coefficient.

Our findings are consistent with more recent studies investigating the dependence of muscle fiber type on age-related changes in the kinetic properties of SERCA. Viner et al. (32)
showed an age-dependent loss of SERCA activity in SR preparations isolated from muscles with mixed fiber composition but not from preparations containing predominantly fast-twitch muscle fibers.

It has been shown previously that treatment with the β2-agonist salbutamol for 6 wk increased SERCA1 protein levels in canine latissimus dorsi and vastus intermedius muscles (36). Similarly, our findings show that fenoterol treatment increased SERCA1 protein levels in the RG and WG muscle of both adult and aged rats. Furthermore, we (22) have previously shown that fenoterol treatment can reverse age-related muscle wasting and weakness, a finding that is similar in some respects to another study (10), where salbutamol treatment attenuated the muscle atrophy usually associated with CLFS. The results of the present study show that fenoterol treatment reverses the age-related decrease in both the rate of relaxation and the $V_{\text{max}}$ of SERCA activity, a finding similar to salbutamol treatment attenuating the prolongation of contraction and the depression in SR Ca$^{2+}$ resequestration concomitant with CLFS (10).

SERCA has a relatively long half-life (~14 days) that is extended by ~27% in aged muscle (8). Therefore, SERCA has a high potential for posttranslational modification, particularly in aged muscle. The ATP-binding domain of SERCA appears to be particularly susceptible to posttranslational modification, which may result in altered enzymatic activity. For example, hydroxyl radicals have been shown to inhibit SERCA function in cardiac and skeletal muscle by altering the ATP-binding site (35). Aged skeletal muscle shows an altered ATP-binding site conformation (3). CLFS has been shown to decrease FITC binding, which corresponded to an increase in the number of inactive SERCA enzymes (16). It should be noted that FITC labels Lys515 in the nucleotide-binding domain of SERCA and, as such, is sensitive to changes in the environment around that residue. Our results demonstrate an age-related reduction in FITC-binding affinity, indicating that a similar mechanism may mediate this effect during both aging and CLFS. We have also shown that fenoterol treatment can attenuate the age-related decreases in FITC-binding affinity. This measure could reflect SERCA E2-to-SERCA E1 transitions and/or alterations in the environment of the nucleotide-binding domain (19).

Our findings are consistent with previous reports demonstrating a selective age-related nitration of the SERCA2a isoform (32). It appears that nitration of SERCA2a is, in part, due to a peroxynitrite-mediated process (28). However, the mechanisms linking nitration of SERCA2a to reductions in enzyme activity have not been elucidated. To our knowledge, this is the first report to demonstrate that treatment with a β2-agonist reverses the age-related accumulation of nitrotyrosine residues on the SERCA2a isoform. The mechanism underlying this effect is not known; however, we have shown that fenoterol increases SERCA1 protein levels, and it is possible that the synthesis of SERCA1 protein and/or the consequent increase in the ratio of SERCA1 to SERCA2a could reduce the amount of SERCA2a available for nitration.

Age-related alterations in the CRC, such as DHPR-CRC uncoupling (4), may be associated with skeletal muscle twitch prolongation (4). We have demonstrated that the underlying mechanisms of age-related alterations in the rate of skeletal muscle relaxation are clearly fiber-type specific. Our results demonstrate no age-related changes in SERCA activity in fast-twitch muscle; hence, alterations in excitation-contraction coupling remain a likely explanation for age-related changes in contractile properties in fast muscle (20). However, our results demonstrate that, in slow-twitch muscle, uncoupling of the DHPR-CRC complex is not the only age-related alteration to the proteins involved in excitation contraction and relaxation. Although SERCA activity is not the only factor controlling the rate of skeletal muscle relaxation, it is reasonable to associate decreased SERCA activity with the reduced rate of relaxation in muscles of aged rats. In addition, given the coordination between SR Ca$^{2+}$ resequestration and Ca$^{2+}$ release (23), it is possible that alterations in SERCA activity in slow-twitch muscle may manifest as changes in SR release kinetics and ultimately as changes in contractile parameters, such as force production (20). We have provided evidence that an age-related reduction in SERCA activity may be mediated through SERCA2a nitration and/or alterations in the environment of the nucleotide-binding domain of SERCA. Several other mechanisms may also be important. Phosphorylation of SR-bound calmodulin kinase II (CaMKII) increases the $V_{\text{max}}$ of SERCA2a (34). The protein levels of γ- and δ-CaMKII subunits are conserved through aging of rat muscle (15). However, the enzymatic activity of CaMKII has not been assessed during aging, and this assessment could prove useful. It is unlikely that the actions of fenoterol are mediated through CaMKII phosphorylation, since this pathway is only activated by β1-receptor stimulation in rat cardiac muscle (1).

In summary, we demonstrated that aging was associated with a decrease in the rate relaxation of both slow- and fast-twitch muscles. Aging was also associated with depression in the $V_{\text{max}}$ of rat SERCA in muscle with a mixed fiber type but not in predominately fast-twitch muscle. The mechanism underlying the depression in the $V_{\text{max}}$ of SERCA may be alterations in the environment of the nucleotide-binding domain and/or an age-related selective nitration of SERCA2a. We also demonstrated that treatment with the β2-agonist fenoterol reversed the depression in the $V_{\text{max}}$ of SERCA. We provided evidence that the changes in SERCA activity are related to alterations in SERCA1 protein levels, alterations in the environment of the nucleotide-binding domain, and the accumulation of nitrotyrosine residues on SERCA2a. Taken together, these findings show that the underlying mechanisms responsible for age-related changes in skeletal muscle contractile properties are fiber type specific, whereas the effects of fenoterol are independent of age and fiber type.

GRANTS

This study was supported by research grants from the Muscular Dystrophy Association of the United States, the Rebecca L. Cooper Medical Research Foundation, and the Perpetual Philanthropic Trust (J & R McGauran Charitable Trust).

REFERENCES