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Dihydropyridine Receptor-Ryanodine Receptor Uncoupling in Aged Skeletal Muscle

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Abstract. The mechanisms underlying skeletal muscle functional impairment and structural changes with advanced age are only partially understood. In the present study, we support and expand our theory about alterations in sarcolemmal excitation-sarcoplasmic reticulum Ca^{2+} release-contraction uncoupling as a primary skeletal muscle alteration and major determinant of weakness and fatigue in mammalian species including humans. To test the hypothesis that the number of RYR1 (ryanodine receptor) uncoupled to DHPR (dihydropyridine receptor) increases with age, we performed high-affinity ligand binding studies in *soleus, extensor digitorum longus* (EDL) and in a pool of several skeletal muscles consisting of a mixture of fast- and slow-twitch muscle fibers in middle-aged (14-month) and old (28-months) Fisher 344 Brown Norway F1 hybrids rats. The number of DHPR, RYR1, the coupling between both receptors expressed as the DHPR/RYR1 maximum binding capacity, and their dissociation constant for high-affinity ligands were measured. The DHPR/RYR1 ratio was significantly reduced in the three groups of muscles (pool: 1.03 ± 0.15 and 0.80 ± 0.11, *soleus:* 0.44 ± 0.12 and 0.26 ± 0.10, and EDL: 0.95 ± 0.14 and 0.68 ± 0.10, for middle-aged and old muscles, respectively). These data support the concept that DHPR-RYR1 uncoupling results in alterations in the voltage-gated sarcoplasmic reticulum Ca^{2+} release mechanism, decreases in myoplasmic Ca^{2+} elevation in response to sarcolemmal depolarization, reduced Ca^{2+} supply to contractile proteins and reduced contraction force with aging.

Key words: Skeletal muscle — Ryanodine receptor — Dihydropyridine receptor — Aging — Excitation-contraction coupling — *Soleus* muscle — *Extensor digitorum longus* muscle — Calcium release

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Introduction

Skeletal muscle strength declines with aging as demonstrated in studies on muscle contractility *in vivo* and *in vitro* (Grimby & Saltin, 1983; Edström & Larsson, 1987; Booth, Weeden & Tseng, 1993; Brooks & Faulkner, 1994; for a review *see* Holloszy & Kohrt, 1995). The mechanisms underlying skeletal muscle functional impairment and structural changes with advanced age, termed sarcopenia, are partially understood. Factors that determine hypotrophy and weakness/fatigue with aging can be divided into three main groups: (i) neurogenic, (ii) myogenic, or (iii) a combination of both factors. The former group includes alterations at the spinal cord motor neuron leading to motor unit remodeling. Neuronal alterations determine skeletal muscle denervation and reinnervation (Hashizume, Kanda & Burke, 1988; Einsiedel & Luff, 1992; Doherty et al., 1993; Kadhiresa, Hassett & Faulkner, 1996). In mixed fiber-type muscles, motor unit remodeling leads to changes in fiber-type distribution. Age-related remodeling of motor units appear to involve denervation of fast muscle fibers with reinnervation by axonal sprouting from slow fibers (Lexell, 1995) whereas in muscles consisting of a single fiber-type that phenomenon does not occur (Phillips et al., 1993). When denervation surpasses reinnervation a population of muscle fibers become atrophic and functionally excluded. Although denervation has been suggested as a contributing factor to sarcopenia (Larsson, 1995), the extension of denervated areas in individual muscles and its prevalence on human and animal models of aging remain to be determined. In addition, it is becoming apparent that denervation does not explain a significant deficit in specific maximum isometric tetanic force recorded in aged skeletal muscles (Kadhiresan et al., 1996).

Primary muscular or myogenic factors refer to a group of alterations such as: (i) contraction-induced in-

jury (Brooks et al., 1993), (ii) selective primary muscle fiber atrophy (Coggan et al., 1992), and (iii) alterations in muscle fiber signal transduction (Delbono, O'Rourke & Ettinger, 1995). (i) The phenomenon of contraction-induced injury has been associated with an increased mechanical frailty and to a non-well-characterized decline in muscle restorative capacity with age (for a discussion *see* Carlson, 1995). (ii) A primary atrophy of type II fibers without alterations in fiber type distribution has also been reported, and it had been difficult to determine whether or not these changes are due to aging or sedentariness (Coggan et al., 1992). Although muscle weakness and fatigue in sedentary people resemble muscle impairment associated with aging, early studies support similar rates of decline in muscle performance with aging regardless of the level of physical training (Moore, 1975; for a discussion *see* Faulkner, Brooks & Zerba, 1995). These results suggest that intrinsic alterations of the muscle fiber are not explained by reductions in fiber diameter (hypotrophy) since normalization of muscle force to cross-sectional area still exhibits a tension deficit with aging. (iii) Several mechanisms of signal transduction operate in skeletal muscles. Two of them directly involved in contractility are sarcolemmal excitation-sarcoplasmic reticulum (SR) Ca^{2+} release coupling (ECC) (for a review *see* Melzer et al., 1995) and ATP hydrolysis-myofilaments sliding (Rayment, Rypniewski & Schmidt-Base, 1993). Alterations in ECC may underlie intrinsic modifications of the muscle fiber across ages. We postulated that specific alterations in ECC lead to decreases in muscle contraction force (Delbono et al., 1995). Studies on fibers deprived of sarcolemma (skinned muscle fibers) demonstrated that the force generated per unit cross sectional area does not differ in adult and old mice during isometric and shortening contractions (Brooks et al., 1994). These later data suggest that other factors in addition to reductions in contractile proteins are contributing to weakness in aged muscles (Phillips, Bruce & Woledge, 1991). Since changes in phosphorus metabolites involved in energy transduction (phosphocreatine, ATP, ADP) and percentages of myosin isoforms do not change with aging in muscles composed exclusively of one type of fiber (Phillips et al., 1993; Sullivan et al., 1995), it is likely that early steps in ECC are altered.

Although the skinned muscle fiber preparation has been a useful tool to study the response of myofilaments to Ca^{2+}, the disruption of the sarcolemma-SR interaction prevents studies on the role of alterations in sarcolemmal excitability in sarcopenia. Sarcolemmal depolarization results in a sudden increase in myoplasmic Ca^{2+} concentration. The transduction of the sarcolemmal voltage into a Ca^{2+} signal occurs in a very narrow space between the sarcolemmal infoldings (T-tubule) and the SR terminal cisternae. The dihydropyridine receptor (DHPR), a voltage-gated Ca^{2+} channel, is expressed in the T-tubule and through an hypothetical mechanical interaction with the Ca^{2+} release channel/ryanodine receptor (skeletal muscle isoform-1, RYR1) elicits Ca^{2+} release from the SR (Franzini-Armstrong, 1970; Schneider & Chandler, 1973). The increase in myoplasmic Ca^{2+} concentration triggers muscle contraction. We postulated that a reduction in the absolute number of DHPR, a decrease in functionally active DHPR or a relative more pronounced decrease in the expression of DHPR compared to RYR1, determine a larger number of DHPR-unlinked RYR1 with age (Delbono et al., 1995). An increase in DHPR-unlinked RYR1 results, at least in part, in a decline in skeletal muscle force with aging. DHPR-RYR1 uncoupling at the T-tubule-SR triadic junction results in an absolute reduction in SR calcium release in response to sarcolemmal depolarization and consequently in a reduced contractile strength in aged skeletal muscle. An important conclusion of this study has been that alterations in ECC occur in nondenervated aged muscle fibers. Therefore we stress the relevance of primary alterations in innervated muscle fibers in the decline of the whole muscle mechanical output at later stages of ontogenic development (Delbono et al., 1995).

In the present work, we applied methods used previously to quantitate the amount of DHPR-linked RYR1 in amphibian and mammalian species (Anderson et al., 1994) and in fast- and slow-twitch muscles of the rat (Delbono & Meissner, 1996). We determined the number of DHPR and RYR1, the coupling between both receptors, and their dissociation constant for high-affinity ligands in an almost pure type-I fiber muscle (*soleus*), in an almost pure type-II muscle (*extensor digitorum longus*, EDL) (Edström & Larsson, 1987) and in a pool of several skeletal muscles consisting of a mixture of both fiber-types in middle-aged and old rats. The effects of maturation on ECC are ongoing studies in our laboratory. The main conclusion of this study is that a significant increase in DHPR-unlinked RYR1 occurs in older muscles. This finding is consistent with alterations in DHPR activity and SR Ca^{2+} release in aged human muscle fibers (Delbono et al., 1995).

Materials and Methods

Muscle Preparation

Middle-aged (14-month) and old (28-month) Fisher 344 Brown Norway F1 hybrids rats (F344BNF1/NIA) were obtained from the SPF Aging colony at the National Center for Toxicological Research (NCTR). Animals were housed in a pathogen-free area at Bowman Gray School of Medicine (BGSM) until the day of experimentation. Rats had free access to water and food and all were confined to a similar cage space. Animal handling and procedures were approved by the Animal Care and Use Committee of BGSM. *Extensor digitorum longus* (EDL) muscle, *soleus* muscle or a pool of skeletal muscles, not

including EDL and *soleus* muscles, were removed after sacrificing the animal by decapitation. Muscles were immediately frozen and stored at −135°C.

Preparation of Muscle Homogenates

Soleus, EDL and pool of muscles were separately homogenized at 4°C with a Tekmar Tissumizer (Cincinnati, OH) 2 times for 30 sec on medium setting in eight volumes of a medium containing (in mM): 100 NaCl, 20 Pipes-KOH, 2 EDTA, 0.2 EGTA and various protease inhibitors (0.2 mM phenylmethylsulfonyl fluoride, 100 nM aprotinin, 1 μM leupeptin, 1 μM pepstatin, and 1 mM benzamidine) at pH 6.8. After passing through four layers of cheese cloth the homogenates were quickly frozen and stored at −135°C for binding studies. Protein concentration was determined by Coomassie protein assay with bovine serum albumin as the protein calibration standard (Meissner, 1984; Anderson et al., 1995).

Radioligand Binding to DHPR and RYR

DHPR and RYR1 concentrations were determined using the radioligands [^{3}H]PN200-110 and [^{3}H]Ryanodine, respectively. Homogenates (1–2 mg/ml protein) were incubated either with 0.05–5 nM [^{3}H]PN200-110 for 1 hr at 23°C in 50 mM Tris-HCl, 10 μM Ca^{2+}, 1 mM diisopropyl fluorophosphate (DIFP) and 5 μM leupeptin, at pH 7.5, or 0.5–50 nM [^{3}H]ryanodine for 24–48 hr at 10°C in 20 mM pipes-NaOH, 1.0 M NaCl, 100 μM Ca^{2+}, 5 mM AMP, 1 mM DIFP, and 5 μM leupeptin, at pH 7.0. Membrane bound [^{3}H]PN200-110 and [^{3}H]ryanodine were determined by filtration through Whatman GF/B filters using a Millipore unit XX2702550 (Millipore, Bedford, MA). Filters were rinsed three times with 5 ml of ice-cold 200 mM choline chloride, 20 mM Tris-HCl, pH 7.5. Ecoscint H (National Diagnostics) was used as scintillation solution. Nonspecific [^{3}H]PN200-110 and [^{3}H]ryanodine binding were assessed in the presence of 10 μM unlabeled nifedipine or PN200-110 and 10 μM unlabeled ryanodine respectively. Radioligand concentrations used resulted in occupancy of >95% of the high affinity binding sites (Anderson et al., 1994).

Analysis of Binding Studies

The data from nonspecific and total binding were fit to linear regression or nonlinear least square analysis. Specific binding of [^{3}H]PN200-110 and [^{3}H]ryanodine at each concentration was calculated by subtracting the nonspecific binding from the total binding obtained from the above analysis. The following equation (1): $y = (x \cdot a)/((x + b) + (x \cdot c))$, where, a = receptor number, (maximum binding, B_{max}); $b = K_D$, dissociation constant; c = nonspecific binding or low-affinity site, was used to fit the binding isotherm. K_D and B_{max} values were also obtained from Scatchard analysis. Values are expressed in mean ± SD.

Statistical analysis was performed by applying Student *t*-test for unpaired data.

Results

DHPR-RYR1 Ratio in Mixed Fiber-type Skeletal Muscles

The number of DHPR and RYR1 sites and their dissociation constant for high-affinity radioligands were determined by binding assays to three pools of hindlimb mixed fiber-type rat skeletal muscles. EDL and *soleus* muscles were removed and pooled separately (*see below*). The DHPR, which is located at the T-tubule sarcolemma and functions as the voltage-sensor in excitation-sarcoplasmic reticulum Ca^{2+} release-contraction coupling, was assessed using the high affinity probe [^{3}H]PN200-110. In the same homogenates the sarcoplasmic reticulum RYR1, which functions as the Ca^{2+}-release channel, was quantitated by radioligand analysis using the neutral plant alkaloid [^{3}H]ryanodine as a high affinity probe. Figure 1 shows [^{3}H]PN200-110 (*A*) and [^{3}H]ryanodine (*B*) binding to skeletal muscle homogenates in 14-month-old F344BNF1 rats. The insets for both figures are the Scatchard analysis of ligand binding of both probes to the DHPR and RYR1, respectively. B_{max} and K_D values determined by Scatchard analysis or by fitting Eq. 1 to binding data were not significantly different. For the binding assay included in Fig. 1, B_{max} (pmoles/g. muscle) values obtained by Scatchard analysis for PN200-110 and ryanodine were 104 and 100, respectively, while the K_D (nM) was 1.4 and 8.1, respectively. The same parameters obtained by applying Eq. 1 were for B_{max} 101, 100, respectively and for K_D 1.3 and 8.2, respectively. Table 1 shows the statistics corre-

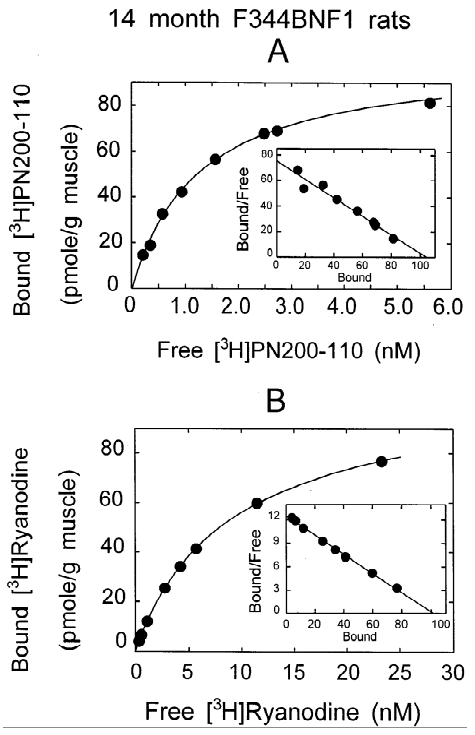

Fig. 1. [^{3}H]PN200-110 (*A*) and [^{3}H]ryanodine (*B*) binding to skeletal muscle homogenates in 14-month-old F344BNF1 rats. K_D and B_{max} values were obtained from Scatchard analysis and fitting of Eq. 1 to binding data (inset). The plots correspond to representative experiments of the total number of studies (n = 12) included in Table 1.

Table 1. High-affinity [^{3}H]PN200-110 and [^{3}H]ryanodine binding to F344BNF1 rat skeletal muscles

	DHPR		RYR1		Ratio
Animal ages	B_{max} (pmoles/g. muscle)	K_D (nM)	B_{max} (pmoles/g. muscle)	K_D (nM)	DHPR/RYR1
14-month old	76 ± 22	1.2 ± 0.5	76 ± 24	5.5 ± 3.4	1.03 ± 0.15
28-month old	37 ± 4.0 ($P < 0.01$)	0.58 ± 0.21 (NS)	47 ± 5.3 ($P < 0.01$)	2.9 ± 0.67 (NS)	0.80 ± 0.11 ($P > 0.01$)

Results are given as means ± SD of 12 experiments done in 3 different preparations. K_D, dissociation constant and B_{max} maximum binding capacity. Statistical significance is given in parentheses; NS, not significant.

sponding to all the experiments included in this part of the study. B_{max} values for DHPR and RYR1, included in Table 1, indicate the presence of high-affinity sites. The B_{max} values corresponded to a PN200-110/ryanodine binding ratio of 1.03 ± 0.15 (12 determinations in 3 different preparations). These results are similar to the values reported for matured rabbit muscles (Anderson et al., 1994) and fast-twitch rat EDL muscles (Delbono et al., 1996). Figure 2 illustrates [^{3}H]PN200-110 (*A*) and [^{3}H]ryanodine (*B*) binding to skeletal muscle homogenates from 28-month-old F344BNF1 rats. For the binding assay included in Fig. 2, B_{max} (pmoles/g. muscle) values obtained by Scatchard analysis for PN200-110 and ryanodine were 38 and 51, respectively, while the K_D (nM) was 0.7 and 4.0, respectively. The same parameters resulting from the application of Eq. 1 gave B_{max} values of 38, 48 for [^{3}H]PN200-110 and ryanodine, respectively, whereas the K_D values were 0.7 and 3.7, respectively. A complete statistics of the total number of determinations (12 in 3 different preparations) were included in Table 1. The reduction in B_{max} for DHPR ($P < 0.01$) determined a reduction in the PN200-110/ryanodine binding ratio in older muscles (0.80 ± 0.11). Changes in the maximum binding capacity cannot be explained by changes in the dissociation constant of both receptors for their ligands because no significant changes in K_D were recorded in both groups (Table 1).

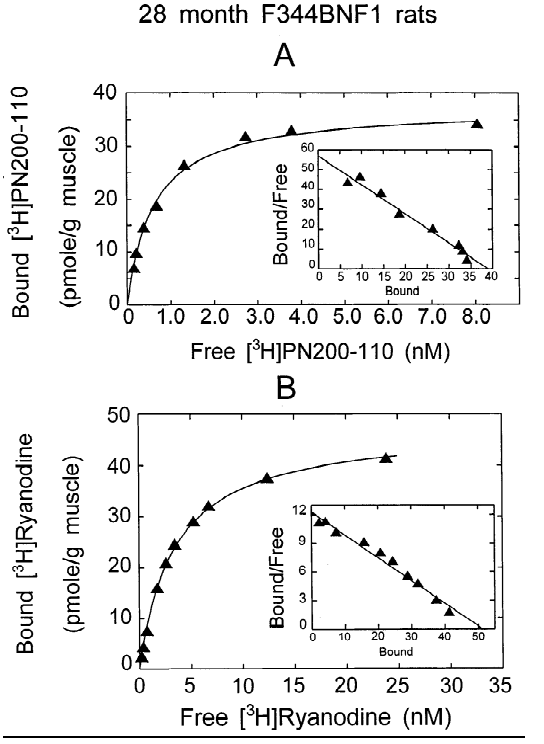

Fig. 2. [^{3}H]PN200-110 (*A*) and [^{3}H]ryanodine (*B*) binding to skeletal muscle homogenates in 28-month-old F344BNF1 rats. K_D and B_{max} values were obtained from Scatchard analysis and fitting of Eq. 1 to binding data (inset). The plots correspond to representative experiments of the total number of studies ($n = 12$) included in Table 1.

DHPR-RYR1 Ratio in *Soleus* and EDL Muscles

To determine whether the increase in DHPR-unlinked RYR1 in the pools of older skeletal muscles was determined predominantly by DHPR-RYR1 uncoupling in fast- and/or slow-twitch muscles, binding studies were performed in homogenates of only EDL or *soleus* muscle pools. Table 2 includes [^{3}H]PN200-110 and [^{3}H]ryanodine binding to *soleus* muscle homogenates from 14- and 28-month-old rats. The total number of determinations (12 in 3 different preparations) were included in Table 2. The number of DHPR-uncoupled RYR1 in *soleus* is larger than in EDL muscles from middle-aged rats. The binding studies in middle-aged rats are consistent with a previous communication (Delbono & Sonntag, 1996). The B_{max} values corresponding to a PN200-110/ryanodine binding ratio for both *soleus* and EDL muscles were significantly lower ($P < 0.01$) than the respective ratios in younger rats. The decline in the DHPR/RYR1 ratios was to 60% and 72% for older *soleus* and EDL muscles, respectively (Table 1 and 2). B_{max} changes in EDL and *soleus* muscles were not associated with statis-

Table 2. High-affinity [^{3}H]PN200-110 and [^{3}H]ryanodine binding to F344BNF1 rat skeletal muscles

Animal ages	*Soleus* muscle					EDL muscle				
	DHPR		RYR1		DHPR/RYR1	DHPR		RYR1		DHPR/RYR1
	B_{max}	K_D	B_{max}	K_D		B_{max}	K_D	B_{max}	K_D	
14 month	24 ± 7.5	1.3 ± 0.3	50.5 ± 5.4	5.2 ± 2.3	0.44 ± 0.12	107 ± 20	0.8 ± 0.4	112 ± 10	4.6 ± 1.8	0.95 ± 0.14
28 month	13.2 ± 3.6	1.0 ± 0.4	50.2 ± 5.2	4.3 ± 1.9	0.26 ± 0.10	45 ± 9	1.0 ± 0.4	67 ± 15	3.6 ± 1.7	0.68 ± 0.1
	($P < 0.01$)	(NS)	(NS)	(NS)	($P < 0.01$)	($P < 0.01$)	(NS)	($P < 0.01$)	(NS)	($P < 0.01$)

Results are given as means ± SD of 12 experiments done in 3 different preparations. K_D, dissociation constant and B_{max} maximum binding capacity. Statistical signifiance is given in parentheses; NS, not significant.

tically significant changes in DHPR and RYR1 K_D for their specific ligands (Table 2). K_D values for DHPR in EDL muscles from 14- and 28-month-old rats were (in nM): 1.3 ± 0.3 and 1.0 ± 0.4, respectively and for RYR1 were 5.2 ± 2.3 and 4.3 ± 1.9, respectively ($n = 6$). Differences between both age groups were not statistically significant. K_D values for DHPR in *soleus* muscles from 14- and 28-month-old rats were (in nM): 0.8 ± 0.4 and 1.0 ± 0.4, respectively and for RYR1 the values were 4.6 ± 1.8 and 3.6 ± 1.7, respectively ($n = 6$). Differences between both age groups for *soleus* muscles were not statistically significant. The significance of the observation that the PN200-110/ryanodine binding ratios for *soleus* and EDL muscles were lower than those for mixed fiber-type hindlimb muscles, is discussed below.

Discussion

In the present study, we found a significant larger increase in DHPR-unlinked RYR1 in *soleus*, EDL and mixed fiber-type muscles from aged than from middle-aged rats. DHPR-RYR1 uncoupling with age results in the impairment of the voltage-gated SR Ca^{2+} release mechanism. The decrease in myoplasmic Ca^{2+} elevation in response to sarcolemmal depolarization results in a reduced Ca^{2+} supply and decline in contraction force. We postulated that EC uncoupling underlies a significant fraction of the decline in skeletal muscle performance with aging (Delbono, 1997). Evidence for the failure of this signal transduction pathway in aged human quadriceps muscle (mixed fiber-type) have been published (Delbono et al., 1995).

The close control of the SR Ca^{2+} release channel by the DHPR suggests a well-defined stoichiometric relationship (Meissner, 1994). Measurement of both DHPR and RYR1 concentration in the same muscle, using high-affinity receptor-specific ligands, allows for determinations of the DHPR/RYR1 ratio (Lamb, 1992). These determinations have been demonstrated to be useful for comparing the magnitude of the receptors coupling in different species (amphibian and mammals) (Anderson et al., 1994) or in different muscle-types in the same species (rat *soleus* and EDL) (Delbono & Meissner, 1996). In these studies, the ratio for fast-twitch muscles (rabbit psoas and rat EDL) was ~1.0. Tetrads, groups of four T-tubule particles, presumed to represent four DHPRs, have been located almost exactly opposite the four subunits of the RYR (Block et al., 1988; Franzini-Armstrong et al., 1991). If there is one high-affinity ryanodine binding site and four DHPR per foot/RYR tetramer, the DHPR/RYR ratio is 4. This assertion is valid as long as all DHPRs and RYRs are located in the T-tubule-SR junction, tetrads interacts with only one RYR and all DHPRs and RYRs bind their specific ligands (Anderson et al., 1994). A binding ratio of ~1 suggests that in EDL muscles every fourth RYR is linked to a group of four DHPRs. In *soleus* muscles a lower ratio of 0.34 suggests that only every twelfth RYR is coupled to a group of four DHPRs. In the present work, we applied the same principle to determine changes in the DHPR/RYR1 coupling with aging.

The reasons for performing the studies in almost 95–97% pure fiber-type muscles, such as *soleus* and EDL are twofold: (i) adult rat muscles (14 month) show significant differences in the number of DHPR-linked RYR1, as recently demonstrated by Delbono and Meissner (1996), and (ii) the aging process seems to affect the muscle fiber-type composition. We showed that the PN200-110/ryanodine ratio for *soleus* and EDL muscles is 0.34 and 0.92, respectively. This means that *soleus*, an almost pure type-I fiber muscle has a larger proportion of DHPR-unlinked RYR1 than the EDL muscle (consisting predominantly of type-II fibers). In EDL muscles, one of every fourth RYR1 is linked to a group of four DHPRs, while in *soleus*, one of every twelfth RYR1 is coupled to a group of DHPRs. In addition, some studies suggest that type-II fibers are more affected than type-I fibers in mixed-fiber type muscles. The more selective alteration may result from primary atrophy or denervation-reinnervation leading to a predominant loss of fast-twitch fibers and increase in slow-fibers motor unit area (Kadhiresan et al., 1996; for a review *see* Lexell, 1995). In muscles consisting predominantly of one fiber subtype such as the mouse EDL, no changes in myosin isoform

were detected with aging (Florini & Ewton, 1989; Phillips et al., 1993). These data suggest that age-related alterations in muscle performance are not fiber-type specific. Based on this information, it can be theorized that an increase in DHPR-unlinked RYR1 may occur only in mixed-fiber type muscles and the maximum number of uncoupled RYR1 induced by aging is that reported for pure type-I muscles (Delbono et al., 1996). In this work, we found a significant decrease in PN200-110/ryanodine binding ratio in muscles consisting predominantly of one fiber-type (*soleus* and EDL). Therefore, this finding does not support a role for motor unit remodeling in the process of DHPR/RYR1 uncoupling with aging. Probably, the ratio for mixed-fiber muscles reported in this study reflects a switch in fiber-type distribution in addition to DHPR-RYR1 uncoupling in motor units not affected by denervation.

An issue that remains to be elucidated is whether muscle fibers undergoing denervation and reinnervation by motor neurons of the same type may underlie substantial changes in recorded DHPR/RYR1 uncoupling. To address this issue, the relative proportion of DHPR and RYR1 in muscles undergoing extensive denervation should be determined (Delbono, 1992; Delbono et al., 1992). To accept that denervation accounts for the increase in DHPR-unlinked RYR1 in a ~30% (as reported in this work), a similar increase in fiber loss should be found in older, compared to middle-aged-muscles. Previous studies do not support this concept. A 10% decrease in the mean number of fibers with a 10% of standard deviation of the mean value has been recorded in older muscles (Edström & Larsson, 1987). The 30% increase in DHPR-unlinked RYR1 is in correspondence with the magnitude of the decrease in the specific muscle strength (maximum force per cross-sectional area) reported previously (Brooks et al., 1988; Einsiedel & Luff, 1992). Recently, reported binding assays on *tibialis anterior* muscle show an apparent discrepancy with our findings (Damiani, Larsson & Margreth, 1996). However, the inclusion of younger animals in the older group, the use of a not completely characterized animal model of aging (Weindruch, 1995), and the use of different muscles in that study make a comparison with the present data difficult.

Alterations in receptor regulation may contribute to changes in muscle contraction kinetics across ages (Larsson et al., 1991; Damiani et al., 1996; Delbono et al., 1996). Force declines in all the muscles to a variable extent with advanced age. Probably, alterations in muscle performance with age are associated with specific function and localization (Fujisawa, 1975). Thus, it may happen that the DHPR/RYR1 uncoupling does not affect muscles from different territories to the same extent. Binding studies in mixed-fiber type muscles show a significant decrease in receptors coupling with aging. However, this decrease is less pronounced than in individual muscles, suggesting that some muscles are less affected by the receptor uncoupling process than others. In this regard, if *tibialis anterior* muscles are unaffected during aging, it might account for the failure of Damiani et al. (1996) to observe a similar change in DHPR/RYR1 ratio as reported here.

The substratum for the increase in DHRP/RYR1 uncoupling with aging is a more pronounced decrease in the expression of DHPR at the sarcolemmal T-tubule than the decrease in the expression of RYR1 at the sarcoplasmic reticulum terminal cisternae. Thus, a larger number of RYR1 are not activated by interaction with DHPRs and probably are only activated through a Ca^{2+}-dependent process (Jacquemond, Kao & Schneider, 1991; Delbono & Stefani, 1993; Delbono, 1995). This concept is valid for older *soleus* muscle. In this muscle, a decrease to 50% of the number of DHPR sites was recorded in the absence of changes in B_{max} for RYR1. The interpretation of alterations in DHPR-RYR1 coupling in EDL muscles seems to be more complex. Similar to *soleus*, the number of DHPRs in EDL muscle declines to ~35% of the value reported for adult muscles, whereas the number of RYR1s decreases to ~65%. This means that, in addition to a decrease in 30% of DHPR-uncoupled RYR1, an absolute reduction in the number of RYR1 in older EDL muscles occurs. This phenomenon contributes to a significant impairment in Ca^{2+} supply to contractile proteins and muscle-force development with aging. In summary, both *soleus* and EDL muscles exhibit an absolute reduction in the number of voltage-sensing molecules at the T-tubule membrane with advanced age. Reasons for differential alterations in the RYR1 expression in aged *soleus* and EDL muscles are not obvious. Alterations in the number of DHPR and/or RYR1 in older muscles may result from decreases in DNA transcription, mRNA translation, modification, sorting, membrane insertion, and/or increase in protein degradation. Ongoing studies in our laboratory are trying to define specific alterations in DHPR and RYR1 expression with aging.

In summary, the present work supports the concept that alterations in DHPR-RYR coupling are major determinants of EC uncoupling in aged skeletal muscles.

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