Age and Sensitivity of Rat Skeletal Muscle Fibres to Calcium and Strontium

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Abstract

Calcium and strontium sensitivity was examined in extensor digitorum longus and soleus muscle fibres from rats aged 5-6 months and 18-19 months. Fibres from the older rats displayed increased sensitivity to calcium, but not strontium, compared to the younger rats, suggesting that modifications occur to the contractile apparatus with age.

Key words: skeletal muscle, ageing, rats, pCa, pSr, normalised tension.

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In mammals, ageing generally is associated with a loss of muscle strength (see review by Vandervoort [19]). A number of changes occur in skeletal muscle with ageing that may contribute to this decline in strength. The number of muscle fibres decreases [2, 7], and an increased proportion of each muscle is occupied by type I fibres. The latter change has been variously attributed to either a selective decrease in the number of type II fibres [15], or to a selective decrease in the size of type II fibres [3]. In addition, a conversion of type II to type I fibres may also occur [12]. Larsson et al. [8] propose that fibre-type conversion follows the pathway IIB → IIX → IIA → I, with IIX fibres representing an intermediate between IIB and IIA fibres. With ageing there also is an increase in both the proportion of extracellular fat and connective tissue [13], and the proportion of intracellular fat [12].

It has been proposed that the observed decrease in muscle strength can be totally explained in terms of an age-related loss of muscle tissue [5, 20]. However other authors, who have observed a decrease in normalised tension (force per muscle cross-sectional area) with ageing, have argued that additional factors must also contribute to this strength loss [1, 11]. It is unclear whether changes occur to the sensitivity of the contractile apparatus to calcium that could contribute to this age-related decrease in normalised tension. Whereas Eddinger et al. [4] found no change to calcium sensitivity with ageing, Lynch et al. [9] observed an increased sensitivity to calcium in type IIB fibres. It is probable that an age-related change in the function of the chemo-mechanical transduction system contributes to the decline in muscle strength observed in old age. Reported here are the results of a preliminary study of age-related changes in the contractile apparatus, using a skinned fibre technique.

Methods

Muscle fibre preparation

The preparation of muscle fibres was undertaken according to the method of Stephenson and Williams [16]. Muscle fibre segments from male Sprague-Dawley rats aged 5-6 months ('young adults'; n = 4) and 18-19 months ('middle-aged adults'; n = 4) were used. The animals were killed by cervical dislocation, following which the soleus and extensor digitorum longus (EDL) muscles were excised and immediately placed in chilled paraffin oil at 4°C until use. Single muscle fibre segments 500-1000 µm in length were then dissected free using fine jeweller's forceps and Vanna scissors, and attached to an AE801 strain-gauge force transducer (SensoNor, Norway). Sarcomere length was adjusted to optimum (2.70-2.85 µm [17]) while viewing the muscle striations under a Leitz Fluovert FU inverted microscope, with a calibrated graticule inserted into the eyepiece, by counting the number of striations visible within the boundaries of the graticule at a total magnification of 200 times. The diameter of each fibre was also measured using this graticule.

Solutions

The components of activating and relaxing solutions (Solution I - high relaxing; Solution II - activating; Solution III - low relaxing) are detailed in Table 1. Varying proportions of solutions I and II were mixed to produce a series of 9 calcium solutions with concentrations in the range $4.90 < pCa < 7.82$ ($pCa = -\log[Ca^{2+}]$), and a series of 8 strontium solutions with concentrations in the range

Table 1. Activating and relaxing solutions.

	Solution I (high-relaxing)	Solution II (activating)	Solution III (low-relaxing)
HEPES	60 mM	60 mM	60 mM
$MgCl_2$	1 mM	1 mM	1 mM
HDTA	-	-	50 mM
EGTA	50 mM	50 mM	-
Sodium Azide	1 mM	1 mM	1 mM
Caffeine	10 mM	10 mM	10 mM
ATP	8 mM	8 mM	8 mM
$CaCl_2$ (or $SrCl_2$)	-	48.5 mM (36.0 mM)	-

Abbreviations
HEPES: N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulphonic acid]
HDTA: 1,6-Diaminohexane-N,N,N',N'-tetraacetic acid
EGTA: Ethylene glycol-bis(β-aminoethyl ether) N,N,N',N'-tetraacetic acid
ATP: Adenosine 5'-triphosphate

$5.11 < pSr < 7.42$ ($pSr = -\log[Sr^{2+}]$). The pCa/pSr of the solutions was determined by titration [10].

Fibre activation

Prior to activation, fibres were placed in solution I (high relaxing) containing 2% Triton X-100 for 10 minutes. The fibres were transferred briefly to solution III (low relaxing), and then to solution II (activating solution). The resultant force output was digitised and logged using software written by the authors. When the force of contraction reached a plateau, the fibre was returned to solution I where it remained until it was again fully relaxed. This cycle was repeated with each solution, beginning with the most concentrated solution and ending with the most dilute, and the force of contraction was logged in all cases. At the end of the series of calcium solutions, the fibre was activated a second time in the most concentrated calcium solution. If the resultant force was less than 80% of that produced from the first activation in this solution, the data was excluded from analysis. The entire process was then repeated for the strontium series. All experiments were conducted at room temperature (21-25°C). Fibre type was determined by the response of each fibre to the strontium solutions [14]. If the pSr_{10} of a fibre was 6.8 or greater it was considered to be a type I fibre. If the pSr_{10} was 6.3 or less, it was classed as a type II fibre. No attempt to subclassify fibres was made. After calibration of the force-transducer, the absolute force of each contraction was calculated in Newtons.

Analysis of data

The response of fibres to each concentration of activating solution was expressed relative to maximal activation. Maximal activation was defined as the response to the most concentrated calcium or strontium solution. These solutions have previously been established in our laboratory as producing maximal activation. The force outputs of fibres immersed in each calcium or strontium activating solution were then averaged and plotted against the pCa or pSr.

Data were fitted to the Hill equation [6], which predicts the response of actin-myosin force output in the presence of differing levels of divalent cations (Equation 1).

$$Pr = K[X^{2+}]^n / (1 + K[X^{2+}]^n) \qquad \text{Equation 1}$$

where Pr = Relative Force, $[X^{2+}]$ is the concentration of either calcium or strontium ions, n (the Hill coefficient) is a constant determined by the slope of the force-pCa or force-pSr curves, and K is a constant related to the lateral shift of the curves.

pCa_{50} and pCa_{10} (the pCa that produced 50% and 10% of maximal activation respectively), and pSr_{50} and pSr_{10} (the pSr that produced 50% and 10% of maximal activation respectively) were calculated for each fibre using software written by the authors. The averages of the two age groups were statistically compared using student's t-test.

Normalised tension was calculated by dividing the maximum force produced by each fibre by its cross-sectional area. The assumption was made that fibres were round in profile when calculating cross-sectional from their diameter. The averages of the two age groups were again compared using student's t-test.

Results

A total of 17 and 20 EDL fibres were sampled from the young and middle-aged rats respectively. All EDL fibres were classed as type II fibres according to their response to strontium activation. A total of 10 and 12 soleus fibres were sampled from the young and middle aged rats respectively. All soleus fibres from the young rats were classed as type I fibres. 10 of the 12 middle-aged soleus fibres were classed as type I fibres, the remaining two were classed as type II fibres. Because of the very small number of soleus type II fibres, and the lack of similar fibres from the young animals, these fibres have been excluded from further analysis.

The force-pCa curves for fibres taken from the 4 'middle-aged' (18-19 months) animals showed a shift to the left when compared to those generated by the younger fibres

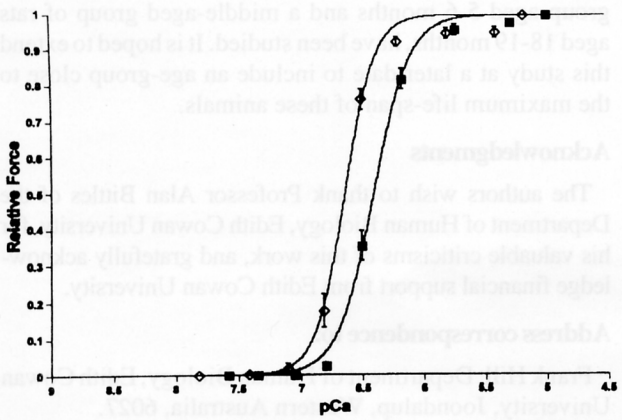

Figure 1a. Force-pCa curves for young adult (squares) and middle-aged adult (open diamonds) EDL fibres. Vertical bars represent standard errors of the mean.

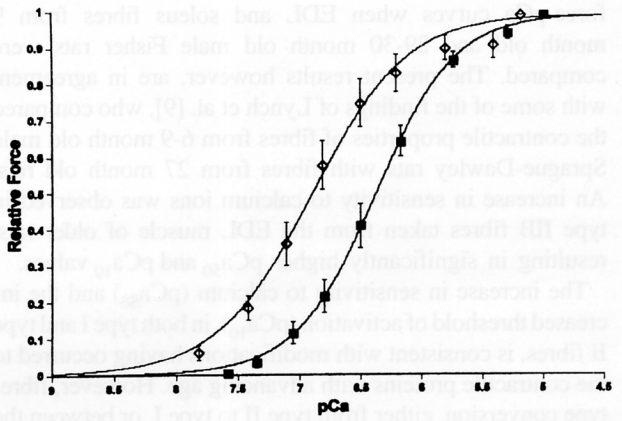

Figure 1b. Force-pCa curves for young adult (squares) and middle-aged adult (open diamonds) soleus fibres. Vertical bars represent standard errors of the mean.

(Figures 1a and 1b). This is seen for fibres from both EDL and soleus muscles, and the observation is supported by the significantly higher pCa_{50} and pCa_{10} values calculated for the older fibres (Table 2). The slope of the force-pCa curves, represented by the n_{Ca} values, showed no significant difference when the two age groups were compared. In contrast to the calcium response data, fibres taken from young and middle-aged rats showed no significant kinetic differences in response to activation by exogenous strontium ions (Table 2).

When activated by either calcium or strontium ions, the average normalised tensions observed in both EDL and soleus fibres were substantially lower in the older fibres. However, although the magnitudes of the differences between young adults and middle-aged adults were similar for both the EDL and soleus fibres, only the EDL data generated statistically significant differences between the age groups (Table 2).

Discussion

With advancing age there was an increase in sensitivity to calcium, but not to strontium ions, in both type I fibres from rat soleus muscle and type II fibres from rat EDL muscle (Figures 1a and 1b; Table 2). This is in contrast to the findings of Eddinger et al. [4], who found no differences in the

Table 2. Contractile characteristics for fibres from young adult and middle-aged rats.

	EDL		Soleus	
	Young (n = 16)	Middle-aged (n = 11)	Young (n = 10)	Middle-aged (n = 10)
n_{Ca}	3.21 ± 0.13	3.46 ± 0.12	1.52 ± 0.10	1.43 ± 0.16
pCa_{50}	6.40 ± 0.03	6.63 ± 0.02***	6.39 ± 0.07	6.87 ± 0.12**
pCa_{10}	6.71 ± 0.02	6.91 ± 0.03***	7.04 ± 0.10	7.62 ± 0.11**
P_o(Ca)	47.33 ± 7.99	18.20 ± 3.67*	49.31 ± 12.71	19.17 ± 4.47
	(n = 17)	(n = 20)	(n = 10)	(n = 9)
n_{Sr}	3.81 ± 0.12	3.57 ± 0.14	1.15 ± 0.13	1.43 ± 0.22
pSr_{50}	5.59 ± 0.03	5.57 ± 0.02	6.56 ± 0.07	6.51 ± 0.12
pSr_{10}	5.83 ± 0.04	5.85 ± 0.03	7.49 ± 0.09	7.33 ± 0.07
P_o(Sr)	54.07 ± 7.39	23.39 ± 4.49**	68.68 ± 12.43	40.15 ± 9.55

pCa_{50}/pCa_{10} : the pCa that produces 50% and 10%, respectively, of maximal activation
pSr_{50}/pSr_{10} : the pSr that produces 50% and 10%, respectively, of maximal activation
P_o : Normalised Tension (N/cm^2)
Values are ± S.E.M.
* Difference between "young" and "middle-aged" significant at $P < 0.05$
** Difference between "young" and "middle-aged" significant at $P < 0.01$
*** Difference between "young" and "middle-aged" significant at $P < 0.001$

force-pCa curves when EDL and soleus fibres from 9 month old and 29-30 month old male Fisher rats were compared. The present results however, are in agreement with some of the findings of Lynch et al. [9], who compared the contractile properties of fibres from 6-9 month old male Sprague-Dawley rats with fibres from 27 month old rats. An increase in sensitivity to calcium ions was observed in type IIB fibres taken from the EDL muscle of older rats, resulting in significantly higher pCa_{50} and pCa_{10} values.

The increase in sensitivity to calcium (pCa_{50}) and the increased threshold of activation (pCa_{10}), in both type I and type II fibres, is consistent with modifications having occurred to the contractile proteins with advancing age. However, fibre-type conversion, either from type II to type I, or between the various subtypes of type II fibres, is unlikely to have occurred, as no changes were noted in the level of cooperativity of the regulatory proteins in the activation of tension (indicated by n_{Ca} and n_{Sr}). The absence of fibre-type conversion may reflect the relatively young ages of the rats used (5-6 and 18-19 months) in this study.

As fibre-type conversion appears unlikely to have occurred in this study, it seems that other changes to the contractile proteins also occur with ageing. A shift of the force-pCa curve to the left at submaximal calcium concentrations has been observed after phosphorylation of the myosin regulatory light chain [18]. A potential, albeit speculative explanation of the modified response to calcium activation with ageing seen here is an increase in, or an increased propensity to, myosin regulatory light chain phosphorylation with ageing.

In this study, ageing was found to result in a decrease in normalised tension in both EDL and soleus fibres, although only the data obtained with EDL fibres attained statistical significance. The reduction in normalised tension with ageing is consistent with the hypothesis that the muscle weakness of old age results both from a loss of tissue, and changes to the contractile apparatus, such as a decreased proportion of the myosin heads forming cross-bridges during muscle fibre activation, or each cross-bridge producing less force. Alternatively, ageing may be associated with a reduction in the amount of contractile protein per cross-sectional area of muscle fibre, which is consistent with the observation that ageing is associated with an increased proportion of intracellular fat in muscle fibres. With the exception of a small subgroup of type I soleus fibres (n = 2), which showed a decrease in normalised tension with ageing, Lynch et al. [9] observed no change in normalised tension at different ages. More surprisingly, Eddinger et al. [4] found that normalised tension in soleus fibres from aged rats was greater than for fibres from adult animals. The decrease in specific tension seen in this study may also reflect the fact that the specific tension for the young (5-6 months) group of animals was considerably higher than that found by other workers [9], which may result from methodological differences between this and other studies. Further work is in progress by us to clarify the reasons underlying these data.

In this study only two age groups of rats, a young adult group aged 5-6 months and a middle-aged group of rats aged 18-19 months, have been studied. It is hoped to extend this study at a later date to include an age-group close to the maximum life-span of these animals.

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