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Short communication

Changes in membrane ionic conductances and excitability characteristics of rat skeletal muscle during aging

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Abstract: Membrane electrical properties, component ionic conductances and excitability characteristics of extensor digitorum longus muscle from 3-4, 16 and 29 months old rats were measured "in vitro". Fiber diameter, membrane resistance (Rm) and membrane capacitance, increased with aging, and the increase was significant at 29 months. The increase of Rm was mostly due to a decrease of chloride conductance (GCl), whereas potassium conductance (GK) increased only slightly, at 16 and 29 months. Due to the lowered GCl, the latency of action potential increased at both ages with a consequent prolongation of the duration of action potential. Nevertheless, a decrease in the firing capability was recorded in the aged fibers. Our results indicate, that during aging, the most affected parameter of skeletal muscle fibers is GCl, although changes of this passive conductance alone cannot entirely account for the changes in the excitability characteristics recorded.
Key words: Aging rat, Skeletal muscle, Cl^- and K^+ conductances, excitability.

INTRODUCTION

During aging, a decrease in muscle performance, with regard to both speed and strenght of contraction, occurs in humans (11) and animals (7). Apart from a loss of muscle mass generally observed (8), the available data about the contractile properties and the histochemical characteristics of muscles from aged rodents are often controversial with respect to the species, the preparations or the methodology used and, therefore, could only in part explain the impairments of the old subjects (2,7). No attempts have been made to investigate whether changes in the membrane electrical properties occur with advancing age. The membrane of adult skeletal muscle have specialized electrical characteristics determining appropriate muscle response to a depolarizing stimulus. In particular a large resting conductance to chloride (GCl) ensures the electrical stability of the membrane; indeed, an abnormally low GCl is responsible for the repetitive firing observed in some forms of naturally occurring (1) and drug induced myotonic syndromes (4). Also, we recently demonstrated that the membrane electrical properties, and in particular GCl, change significantly within the first weeks of rat post-natal life (5), a period during which the maturation of contractile properties also takes place (13). Thus, the present study was aimed at evaluating the passive cable properties, component ionic conductances and excitability characteristics of rat skeletal muscle membrane during aging, to asses whether changes of these parameters that could be related to the impaired performance of aged muscle do occur.

METHODS

All experiments were carried out "in vitro" at 30°C on extensor digitorum longus (EDL) muscles removed under urethane anaesthesia from male Wistar rats aged 3-4, 16 and 29 months. Particular care was taken in housing the aged animals (16 and 29 months) and to ensure that those used for the experiments (3 and 4 rats of 16 and 29 months, respectively) were generally in good healt. In the 29 months old animals (named R_1 to R_4) a tendency to a more restricted locomotor activity was observed by comparison with the younger rats, although they showed no signs of paralysis nor serious impairment of hind limb movements that could arise from a pathological state of motoneurones. A pair of microelectrodes, one for recording potentials and the other for passing current, was used for making intracellular measurements of membrane potentials, cable properties and membrane excitability characteristics, (3,4). In each preparation, by using standard cable analysis and assuming a myoplasmic resistivity, Ri, of 125Ω.cm, the calculated fiber diameter, dcalc, the membrane resistance, Rm and the membrane capacitance, Cm, were estimated in chloride containing and in chloride-free medium. The value of Ri, the "apparent" resistivity of the myoplasm, dependes on concentration of conducting ions and to some degree on the sarcomere lenght (6), that was kept constant in all the experiments. It is possible for intracellular ions concentration to change with age; but the intracellular ions are in osmotic equilibrium with the extracellular ions and the osmotic pressure of the plasma does not change appreaciably during aging. Then, great changes of Ri during aging, can not be expected by this effect; therefore we assumed that Ri remains constant with age.

In addition the reported values of Rm depends on the square root of Ri which decreases the amount of error due to possible changes of Ri. The total membrane conductance Gm was 1/Rm in the normal physiological solution. The potassium conductance, GK, was 1/Rm in the chloride-free medium. The mean chloride conductance, GCl, was calculated as the mean Gm minus the mean GK. The excitability characteristics of the sampled fibers were determined by recording the intracellular membrane potential response to square-wave constant current pulses. In each fiber the membrane potential was set by a steady holding current to -80mV, before passing the depolarizing pulses. The normal

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physiological solution had the following composition in mM: NaCl 148; KCl 4.5; $CaCl_2$ 2.0; $MgCl_2$ 1.0; $NaHCO_3$ 12 and glucose 5.5. The chloride-free medium was made up by equimolar substitution of methylsulfate salts for NaCl and KCl and nitrate salts for $CaCl_2$ and $MgCl_2$.

The solutions were bubbled with 95% O_2 and 5% CO_2 (pH=7.2-7.4). The values are expressed as mean±SEM. Significance of differences between group means was calculated by unpaired Student's t test. The estimates for SEM of GCl were obtained from the variance of Gm and GK, assuming no covariance, using standard methods (4,5).

RESULTS

Passive cable properties and component conductances

In normal physiological solution the mean value of membrane resistance(Rm) at 3-4 months of age was 297± 23Ω.cm^2(n=12). Rm increased during aging and it was 348±39Ω.cm^2(n=9) and 613±75Ω.cm^2(n=35) at 16 and 29 months, respectively. At 29 months the increase of Rm was statistically significant, but at this age a certain degree of variability was found from preparation to preparation. A statistical analysis performed for the mean Rm value of each of the 29 months old rats versus the mean Rm value at 3-4 months revealed that the increase of Rm was a general feature of the aged muscles and was highly significant in two preparations (Table 1).

PREPARATION	AGE (months)	n	Rm (Ω.cm^2)	n'	GCl (μS/cm^2)	GK (μS/cm^2)
Mean of 3 rats	3-4	12	297±23	19	3287±179	308±88
R_1	29	12	410±54	7	2455±254[a]	412±64
R_2	29	8	690±141[a]	13	1538±263[a]	435±79
R_3	29	10	854±191[a]	7	1340±306[a]	453±74
R_4	29	5	494±179	10	2223±299[a]	522±52

Table 1. From left to right: Preparation, where R_1-R_4 are preparations each from a different rat; Age, of the rats in months; n, number of fibers for membrane resistance (Rm); n', number of fibers for the component conductances to chloride (GCl) and potassium (GK) ions. [a]Significantly different from mean of 3-4 months old rats; p<0.05 or less.

The mean calculated diameter(dcalc) and the membrane capacitance(Cm) showed an homogeneous tendency to increase during aging. The mean dcalc was 48.5±5.1 μm (the no fibers was the same of Rm) at 3-4 months and 62.7±4μm and 60.4±3.7μm at 16 and 29 months, respectively. The mean Cm that was 4±0.9μF/cm^2 at 3-4 months, slightly increased at 16 months(4.9±1.3μF/cm^2) and its increase was significant at 29 months (6.7±0.6μF/cm^2).

In chloride-free medium, the mean values of dcalc and Cm at 16 and 29 months were similar to the age-matched values recorded in normal medium. In these experimental conditions Rm was 4372±615Ω.cm^2(n=12) at 3-4 and 16 months, respectively. A decrease of Rm was recorded at 29 months(2802±281Ω.cm^2; n=37). The calculation of resting component conductances showed a slight, but not significant decrease of GCl at 16 months, while at 29 months the mean value of GCl was significantly lower with respect to that of the 3-4 months old rats (Fig 1). As for the Rm value, also for GCl we observed a certain degree of variability among the preparations from the 29 months old rats. However, the reduction of GCl of each preparation was always statistically significant from the 3-4 months controls (Table 1). The mean GK values showed a slight but not significant tendency to increase at both 16 and 29 months (Fig. 1). A less variability among the 29 months old rats was recorded for GK (Table 1).

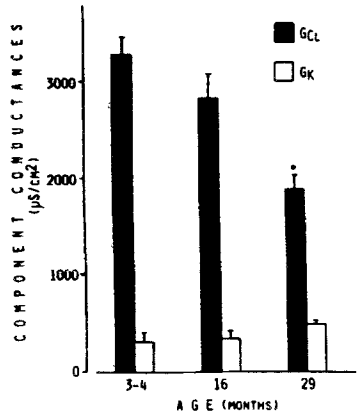

Fig 1. Each group of bars represents the mean values of resting conductances to chloride (GCl) and potassium (GK) ions at different ages. From left to right the groups are from 3-4, 16 and 29 months, respectively. Each value is the mean±SEM from 9-35 fibers. *Significantly different from mean of 3-4 months old rats; p<0.05 or less

Resting membrane potentials and excitability characteristics

The mean RP value at 3-4 months of age was -75±2.3 mV(n=25). A significant depolarization of the sampled fibers was recorded with progressive aging: RP was -60±1.4 mV(n=20) and -55±0.8 mV(n=60) at 16 and 29 months, respectively. The excitability characteristics underwent age-dependent changes. In particular, as would be expected from fibers with reduced GCl values(1), the delay (latency, Lat) between the onset of the current pulse and initiation of the action potential showed an increase of 110% and 175% at 16 and 29 months, respectively(Table 2). A prolongation with age of the duration of the action potentials (Dur: measured as the duration of all the process, including the electrotonic potential duration up to the point of all or none response and the recovery back to RP), was also observed; this parameter was significantly increased at 29 months (Table 2). Again as for Rm and GCl, also Lat and Dur vary among each preparation at 29 months(Table 2); as expectable, the highest values of Lat and Dur were found in the muscles with the lowest GCl. In spite of the above described changes that would be expected to be accompanied by an increase in fiber excitability, we did not observe any change in the current necessary to evoke the first action potential (Ith), and the ratio between the threshold current and the current to evoke the second action potential(Ith/I_2) even decreased by 19% and 31% at 16 and 29 months, respectively(Table 2). The decrease in the ratio Ith/I_2 was mirrored by a decrease in the number of action potentials evoked by a standard stimulus(Table 2). The amplitude of the first action potential (AP) did not change (Table 2).

DISCUSSION

It has been proposed that the muscle weakness in old age is not strictly caused by changes in the types of contractile proteins, but rather by other processes such as morphological and functional changes in sarcoplasmic reticulum and transverse tubules(7) or by

	Mean of 3 rats	Mean of 3 rats	Mean of 4 rats(R_1-R_4)
AGE (months)	3	16	29
Ith(nA)	92±15	136±24	89±11
Ith/I_2	0.87±0.07	0.7±0.07	0.6±0.06[a]
AP_1(mV)	98±2.8	102±2.1	96±2.1
Lat(msec)	8.9±1.4	18.9±8.8	24.6±3.3[a]*
Dur(msec)	13.5±1.6	23.3±8.8	30.6±3.3[a]*
N spikes	7.0±1.0	4.8±0.9	2.9±3.1
N fibers	13	9	37

Table 2. Ith: threshold current; Ith/I_2: ratio between threshold current and the current necessary to evoke the second action potential; AP_1, Lat and Dur: respectively, amplitude, latency and duration of the action potential; N spikes: maximum number of action potentials elicited and N fibers: number of fibers sampled. [a]Significantly different from mean of 3-4 months old rats; $p<0.05$ or less. *Variability among each prparations at 29 months:

Lat: R_1= 15.7±3.2; R_2= 40.6±7.7[a]; R_3= 39.4±7.3[a]; R_4= 13.6±0.5

Dur: R_1= 21.8±3.6; R_2= 49.0±8.3[a]; R_3= 45.1±7.0[a]; R_4= 17.5±0.6

alterations at the neuromuscular junction(12). However, a significant population of small atrophic fibers that would be involved in the observed decrease of developed tension, was observed in aged mice(2). From electrophysiological recordings the EDL fiber diameter of aged rats was even slightly greater than that of 3-4 months old rats and this increase was paralleled, as already described, by an increase in Cm (9), suggesting that the electrical parameters we detected were not from atrophic fibers. The most interesting observation of the present work is the age-related increase of Rm, almost entirely due to a decrease of GCl. In adult mammalian muscle a lowered GCl either occurring naturally(1) or induced by drugs(4) leads to an increase in membrane excitability, with lengthening of the latency of the action potential and tendency to fire repetitively(1). Accordingly, we observed that in the aged EDL fibers the latency of action potential was prolonged in a manner that strictly reflected the lower GCl. Nevertheless, the firing capability of the aged fibers was unexpectedly reduced with respect to the adult controls, suggesting a possible modification of other factors important for determining action potential characteristics and its conduction along the sarcolemma. Since in our experiments the fibers excitability was measured at constant membrane potential (see methods), its reduction suggest that there is lower density of Na^+ channels in the aged rats, possibly due to a decreased protein synthesis. Moreover, alteration of the surface charges, as well as changes in the activity of the membrane pumps could justify the decrease of RP generally observed in the aged fibers. These changes could also be involved in the decreased mechanical activity found in aged animals(2).

The main factor responsible for the selective impairment of GCl in aged muscle remains to be elucidated. The resting voltage is not responsible for this feature, since a depolarization usually leads to an increase of GCl(10). It is known that the expression of the chloride channels responsible for the macroscopic GCl is controlled by motor nerve; indeed we have demonstrated that denervation of EDL muscle leads to a drastic fall of GCl, in adult(3) and seriously prevent GCl maturation in newborn rats(5). Moreover it has been documented that in aged rats changes occur at the neuromuscular junction, such as a reduced acetylcholine availability(12); of course, this could create a functional denervation of the muscle fibers that could cause a drop of GCl. Obviously, further studies are necessary to verify this hypothesis, as it remains to be clarified whether the decrease in GCl is due to a reduction in the number of chloride channels or to other processes. The use of drugs highly specific for muscle chloride channels(4) would be useful at this regard.

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