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Actions of Lyotropic Anions on the Mechanical Properties of Fast and Slow Twitch Rat Muscles at Different Temperatures

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Summary

The effects of lyotropic (swelling) anions (Cl^- , Br^-, NO_3^- and I^-) on contractile properties of fast-twitch extensor digitorum longus (EDL) and slow-twitch soleus (SOL) muscles were investigated *in vitro* at 20 °C and 35 °C. Isolated muscles bathed in anionic Tyrode solution were stimulated directly and isometric single twitches and fused tetanic contractions were recorded. In a Cl^- Tyrode solution a decrease of the bathing temperature led to a cold potentiation of the twitch tension (P_t) in EDL muscles, however, to a cold depression in SOL muscles, in both muscles combined with a prolongation of contraction (CT) and half relaxation (HRT) times. The extent and order of the potentiating effect of lyotropic anions on the P_t, CT and HRT in EDL and SOL were quite similar and increased in the order: $Cl^- < Br^- < NO_3^- < I^-$. Since the lyotropic anions did not influence tetanic tensions, the twitch-tetanus ratio (TTR) was increased in NO_3^- and I^- solutions. All effects of the anions were rapidly and completely reversed in both muscles when the test solution was replaced by the normal one. The temperature decrease caused no significant alteration in the potentiation capacity of the anions or in the kinetics of their action and reversibility.

Key words

Slow and fast muscles • Contractile properties • Twitch potentiation • Lyotropic anions • Temperature • Rat

Introduction

Temperature variations alter the isometric twitch responses in functionally different slow and fast types of mammalian skeletal muscle fibers (Close and Hoh 1968, Bennett 1984, Buller *et al.* 1984, Ranatunga 1984, Asmussen and Gaunitz 1989, Ranatunga and Wylie 1989). In muscles mainly composed of slow-twitch type I fibers (Asmussen and Soukup 1991, Soukup *et al.* 2002a,b, Vadászová *et al.* 2002), a decrease in muscle temperature reduces twitch tension (cold depression), whereas in muscles containing a majority of fast-twitch (type IIA, IIX/D or IIB) fibers, the twitch tension is enhanced (cold potentiation). The temperature response can thus be used to classify a given muscle (Asmussen and Gaunitz 1989). The maximum tetanic force, on the other hand, progressively diminishes with decreasing temperature in all muscles regardless of their fiber type composition (Rall and Woledge 1990).

The mechanical properties of a muscle can also be altered by some chemical agents, including lyotropic (swelling) anions (Cl^- , Br^-, NO_3^-, and I^-), which belong to the most important ones. It has been accepted that these anions with a low free energy of hydration are able

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to act on ionic channels (Klemm *et al.* 1998, Gong *et al.* 2002). The high permeability of muscle plasmatic membrane to chloride thus contributes to membrane potential stability, however, anion channels are generally considered to be more permeable for I^-, NO_3^- and Br^- than for Cl^- anions. It was found that in amphibian muscle fibers, these anions prolong the action potential, increase the duration of the active state and enhance twitch tension according to their lyotropic order (Ritchie 1954, Kahn and Sandow 1950, 1955). However, all these studies were performed at low temperatures (0 °C – 25 °C) and no detailed study was carried out in mammalian muscles.

Therefore the aim of the present study was to investigate the effects of lyotropic anions on twitch force, twitch duration and tetanic tension at 35 °C (and at 20 °C to analyze the effect of a lower temperature) in mammalian slow- and fast-twitch muscles. Furthermore, the reversibility of the altered responses was analyzed after withdrawal of the anions.

Methods

Twenty-three Wistar rats of either sex (aged 21 to 30 days, body weight 90-120 g) were used. The animals were anesthetized for several hours with urethane (1.5 g/kg body weight i.p.) and the slow-twitch SOL and the fast-twitch EDL muscles were excised successively for the experiments. The arrangement of the muscles *in vitro*, the composition of the Tyrode solution, direct stimulation (between two platinum electrodes) and the tension recordings were the same as those previously used (Asmussen and Gaunitz 1978, 1989). In the experimental anionic solutions, the chloride anions were replaced by either nitrate, bromide or iodide anions. Their isotonicity corresponded to the standard Cl^--Tyrode solution and they contained cations in an identical ratio.

The Ethical Principles and Guidelines for Scientific Experiments on Animals were respected throughout the studies. The maintenance and handling of experimental animals followed the recommendations of the EU and the animals were treated in accordance with principles of the Care and Use of Animals. After the experiments, the rats were killed by an overdose of the anesthetic (Soukup *et al.* 2001).

The excised muscles were set up for recording at a temperature alternating between 35 °C or 20 °C. Before starting stimulation, the muscles were equilibrated for 10 min in bubbled (95 % O2, 5 % CO2) standard Cl^--Tyrode solution. The muscles were stretched until twitch tension was maximal (optimal muscle length, L_0). Thereafter, each muscle was activated using the following multistep protocol: 1) control twitches at regular 10 min intervals at a given temperature; 2) twitch recordings in the 1st, 2nd, 3rd, 4th, 6th, 8th, 10th, 12th, 15th, 20th min in one of the anionic solutions, namely nitrate (NO_3^-), bromide (Br^-) or iodide (I^-), to evaluate twitch potentiation by the anions; 3) recordings in 1-3 min intervals for 15 min in a standard Tyrode solution to assess the recovery after action of anionic solutions; 4) warming or cooling the muscle bath and repeating steps in the regimen 1, 2, and 3. At the beginning and at the end of each stimulation protocol a single tetanus was evoked by a train of stimuli at a fusion frequency for 500 ms. From the recordings, the maximum twitch (P_t) and tetanic (T_0) tensions, the twitch-tetanic-ratio (TTR) and the twitch contraction (CT) and half-relaxation (HRT) times were determined. The data are presented as means ± S.D. The paired Student's t test was used for comparison of the control and experimental data (Weber 1967).

Results

Action of lyotropic anions on the contractile parameters of EDL and SOL muscles at 35 °C

The characteristic potentiating effect of a lyotropic anion (iodide) is illustrated in Figure 1. It is clearly shown that at 35 °C the amplitude as well as the duration of a single twitch are increased in both muscles. The mean augmentation in P_t of EDL (Fig. 2A) and SOL (Fig. 2B) in Br^-, NO_3^- and I^- solutions compared to a normal Cl^--Tyrode solution, showed an increasing effect of these anions in the order: $Cl^- < Br^- < NO_3^- < I^-$. The P_t in Br^- was higher than in the Cl^--Tyrode solution ($p<0.05$), NO_3^- showed a stronger potentiation effect ($p<0.02$) than Br^- and the effect of I^- on P_t was even stronger than that of Br^- and NO_3^- ($p<0.01$). The extent as well as the order of P_t potentiation were similar in the EDL and SOL muscles. Furthermore, the twitch records of these muscles (Fig. 1) indicated changes in CT and HRT (Table 1). Compared to the control Cl^--Tyrode solution, the effect of lyotropic anions on the slowing of the twitch increased in the order: $Br^- < NO_3^- < I^-$. Similarly as in the case of P_t, this increase was maximal in the I^--solution ($p<0.01$) and moderately enhanced in the NO_3^- solution ($p<0.05$), whereas Br^- caused a borderline non-significant alteration of CT and HRT (Table 1). The effects on P_t, CT and HRT were rapidly

reversed to the pre-treatment levels by replacing the test solutions with Cl^--Tyrode solution (Fig. 2).

As compared to the controls, no augmentation of the T_0 value was induced by exchanging different anions (Table 1). The TTR was slightly increased following NO_3^- ($p<0.05$) and moderately increased after I^- ($p<0.02$) solutions (Table 1).

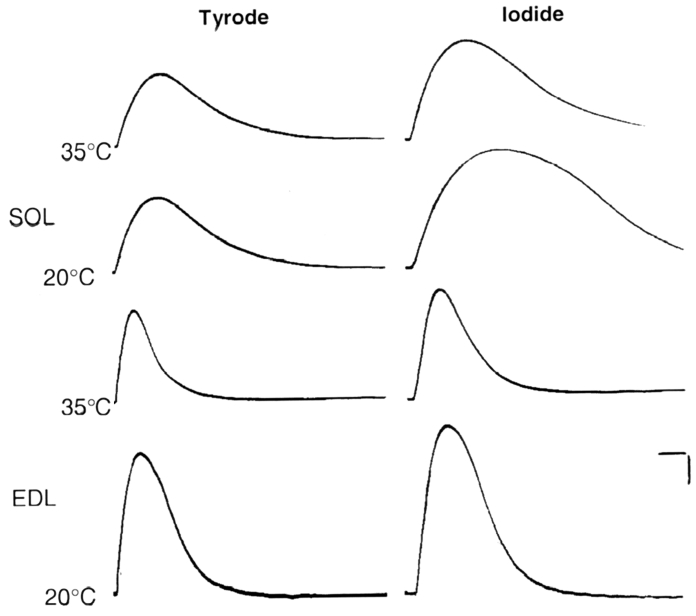

Fig. 1. *Representative records of single twitches of soleus (SOL) and extensor digitorum longus muscles (EDL) at different temperatures (35 °C and 20 °C) evoked by direct stimulation in standard chloride Tyrode and experimental iodide solutions. These recording demonstrate the small cold depression (about 10 %) in the SOL, the cold potentiation in the EDL and the potentiation by the lyotropic iodide anion in both muscles. Note that the calibration for the twitch force is the same for both muscles and both temperatures; namely 40 mN. However, the time scale is 20 ms at 35 °C and 50 ms at 20 °C.*

Action of lyotropic anions on the contractile parameters of EDL and SOL muscles at 20 °C

At a temperature of 20 °C of the bath, a three to four fold prolongation was found for the CT and HRT values of the EDL muscle and the P_t showed a cold potentiation of about 60 % (Fig. 1, Table 1). Starting from that level, both time parameters and maximum twitches were increased by the application of lyotropic anions. The proportion of this potentiation followed the lyotropic order ($Cl^- < Br^- < NO_3^- < I^-$) and was comparable to that found at 35 °C (Fig. 2C, Table 1).

The SOL muscle also exhibited an increase of the CT and HRT at 20 °C by a factor of three, however, the P_t showed a small cold depression of about 10 % (Fig. 1, Table 1). As in the EDL muscle, lyotropic anions enhanced the CT and HRT as well as the P_t of the SOL muscle. The effect of Br^- was statistically not significant (as it only reversed the cold depression), but the lyotropic potentiation of NO_3^- ($p<0.05$) and I^- ($p<0.01$) was higher at 20 °C than at 35 °C (Fig. 2D, Table 1).

The effects on P_t, CT and HRT in both EDL and SOL muscles at 20 °C were quickly reversed to pre-treatment levels by replacing the test solutions with the standard Tyrode solution (Fig. 2). The T_0 values of both muscles were about 10 % lower at 20 °C compared to 35 °C. The cold depression of the tetanus and the cold potentiation of the twitch in the EDL muscle were followed by an increase of TTR. In the SOL muscle, however, the cold depression of the tetanus was combined with a cold depression of the twitch in the same proportion so that the TTR was practically unchanged (Table 1). Lyotropic anions had no influence on T_0, but did affect P_t, hence the TTR values were increased in both muscles in the same order as the twitch potentiation (Br^-: not significant; NO_3^-: $p<0.05$; I^-: $p<0.01$; Table 1).

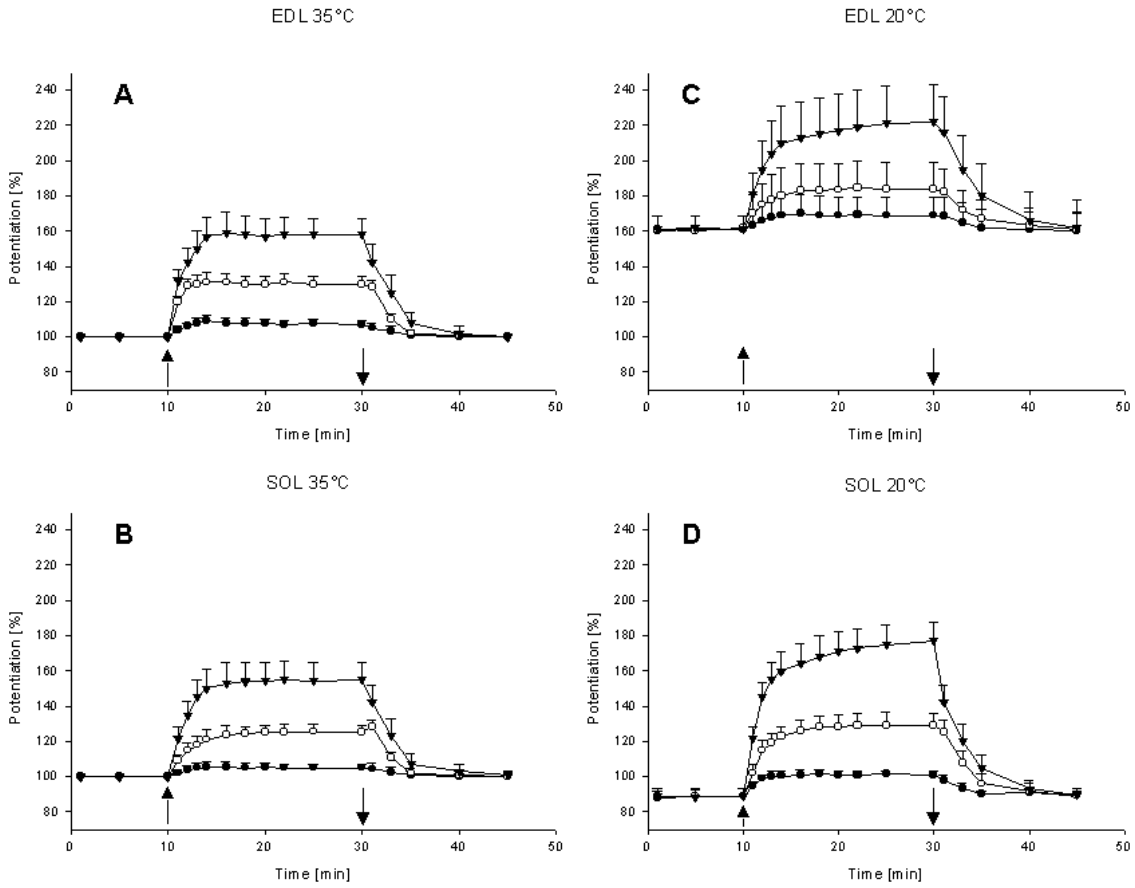

Fig. 2. *Average changes of maximal twitch tension of extensor digitorum longus (EDL; A, C) and soleus (SOL; B, D) muscles at different bathing temperatures (35 °C: A, B and 20 °C: C, D) expressed as a function of time in bromide (Br^-, filled circles), nitrate (NO_3^-, open circles), and iodide (I^-, filled triangles) Tyrode solutions. The 100 % level represented the peak isometric P_t developed by the muscle in Cl^--Tyrode solution at 35 °C and the experimental values are plotted as percentage (%) of the control tension. ↑ = change to the test solution; ↓ = return to the standard Tyrode solution. Data are presented as mean ± S.D. (n = 6-8).*

Discussion

Earlier studies on the effects of lyotropic anions had mainly focused on amphibian muscles (Ritche 1954, Kahn and Sandow 1955). In the present study we described the influence of these anions at different muscle temperatures on twitch force and twitch time tension in slow- and fast-twitch muscles of a warm-blooded animal, namely the rat. We have shown that compared to normal Cl^- Tyrode solution, Br^-, NO_3^- and I^- profoundly alter the contractile behavior of the SOL and EDL muscles as they prolong the CT and HRT, although to a different extent (Table 1), and enhance the twitch force (Fig. 2). Comparable results have also been found in the mouse gastrocnemius and soleus muscles at 15 ^oC (Brust 1966). Unlike the potentiation by caffeine (Wondmikun *et al*., data to be published) lyotropic twitch potentiation resulted in similar changes of the profiles of force and time parameters in the SOL and EDL muscles (see Table 1, Fig. 2). Strikingly, no difference in the extent or pattern of potentiation was observed by changing the bathing temperature in EDL and SOL with

the exception of I^- in the SOL muscle (Fig. 2D). The relative effectiveness of the modified Tyrode's solutions (bromide-, nitrate-, iodide-Tyrode) in producing twitch tension potentiation is in agreement with the usual lyotropic series of these anions replacing the chloride anion. Besides the parameters tested here, changes in the shortening of the latent period and an increase in the latency relaxation has been shown in sartorius muscles of the frog (Kahn and Sandow 1955).

The effect of the anions was evident within a few minutes. In our experiments, the full effect of Br^-, NO_3^- and I^- on both muscles and at both test temperatures on the CT, HRT and P_t was attained within about 5 min (Fig. 2). Considering the relatively slow diffusion rate of the anions (for NO_3^-, see Conway and Moore 1945), it is less likely that the whole effect of the anions is intracellular. Ritche (1954) found that NO_3^- prolonged the duration of the active state of frog sartorius muscle at 0 °C. It was therefore suggested that the muscle membrane is the primary site of lyotropic anion action (Kahn and Sandow 1950). This assumption is supported by the fact that the half-renewal times of the chloride ions in the intercellular space and inside the cell are 2 and 10 min, respectively (Levi and Ussing 1949).

Table 1. Effects of lyotropic anions (Br^-, NO_3^-, I^-) and bathing temperature (35 °C and 20 °C) on contractile properties of fast extensor digitorum longus (EDL) and slow soleus (SOL) muscles of the rat.

	Test solutions			
Parameter	**Cl^- (normal)**	**Br^-**	**NO_3^-**	**I^-**
EDL 35 °C				
CT [ms]	13.1±3.4	13.5±4.0	15.1±2.9*	20.3±3.4***
HRT [ms]	14.5±4.0	15.1±4.2	17.2±3.9*	23.8±4.8***
P_{T35}/P_{T35} [1]	1	1.08±0.02*	1.30±0.04**	1.58±0.12***
T_{O35}/T_{O35} [2]	1	1.00±0.01	1.01±0.02	1.01±0.02
Twitch/Tetanus	0.15±0.06	0.16±0.05	0.20±0.06*	0.25±0.06**
EDL 20 °C				
CT [ms]	46.1±5.1	46.5±5.5	49.4±7.9*	54.3±4.9**
HRT [ms]	57.2±6.0	57.6±6.6	60.6±8.9*	65.9±7.8**
P_{T20}/P_{T35} [1]	1.61±0.18	1.69±0.10	1.84±0.15**	2.22±0.21***
T_{O20}/T_{O35} [2]	0.87±0.04	0.89±0.04	0.87±0.03	0.88±0.03
Twitch/Tetanus	0.26±0.08	0.26±0.09	0.30±0.09*	0.39±0.10**
SOL 35 °C				
CT [ms]	31.0±7.4	31.5±5.2	42.1±6.2*	52.3±8.8**
HRT [ms]	39.3±8.0	41.1±7.2	50.2±8.9*	63.8±8.8**
P_{T35}/P_{T35} [1]	1	1.05±0.02*	1.25±0.05**	1.55±0.11***
T_{O35}/T_{O35} [2]	1	1.01±0.01	0.99±0.02	0.98±0.03
Twitch/Tetanus	0.15±0.06	0.16±0.05	0.20±0.06*	0.25±0.06**
SOL 20 °C				
CT [ms]	84.9±14.3	88.4±14.5	114.0±9.9*	125.3±14.9**
HRT [ms]	134.3±16.1	141.6±14.6	156.6±18.9*	190.9±27.8**
P_{T20}/P_{T35} [1]	0.89±0.04	1.01±0.05*	1.29±0.07**	1.77±0.11***
T_{O20}/T_{O35} [2]	0.90±0.04	0.88±0.05	0.88±0.03	0.89±0.05
Twitch/Tetanus	0.14±0.02	0.16±0.04	0.20±0.04*	0.28±0.06***

*Data are expressed as means ± S.D., n = 6-8. CT – contraction time. HRT – half-relaxation time. P_T – twitch tension, [1] expressed as a fraction of the twitch tension developed at 35 °C (the proportion in normal Cl^--Tyrode solution is considered as 1). T_O = tetanic tension, [2] expressed as a fraction of the tetanic tension developed at 35 °C (the proportion in normal Cl^--Tyrode solution is considered as 1). Significant actions of lyotropic anions in comparison to Cl^--Tyrode solution: * $p < 0.05$, ** $p < 0.02$, *** $p < 0.01$).*

Other studies (Bianchi 1961, 1965, Carvalho 1968) have shown that the used anions may affect the twitch not only by acting on the surface membrane by prolonging the action potential and lowering its mechanical threshold, but also by depressing the relaxing activity of the sarcoplasmic reticulum (SR); it was found that the uptake of Ca^{2+} by isolated SR preparation is depressed by Br^-, NO_3^-, I^- and SCN^-. This effect is similar to the effects of caffeine (Weber and Herz 1969), but caffeine, unlike the lyotropic anions, readily penetrates into the muscle fiber (Isaacson and Sandow 1967, Bianchi 1968, Fryer and Neering 1989). It is thus possible that the anions influence the mechanical parameters of muscles, both by their effect on the sarcolemma, leading to a lowered threshold, and by a depression of the Ca^{2+} uptake by the SR, resulting in a prolongation of the active state with a decreasing relaxation. However, it is probable that other mechanisms also contribute to the action of lyotropic anions. It is known that the muscle plasmatic membrane is highly permeable to chloride anions and that this permeability contributes to membrane potential stability. However, anion channels are generally considered to be more permeable for I^-, NO_3^- and Br^- than for Cl^- and the replacement of anions reflecting different membrane anion selectivity probably influences the excitation threshold, the membrane potential or its stability. Interestingly, the same permeability ratios as we have found in muscles have been described for voltage-sensitive Cl^- channels in rat neurons (Franciolini and Nonner 1987) and for other types of receptors (for review see e.g. Hille 2001). Furthermore, the fact that these anions cause swelling of muscle cells indicates that the replacement could affect not only SR membrane Ca^{2+} reuptake, but also directly or indirectly mechanisms regulating cell volume and intracellular composition (Na^+,K^+-ATPase, Na^+,K^+,$2Cl^-$-cotransport, e.g. van Mil *et al.* 1997).

The rapid return of the peak P_t outputs to nearly normal values irrespective of the temperature, anionic form or muscle type can be achieved when the anionic experimental solutions are abruptly replaced by normal Tyrode solution (see Fig. 2 after 30 min). Each reversal to the original P_t level is essentially attained in 2-3 min and completed within 10 min. We can therefore conclude that the potentiating effect of lyotropic anions is similar in both fast EDL and slow SOL muscles, it can be rapidly reversed by the chloride solution and that it does not depend (with exception of iodide) on temperature changes.

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